IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ELAN PHARMA INTERNATIONAL LIMITED,	)	
Plaintiff,	)	C.A. No. 06-438-GMS
v.	)	
ABRAXIS BIOSCIENCE, INC.,	)	
Defendant.	)	

PLAINTIFF ELAN PHARMA INTERNATIONAL LIMITED'S OPENING CLAIM CONSTRUCTION BRIEF

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Plaintiff Elan Pharma International Limited ("Elan") respectfully submits its Opening Claim Construction Brief in support of its proposed claim constructions of the disputed terms in the patents-in-suit, U.S. Patent No. 5,399,363 ("the '363 Patent") and U.S. Patent No. 5,834,025 ("the '025 Patent").

I. NATURE AND STAGE OF THE PROCEEDINGS

Elan filed its Complaint on July 19, 2006 alleging that Defendant Abraxis BioScience, Inc. ("Abraxis") infringes the '363 Patent and the '025 Patent under 35 U.S.C. § 271 by making, using, selling, and/or offering to sell Abraxane®, contributing to the use of Abraxane® by others, and/or inducing others to use Abraxane®. (D.I. 1). Abraxis filed an Answer and Counterclaims to Elan's Complaint requesting a declaration of invalidity and non-infringement of the '363 and '025 Patents. (*See* D.I. 7, D.I. 84). This matter is set for trial on June 2, 2008.

In order to evaluate the merits of the Parties' claims, the Court is tasked with construing the disputed claim terms at issue. Elan and Abraxis submitted a Joint Claim Chart on April 20, 2007 (D.I. 99), as required by the Court's Scheduling Order. (D.I. 20). Elan submits this brief in support of its proposed claim constructions.

II. SUMMARY OF ARGUMENT

Elan's proposed claim constructions are well-grounded in the principles recently re-affirmed by the United States Court of Appeals for the Federal Circuit ("Federal Circuit") in *Phillips v. AWH Corp.*, 415 F.3d 1303 (Fed. Cir. 2005) (*en banc*). In accordance with those principles, Elan's proposed claim construction primarily relies on the intrinsic evidence, *i.e.*, the claims, the specifications, and the file histories of the patents-in-suit and of the parent patent to the '363 Patent.

To the extent that Elan's proposed claim constructions refer to extrinsic evidence, such evidence wholly supports the intrinsic evidence. Accordingly, Elan employs the correct canons of claim construction articulated by the Federal Circuit.

Abraxis's proposed claim constructions, on the other hand, often include definitions that differ from the ordinary and customary meaning given a term interpreted by one skilled in the pertinent art. At other times, Abraxis proposes constructions that are neither based on the intrinsic evidence nor on relevant extrinsic evidence. Accordingly, Abraxis's proposed claim constructions are inconsistent with the Federal Circuit precedent and must.

III. INTRODUCTION

The patents-in-suit relate to nanoparticle technology.[1] The inventors' discoveries permit (1) formulation of poorly water soluble chemotherapeutic drugs so they are stable during storage, more effective, and less toxic; and (2) a safer way to administer nanoparticles[2] intravenously. These pioneering discoveries, which resulted in grant of the '363 and '025 patents, respectively, lay the foundation for cancer patients to receive more effective, safer treatment for their cancer.

Abraxis's product, Abraxane® employs Elan's important advances in formulating and delivering poorly soluble chemotherapeutic drugs. The Abraxane® product is nanoparticle paclitaxel, a potent chemotherapeutic agent, coated with human

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[1] *See generally* the '363 Patent (Ex. A), and the '025 Patent (Ex. B).

[2] Nanoparticles in the context of these patents are particles that are less than 1000 nanometers ("nm") (and preferably less than 400 and 300 nm) in size. For perspective, a "nanometer" is defined as "one-billionth of a meter." *Webster's Ninth New Collegiate Dictionary* 786 (1991) (Ex. C).

serum albumin, as claimed in the '363 patent. Abraxis instructs others to administer these nanoparticles at a rate that avoids or reduces adverse hemodynamic effects, as claimed in the '025 patent.

A. *The '363 Patent*

1. The Problem

The delivery of drugs that do not dissolve well in water, otherwise known as poorly water soluble drugs, has always been problematic. Poor solubility hampers drug dissolution in the body, which is necessary for drug bioavailability. "Bioavailability" refers to the extent to which a drug reaches its site of action.[3] When a drug does not reach its site of action, it cannot be effective. Most anticancer drugs are poorly water soluble and consequently have correspondingly lower therapeutic value because of their reduced bioavailability.

One approach to enhancing the bioavailability, and therefore the effectiveness of anticancer agents, is to use organic solvents. The organic solvents increase the solubility/bioavailability of the anticancer agent, but are often toxic to the patient. Thus, although organic solvents can help the solubility of drugs, they also may be harmful to patients.

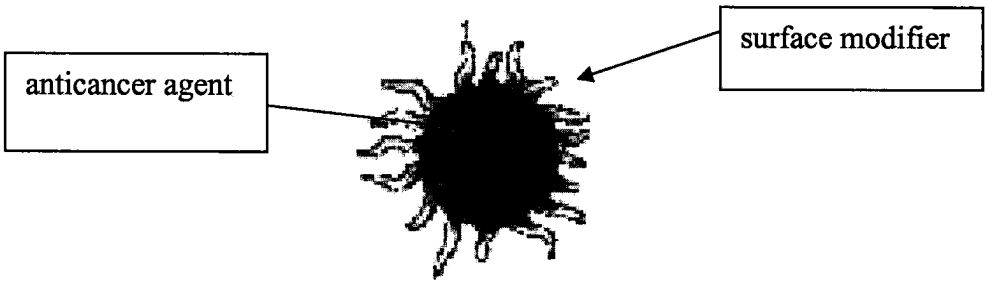
2. The Solution

The inventors of the '363 Patent developed a nanoparticle technology useful for the delivery of poorly water soluble anticancer drugs, and they used this technology to

[3] *The Pharmacological Basis of Therapeutics* 5 (7th ed. 1985) (Ex. D).

successfully create an anticancer drug particle with improved bioavailability that avoids solvent-related toxicity.[4]

The novel nanoparticles, depicted below, have two main components (1) a drug substance; and (2) a surface modifier, which is coated onto the surface of the drug substance.[5]



Typically, nanometer-sized drug particles combine with each other to form larger drug particles. This process is known as aggregation or flocculation. Importantly, the inventors discovered that the nanometer-sized drug particles could be stabilized or maintained by the surface modifier coating on the drug surface, such that aggregation or flocculation is hindered.

The discovery described in the '363 Patent has significant advantages over then-existing technology. The maintenance of the very small sized particles enhanced the effectiveness of the poorly water soluble anticancer agent by increasing its bioavailability. To illustrate, consider how sugar dissolves in a cup of tea. Given a sugar cube and an equal amount of granular sugar, the granular sugar dissolves more

[4] The '363 Patent, col. 1, ll. 44-47; col. 2, ll. 18-27 (Ex. A).

[5] *Id.* col. 1, ll. 48-54.

easily. When granular, the sugar is more soluble because it has a much larger surface area.

Equally important to the discovery was that the inventors' approach avoided solvent-related toxicity. Advantageously, the nanoparticles described by the '363 Patent are essentially free of solvent contamination and therefore do not magnify the toxicity of the anticancer drug.

B. *The '025 Patent*

1. The Problem

For some unknown reason, intravascular administration of nanoparticles was observed to cause significant cardiovascular dysfunction in a sensitive mammal receiving the nanoparticles.[6] The significant cardiovascular dysfunction, known as hemodynamic effects, encompasses reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility.[7]

2. The Solution

The invention of the '025 Patent is based on the discovery that the significant cardiovascular dysfunction observed with intravenous administration of nanoparticles is associated with the surface modifier-particle combination.[8] Armed with this knowledge, the inventors developed a method for administering nanoparticles such that the adverse hemodynamic effects are eliminated or reduced. The inventive method of administration is based on the discovery that adverse hemodynamic effects could be

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[6] The '025 Patent, col. 1, ll. 49-52.

[7] *Id.*

[8] *Id.* col. 6, ll. 61-62.

eliminated or reduced by controlling the intravenous infusion rate of the nanoparticles at less than 10 milligrams per minute.[9]

IV. LEGAL STANDARDS

The basic principles of claim construction were recently restated by the Federal Circuit in *Phillips,* 415 F.3d 1303. There, the court reaffirmed the principles of claim construction outlined in three prior decisions, namely, (1) *Markman v. Westview Instruments, Inc.,* 52 F.3d 967 (Fed. Cir. 1995) (*en banc*), *aff'd* 517 U.S. 370 (1996); (2) *Vitronics Corp. v. Conceptronic, Inc.,* 90 F.3d 1576 (Fed. Cir. 1996); and (3) *Innova/Pure Water, Inc. v. Safari Water Filtration Sys., Inc.,* 381 F.3d 1111 (Fed. Cir. 2004).[10]

Claim construction begins with the intrinsic evidence of the subject patent, *i.e.*, the claims, specification, and prosecution history.[11] "Because claim terms are normally used consistently throughout the patent, the usage of a term in one claim can often illuminate the meaning of the same term in other claims."[12] Further, differences among claim terms can be a useful guide in understanding the meaning of a particular claim

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[9] *Id.* col. 1, ll. 62-65.

[10] *Phillips,* 415 F.3d at 1312 ("What we said in those cases bears restating, for the basic principles of claim construction outlined there are still applicable, and we reaffirm them today.").

[11] *Vitronics Corp.*, 90 F.3d at 1582.

[12] *Phillips,* 415 F.3d at 1314 (citing *Rexnord Corp. v. Laitram Corp.*, 274 F.3d 1336, 1342 (Fed.Cir. 2001); *CVI/Beta Ventures, Inc. v. Tura LP*, 112 F3d 1146, 1159 (Fed. Cir. 1997).

term.[13] Therefore, the starting point for proper claim construction is a review of all of the claims of the subject patent.[14]

Although the words of a claim are given their ordinary and customary meaning to a person of ordinary skill in the pertinent art in question at the time of the invention, it would be legal error to construe a claim by considering it in isolation.[15] Rather, a claim must be read in view of the specification of which it is a part.[16] In this regard, the Federal Circuit in *Phillips* noted that:

> Importantly, the person of ordinary skill in the art is deemed to read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification.[17]

Additionally, the person of ordinary skill in the art is deemed to have knowledge of any special meaning to the claim terms.[18]

"[T]he prosecution history can often inform the meaning of the claim language by demonstrating how the inventor understood the invention and whether the inventor limited the invention in the course of prosecution . . .".[19] During prosecution, a patent

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[13] *Id.*

[14] *See id.*

[15] *Bell Commc'ns Research, Inc. v. Vitalink Commc'ns Corp.*, 55 F.3d 615, 620 (Fed. Cir. 1995).

[16] *Id.*

[17] *Phillips,* 415 F.3d at 1313.

[18] *Id.*

[19] *Nystrom v. Trex Co.*, 424 F.3d 1136, 1142 (Fed. Cir. 2005) (quoting *Phillips*, 415 F.3d at 1317).

applicant may consistently and clearly use a term in a manner either more or less expansive than it is used in the relevant art, thereby expanding or narrowing the scope of the term in the context of the patent claims.[20] Notably, "in order to disavow claim scope, a patent applicant must clearly and unambiguously express surrender of subject matter during prosecution." [21]

In addition to the intrinsic evidence, a court may look to extrinsic evidence to discern the meaning of a claim term.[22] Such evidence includes dictionaries, learned treatises and expert testimony.[23] The use of extrinsic evidence is especially helpful when the ordinary meaning of an art-specific term is not immediately apparent to judges and others outside the pertinent field.[24] Since extrinsic evidence is potentially less reliable in interpreting claim terms than intrinsic evidence, the Federal Circuit has endorsed the use of extrinsic evidence when it serves as an aid to "help educate the court regarding the field of the invention." and to "determine what a person of ordinary skill in the art would understand claim terms to mean."[25] In this manner, the Federal Circuit has enunciated that the extrinsic evidence should be viewed in the context of the intrinsic evidence.[26]

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[20] *Sorenson v. Int'l Trade Comm'n*, 427 F.3d 1375, 1378 (Fed. Cir. 2005).

[21] *Id.*

[22] *Phillips*, 415 F.3d at 1317.

[23] *Id.*

[24] *Id.* at 1214 (citing *Innova/Pure Water, Inc. v. Safari Water Filtration Systems, Inc.* 381 F.3d 111, 116 (Fed. Cir. 2004)).

[25] *Id.* at 1319.

[26] *Id.*

V. CLAIM CONSTRUCTION

A. *Disputed Claim Terms Common to Both Patents-in-Suit*

1. "consisting essentially of"

The disputed phrase "consisting essentially of" is found in claim 1 of the '363 Patent and in claims 1 and 13 of the '025 Patent. The contentions of the parties regarding the proper construction is shown below.

Claim 1 of the '363 Patent	
Elan's Construction	*Abraxis's Construction*
The particles, which include a "crystalline medicament useful for treating cancer" and "surface modifier," are essentially free of solvent contamination.	The particles are composed only of "crystalline medicament," "surface modifier," and at most trace amounts of solvent or other contamination.
Claim 1 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
The particles, which include a "crystalline organic drug substance" and a "surface modifier," are essentially free of solvent contamination.	The particles are composed only of a "crystalline organic drug substance," a "surface modifier," and at most a trace amount of solvents or other contaminants that would not change the basic and novel characteristics of the particles.
Claim 13 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
The particles, which include an "organic drug substance" and "liposome or colloidal polymeric material," are essentially free of solvent contamination.	The particles are composed only of a "crystalline organic drug substance," a "surface modifier," and at most a trace amount of solvents or other contaminants that would not change the basic and novel characteristics of the particles.

"Consisting essentially of" is a standard transitional phrase in patent law. Specifically, this transition allows for elements (such as materials) not listed in a patent claim "provided that they do not materially affect the basic and novel properties of the invention."[27]

[27] *AK Steel Corp. v. Sollac and Ugine*, 344 F.3d 1234, 1239 (Fed. Cir. 2003) (quoting

During prosecution of the parent patent application to the '363 Patent, U.S. Patent Appln. No. 07/647,105, which issued as U.S. Patent No. 5,145,684 ("the '684 Patent"),[28] the patentee indicated what "basic and novel properties" were relevant.

> The particles are free of unacceptable solvent contamination and consist essentially of the crystalline drug substance and the surface modifier adsorbed on the surface thereof.[29]

The resulting particles "consist essentially of" drug and modifier because they are free of contaminating solvents. Such contaminants are the *only* materials that an ordinary artisan would conclude are excluded, based on the file history.

The Summary of the Invention emphasizes reduced toxicity.[30] The first sentence describing the preferred embodiments identifies reduced toxicity as a basis for the invention.[31] Additionally, the prosecution history of the '363 Patent specifies that prior art particles made from conventional solvent precipitation techniques are contaminated with toxic solvents.[32] The specification and file history indicate which

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PPG Indus. v. Guardian Indus. Corp., 156 F.3d 1351, 1354 (Fed. Cir. 1998)).

[28] *See* the '684 Patent (Ex. E). The '363 Patent was filed as a continuation-in-part application of the application that issued as the '684 Patent. *See* the '363 Patent (Exh. A).

[29] The '684 Patent Prosecution History, Paper No. Paper No. 10/a, Nov. 18, 1991 Amendment at 7; *see also id.* at 9 ("By 'consisting essentially of' it is meant that the claimed particles are essentially free of solvent contamination and other toxic materials, e.g., monomer or initiator, resulting from solvent precipitation and polymerization methods of particle preparation.") (Ex. F).

[30] The '363 Patent, col. 1, ll. 24-27; col. 1, ll. 44-47; col. 1, ll. 60-65 (Ex. A).

[31] *Id.* col. 2, ll. 18-21.

[32] The '363 Patent Prosecution History, Paper No. 2, Information Disclosure Statement at 2; Paper No. 6/a, Sept. 9, 1993 Amendment; Paper No. 7, Liversidge Decl. ("The crystalline particles described in [this application] are essentially free of solvent

materials should be excluded from the compositions of the invention—solvent contamination.

The specification of the '025 Patent refers to the disclosure of the '684 Patent for a description of nanoparticles.[33] Thus, the particles of the '684 Patent are relevant to the nanoparticles claimed in the '025 Patent. Those nanoparticles are essentially free of solvent contamination. The term "consisting essentially of" should be construed to have the same meaning in both patents, as both parties have proposed.

Abraxis's construction would allow solvent contaminants as the *only* permissible ingredient beyond those listed in the claims. As emphasized in the specification and file history, this construction is *opposite* the purpose of the invention, which is to avoid contaminating solvents. It would also exclude the preferred embodiment of the '363 Patent, which includes material other than the recited drug and modifier—namely, a dispersion medium.[34]

Abraxis's construction is more consistent with another transitional phrase the patentee chose not to employ: "consisting of." That phrase, in contrast to "consisting essentially of," signifies that the scope of the claim is closed, *i.e.,* precludes additional components with the exception of impurities.[35] In effect, Abraxis is reading "essentially" out of the claims.

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contamination") (Ex. G).

[33] The '025 Patent, col. 1, ll. 23-27 (Ex. B).

[34] *Vitronics Corp.*, 90 F.3d at 1583 (claim construction excluding preferred embodiment is "rarely, if ever, correct and would require highly persuasive evidentiary support").

[35] *Conoco, Inc. v. Energy & Environ. Int'l L.C.*, 460 F.3d 1349, 1360-61 (Fed. Cir. 2006).

2. "surface modifier"

The term "surface modifier" is used in claim 1 of the '363 Patent and in claim 1 of the '025 Patent. The contentions of the Parties are shown below.

Claim 1 of the '363 Patent	
Elan's Construction	*Abraxis's Construction*
a stabilizing agent capable of hindering the aggregation, flocculation and/or agglomeration of the particles.	a substance that physically adheres to the surface of the crystalline medicament but does not chemically bond to it, and which modifies the surface properties of the crystalline medicament.
Claim 1 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
a surface active agent or stabilizing agent capable of hindering the aggregation, flocculation and/or agglomeration of the particles.	a substance that physically adheres to the surface of the crystalline medicament but does not chemically bond to it, and which modifies the surface properties of the crystalline drug substance.

The term "surface modifier" defines a property for these materials. To determine what that property is, we look to the claims themselves and the specifications. The claims of each patent explicitly state that the surface modifier maintains the effective average particle size.[36] Moreover, the specifications explain that the "surface modifier" stabilizes the particle by hindering or preventing flocculation, agglomeration, and/or congregation of the particles.[37] Notably, small particles were typically known to combine or agglomerate to form larger particles.

The specification of the '684 patent explains that the surface modifier minimizes or hinders the close contact between the particles necessary for agglomeration or flocculation to occur. This is achieved by the surface modifier functioning as a barrier

[36] The '363 Patent, claim 1 (Ex. A); the '025 Patent, claim 1 (Ex. B).

[37] The '363 Patent, col. 4, ll. 53-56; col. 8, ll. 31-32; col. 9, ll. 44-45 (Ex. A); the '025 Patent, col. 1, ll. 37-38 (Ex. B).

between drug particles thereby minimizing close contact or alternatively by preventing the close contact between the particles by repulsion.[38] Elan's construction gives effect to these defined properties of the "surface modifier."

On the contrary, Abraxis's construction does not address the basis properties of a "surface modifier." Rather, Abraxis merely exemplifies how a surface modifier might interact with a drug substance, whether it is the "crystalline medicament" of the '363 Patent or the "crystalline organic drug substance" of the '025 Patent. This construction of improperly collapses two distinct claim terms, *i.e.*, "surface modifier" and "adsorbed on the surface."

3. "adsorbed on the surface"

The term "adsorbed on the surface" is used in claim 1 of the '363 Patent and in claim 1 of the '025 Patent. The contentions of the Parties are shown below.

Claim 1 of the '363 Patent	
Elan's Construction	*Abraxis's Construction*
means that the surface modifier physically contacts the surface of the "crystalline medicament useful for treating cancer" and does not chemically react with such medicament.	means that the surface modifier physically adheres to the surface of the crystalline medicament and does not form a chemical bond with the crystalline medicament.
Claim 1 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
means that the surface modifier physically contacts the surface of the drug substance and does not chemically react with such drug substance.	means that the surface modifier physically adheres to the surface of the crystalline drug substance and does not form a chemical bond with the crystalline drug substance.

The sole dispute is whether this term is limited to "adherence" to a surface, or is broad enough to encompass any contact. The specification of the '363 Patent teaches

[38] The '684 Patent, col. 8, ll. 22-29 (Ex. E).

that "[u]seful surface modifiers are believed to include those which physically adhere to the surface of the anticancer agent but do not chemically bond to the anticancer agent."[39] Thus, "adsorbed on the surface" must cover more than adherence. And the specification teaches there are other ways in which the surface modifier interacts with the medicament or anticancer agent. For example, "particles can be contacted with a surface modifier after attrition."[40] Accordingly, the proper construction of the term "adsorbed on the surface" in the '363 Patent is not limited to adhesion.

Regarding the '025 Patent, neither the claims nor specification explicitly define "adsorbed on the surface." The specification does, however, indicate that surface modified nanoparticles are "described in U.S. Patent No. 5,145,684."[41] Like the '363 Patent, the specification of the '684 Patent states "useful surface modifiers include those which physically adhere to the surface of the drug substance"[42] and that the "particles can be contacted with a surface modifier after attrition."[43] Finally, extrinsic evidence of this term suggests "collection of one substance on the surface of another."[44]

Rather than applying sound claim construction procedures, Abraxis limits the term to one embodiment even though the specification was drafted expressly to include others.

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[39] The '363 Patent, col. 3, ll. 57-60 (Ex. A).

[40] *Id.* col. 5, ll. 28-31.

[41] The '025 Patent, col. 1, ll. 23-25 (Ex. B).

[42] The '684 Patent, col. 4, ll. 28-33 (Ex. E).

[43] *Id.* at col. 5, ll. 16-19.

[44] Steven S. Zumdahl, *Chemistry* A25 (1986) (Ex. H).

4. "sufficient to maintain an average effective particle size"

The term is used in claim 1 of both patents-in-suit. The Parties' positions are summarized below.

Claim 1 of the '363 Patent	
Elan's Construction	*Abraxis's Construction*
means an amount of surface modifier that maintains the size of the particles such that at least 90 percent of the particles have a number average particle size of less than 1000 nanometers.	means that the particles contain an amount of surface modifier sufficient to maintain the effective average particle size of the particles for an unspecified period of time and under unspecified conditions, wherein the effective average particle size means that at least 90% of the particles have the number average particle size specified in the claim.
Claim 1 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
means sufficient surface modifier to maintain the size of the particles at an effective average particle size from about 50 to about 1000 nanometers.	means that the particles contain an amount of surface modifier sufficient to maintain the effective average particle size of the particles for an unspecified period of time and under unspecified conditions, wherein the effective average particle size means that at least 90% of the particles have the weight average particle size specified in the claim.

The claims and the specification of the '363 Patent require the surface modifier be "adsorbed on the surface" of the anticancer agent in an amount "sufficient to maintain an effective average particle size of less than 1000 nm."[45] The specification expressly defines "effective average particle size less than 1000 nm." as "at least 90% of the particles have a number average particle size of less than about 1000 nm."[46]

This is a clear instance of the patentee acting as his own lexicographer. Elan's construction is based on this definition in the specification. Abraxis's definition

[45] The '363 Patent, col. 1, ll. 50-54; claim 1 (Ex. A).

[46] *Id.* col. 4, l. 60 - col. 5, l. 10.

includes a number of extraneous limitations not found in the patentees own, express definition of this term.

B. *Disputed Terms of the '363 Patent*

Elan has asserted claims 1-3, 5 and 8-12 of the '363 Patent against Abraxis. In addition to the terms discussed above, the parties dispute the construction of three terms and phrases.

1. "crystalline medicament useful in treating cancer"

Elan's Construction	*Abraxis's Construction*
an anticancer drug as recited in the Markush group of the claim in a nanoparticulate phase different from an amorphous phase.	"crystalline" means having a regular arrangement of atoms in a space lattice, distinguished from amorphous. "medicament useful in treating cancer" means a medicine suitable for treating cancer.

Rather than construing the phrase "crystalline medicament useful in treating cancer," Abraxis breaks it into two separate terms: "crystalline" and "medicament useful in treating cancer." The intrinsic evidence supports Elan's construction, that is, "an anticancer agent as recited in the Markush group of the claim in a nanoparticulate phase, different from an amorphous phase."[47]

The claim itself defines the term "medicament" as specific, listed anticancer agents.[48] This is consistent with the use of "anticancer agent" throughout the specification of the patent.

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[47] "A Markush group lists specified alternatives in a patent claim, typically in the form: a member selected from the group consisting of A, B, and C." *Gillette Co. v. Energizer Holdings, Inc.*, 405 F.3d 1367, 1372 (Fed. Cir. 2005) (citing *Manual of Patent Examining Procedure* § 803.02 (2004)).

[48] The '363 Patent, claim 1 (Ex. A).

The prosecution history is also consistent with Elan's construction. The claims were amended from "crystalline anticancer agent" to "crystalline medicament useful in treating cancer" because the Examiner thought the former term suggested a cure for cancer and requested that the term "medicament" or "drug" be substituted for it.[49] In response, the claim was amended to include a Markush group of anticancer agents and further to recite a "medicament useful in treating a cancer susceptible to treatment with such medicament."[50] Additionally, the inventors noted that the "invention can be practiced with poorly soluble crystalline anticancer agents known in the art to be useful in treating susceptible cancer."[51] The express words of the claim require a construction of "medicament" as limited to the anticancer agents recited in the claim's Markush group.

Abraxis's proposed construction of "a medicine suitable for treating cancer" is not consistent with the claim's recitation of specific anticancer agents. These agents are not "medicines suitable for treating cancer," because they could not be administered to a patient without some form of carrier or excipient. The claim language requires that the "medicament useful in treating cancer" is a single component, selected from a group of specific possible compounds, of a multi-component particle. An ordinary reading of the claim language compels the conclusion that the "medicament useful in treating cancer" is only one component of a multi-component particle.

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[49] The '363 Patent Prosecution History, Paper No. 9, Dec. 22, 1993 Office Action at 3 (Ex. G).

[50] *Id.* Paper No. 12B, June 22, 1994 Amendment at 2.

[51] *Id.* Paper No. 12B, June 22, 1994 Amendment at 3.

Differences between claims 1, 8 and 9 support a construction that "crystalline medicament useful in treating cancer" is one component of a multi-component article. Claim 8 recites an anticancer composition comprising the particles of claim 1, *i.e.,* a composition comprising the medicine;[52] claim 9 recites a method of treating a mammal comprising administering the anticancer composition (of claim 8).[53] It is well established that different words or phrases used in separate claims are presumed to indicate that the claims have different meanings and scope.[54] Taken in context, differences among claims 1, 8, and 9 indicate that the term "anticancer composition" is a "medicine suitable for treating cancer" because it is administered to a mammal, and this medicine, which contains the nanoparticles, includes the "crystalline medicament useful in treating cancer," *i.e.,* an anticancer agent, as one component of the nanoparticle.

Abraxis's construction of the term "crystalline" is based on an isolated dictionary definition and unsupported by the intrinsic evidence. Throughout the '363 Patent, nanoparticles are identified as for the delivery of poorly water soluble anticancer agents. The specification describes "crystalline"[55] in contradistinction to amorphous materials that "result[ ] from conventional solvent precipitation techniques."[56] Elan's

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[52] The '363 Patent, claim 8 (Ex. A).

[53] *Id.* claim 9.

[54] *Karlin Tech. v. Surgical Dynamics, Inc.*, 177 F.3d 968, 971-72 (Fed. Cir. 1999) ("The doctrine of claim differentiation . . . which is ultimately based on the common sense notion that different words or phrases used in separate claims are presumed to indicate that the claims have different meanings and scope . . .").

[55] *Id.* col. 1, ll. 48-50.

[56] *Id.* col. 2, ll. 30-31.

construction is based upon this distinction drawn in the specification. It is also consistent with statements in lines 28-29 of column 2, that "the anticancer agent is present in one or more discrete crystalline phases."[57]

"Crystalline" has a special meaning in the nanoparticulate art and in this patent. In the 1980's, persons of ordinary skill in this art recognized that the "crystallinity was a variable property that ranged from 100% (a perfect crystal) to 0% (amorphous) depending on the state of order/disorder in the lattice.[58] It was also recognized that milling or grinding processes reduced a material's crystallinity.[59] The '363 Patent teaches these processes as the way to reduce the anticancer agent to nanoparticulate sizes.

The phrase "crystalline medicament useful in treating cancer" properly interpreted, in the context of the intrinsic evidence,[60] means "an anticancer agent as recited in the Markush group of the claim in a nanoparticulate phase, which is different from an amorphous phase."

2. "non-crosslinked"

This term is used in claim 1 of the '363 Patent. The Parties' contentions are found below.

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[57] *Id.* col. 2, ll. 28-29.

[58] R. Suryanarayanan and A.G. Mitchell, *Evaluation of Two Concepts of Crystallinity Using Calcium Glucepate as a Model Compound,* 24 Int'l J. of Pharmaceutics 1, 2 (1985) (Ex. I); *see also* R. Cahn, *Strategies to Defeat Brittleness,* 332 Nature 112 (Ex. J); A. Saleki-Gerhardt *et al.*, *Assesment of Disorder in Crystalline Solids,* 101 Int'l J. of Pharmaceutics 237 (1994) (Ex. K).

[59] *See id.*

[60] *Medrad, Inc. v. MRI Devices Corp.,* 401 F.3d 1313, 1317 (Fed. Cir. 2005).

Elan's Construction	*Abraxis's Construction*
means that the surface modifier does not chemically react with the anticancer agent or itself.	means that the surface modifier molecules are individually adsorbed on the surface of the crystalline medicament and are essentially free of intermolecular crosslinkages between surface modifier molecules.

The specification of the '363 Patent notes that the "surface modifier does not chemically react with the anticancer agent or itself."[61] As no significant chemical reaction occurs between the anticancer agent and the surface modifier or with the surface modifier itself, both the "medicament" and the "surface modifier" are essentially chemically unaltered by the physical adsorption of the surface modifier to the medicament. This is consistent with the specification, which states that the "individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages."[62]

Abraxis contends that the term "noncrosslinked" means that the surface modifier is essentially free of cross linkages between surface modifier molecules. This definition is circular; it attempts to define a term by using the words of that term itself. In other words, Abraxis defines the term "noncrosslinked" by suggesting that it means "free of cross linkages" without further defining crosslinkage. Accordingly, the term "non-crosslinked" remains undefined by Abraxis and is inconsistent with the express definitions in the specification of the '363 Patent.

[61] The '363 Patent, col. 4, ll. 56-57 (Ex. A).

[62] *Id.* col. 4, ll. 58-59.

3. "surfactant"

The Parties' contentions regarding the proper construction of the term "surfactant" as found in claim 12 of the '363 patent is found below.

Elan's Construction	*Abraxis's Construction*
a stabilizing agent that reduces interfacial tension between or among immiscible substances.	a molecule that consists of two basic parts: a hydrophobic part (a non-polar hydrocarbon chain) and a hydrophilic part (a polar functional group).

"Surfactant" is defined in a general-purpose dictionary as a "surface-active substance,"[63] which in turn is defined as "altering the properties and esp. lowering the tension at the surface of contact between phases."[64] Regarding "surfactants," the specification states only that "preferred surface modifiers include nonionic and anionic surfactants."[65] Nothing in the specification or file history limits these "surfactants" to the ones identified by Abraxis—*i.e.*, molecules having hydrocarbon chains and polar functional groups.

C. *Disputed Terms of the '025 patent*

Elan has asserted claims 1-3 and 13-15 against Abraxis. In addition to the terms discussed above, the parties dispute the construction of the following terms and phrases:

1. "without eliciting adverse hemodynamic effects"

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[63] *Webster's Ninth New Collegiate Dictionary* 1187 (1991) (Ex. C); *see also* H. Schott, *Colloidal Dispersions,* Remington's Pharmaceutical Sciences 286 (17th ed.) (Ex. L) (cited on the face of the '363 Patent); *see also Grant & Hackh's Chemical Dictionary* 564 (5th ed. 1987) (Ex. M).

[64] *Id.*

[65] The '363 Patent col. 3, ll. 65-66. Further, Claim 12 of the '363 Patent illuminates that a "surfactant" is a "surface modifier."

Elan's Construction	*Abraxis's Construction*
means the claimed method of intravenous administration, reduces, eliminates, or does not elicit cardiovascular dysfunction, such as reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility in species of mammals that are sensitive to hemodynamic effects..	means that the claimed compositions do not elicit undesired effects on the physical aspects of blood circulation (such as reduction in arterial blood pressure and cardiac function) in substantially all of the mammals to whom the claimed compositions are administered.

The claims of the '025 Patent are directed to a method of administering a nanoparticulate composition and not to the composition *per se*.[66] Abraxis's is, in effect, construing these as composition claims, by requiring that the composition achieve specific results in all instances. As a method, the claim is satisfied in each case where the result (avoiding hemodynamic effects) is achieved.

From the plain ordinary meaning of the word "elicit," the claimed method of administration does not "draw forth" adverse hemodynamic effects.[67] These "hemodynamic effects" are defined in the specification: "cardiovascular dysfunctions, such as reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility."[68] Tables 1-7 report the "hemodynamic and hematological response" of dogs to the claimed methods. Those tables show variations in the mean arterial blood pressure of the test subjects.

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[66] *See, e.g.*, the '025 Patent Abstract ("Disclosed are methods of intravenous administration of nanoparticulate drug formulations . . .") (Ex. B).

[67] The word "elicit" means "to draw forth or bring out (something latent or potential)." *Webster's Ninth New Collegiate Dictionary* 404 (1991) (Ex. C).

[68] "We have observed that intravascular administration of [] suspensions to dogs causes significant cardiovascular dysfunction, such as reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility." The '025 Patent, col. 1, ll. 49-52 (Ex. B).

Elan's construction is consistent with the prosecution history, in which United States Patent and Trademark Office was told that:

> [a]pplicants unexpectedly discovered that [] hemodynamic effects associated with intravenous administration of nanoparticulate formulations can be eliminated, or substantially reduced by maintaining the intravenous infusion rate at less than 10 mg/ml, or by pretreating the patient with an antihistamine.[69]

Abraxis's construction—requiring results "in substantially all of the mammals to whom the claimed compositions are administered"—is not. The patentees indicates they had made the invention based solely on tests in dogs. Based on this discovery, the inventors recognized that reducing the rate of administration of nanoparticle compositions below the stated threshold would benefit other sensitive species, such as humans.

2. "infusion rate not exceeding 10 mg/min"

The Parties' contentions for this term as found in the '025 Patent are found below.

Claim 1 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
means intravenously administering the nanoparticles, which are comprised of the "organic drug substance" and the "surface modifier," at a rate not exceeding 10 milligrams per minute.	means intravenously administering the composition by means of gravity flow, at or below a rate of 10 milligrams per minute of the entire composition, including: (a) the drug, (b) the surface modifier, and (c) the pharmaceutically acceptable carrier.
Claim 13 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
means intravenously administering the nanoparticles, which are comprised of	means intravenously administering the composition by means of gravity flow, at or

[69] The '025 Patent Prosecution History, Paper No. 9, Feb. 4, 1998 Amendment at 4 (Ex. N).

the "organic drug substance" and the "liposome or colloidal polymeric material," at a rate not exceeding 10 milligrams per minute.	below a rate of 10 milligrams per minute of the entire composition, including: (a) the drug, (b) the liposome or colloidal polymeric material, and (c) the pharmaceutically acceptable carrier.

The focal point of the dispute seems to be what the term infusion rate refers to, *i.e.,* the subject matter being infused at a rate not exceeding 10 milligrams per minute ("mg/min"), although Elan is also uncertain as to what is meant by "means of gravity flow" and where Abraxis finds support for such construction. Abraxis contends that the nanoparticulate composition, *i.e.*, the nanoparticles plus the pharmaceutically acceptable carrier, is infused at a rate not exceeding 10 mg/min. This construction, however, is inconsistent with the plain meaning of the claim terms, specification, including the examples, and the prosecution history.

The claims recite a method of infusing a composition. This composition comprises drug particles or a drug, a surface modifying agent or a liposome or colloidal polymeric material, and a pharmaceutically acceptable carrier, at a particular infusion rate: at or less than 10 mg/min. The teaching of the specification and subject matter of the claims concerns the rate at which the *nanoparticles*, *i.e.*, the organic drug substance coated by a surface modifying agent or the organic drug substance in a liposome or colloidal polymeric material, are infused. While the nanoparticles are infused in suspension in a pharmaceutical carrier, the rate of infusion is not tied to the mass of the carrier. The specification notes that "[i]t has now been discovered that hemodynamic effects of suspensions can be eliminated or at least substantially reduced by *controlling the rate of infusion and/or the concentration of the active drug in the suspensions*."[70]

[70] '*See id.* col. 1, ll. 62-65 (emphasis added).

The specification further points out that the "[h]emodynamic effect is associated with the surfactant-particle combination."[71] Thus, it is the infusion rate of administration of the nanoparticles, which includes the drug, that is determinative.

To suggest otherwise would essentially eliminate the contribution of the nanoparticles to the recited administration rate, since the suspension liquid forms a much greater part of the whole composition by weight. This requires ignoring that the claims are directed to reducing hemodynamic affects caused by infusion of the nanoparticles above the threshold rate.

The examples disclosed by the inventors convey that one cannot measure the rate of infusion based on the total amount of the composition including the carrier because the results experiments are analyzed in terms of the dry weight of the nanoparticles, *i.e.*, milligrams per minute.[72] The values in Table 7 are determined by taking the concentration of nanoparticles in suspension, provided as percent solids, converting that to a weight to volume value, e.g., mg/ml, and multiplying that by the infusion rate of the suspension, provided in milliliters per minute.[73] Thus, the term is explicitly defined as the rate of administration of the nanoparticle.

Furthermore, any contrary definition, *e.g.*, the definition proposed by Abraxis, would exclude the examples from within the claim scope, since the examples describe the rate of administration of the entire suspension in ml/min. Since water is known to weigh about 1000 mg per ml, and infusion rate of suspension of 1 ml/min corresponds

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[71] The '025 Patent col. 6, ll. 61-62 (Ex. B).

[72] *Id.* Table 7.

[73] *Id.*

to 1000 mg/min; 0.5 ml/min corresponds to 500 mg/min. It is abundantly clear that the infusion rate provided in mg/min refers to the solids infusion rate, and not the suspension infusion rate. The cannons of claim construction disfavor a construction that excludes the specific embodiments.[74]

The prosecution history confirms this construction.

> After identifying the problem to be solved (i.e., adverse hemodynamic reactions associated with intravenous administration of nanoparticulate compositions), Applicants developed experiments designed to distinguish and separate effects arising from components of nanoparticulate formulations and effects of intravenous injections. The experiments comprised using inert polystyrene nanoparticles, surfactant-coated polystyrene nanoparticles, and surfactant alone in *in vivo* tests...Applicants discovered that hemodynamic effect is associated with the surfactant-particle combination.[75]

One skilled in the art would also recognize that the infusion rate not exceeding 10 mg/min refers to the nanoparticles. As noted by John D. Slack *et al.*, intravenous administration of particulate matter, *i.e.*, microspheres, may be associated with significant hemodynamic deterioration effects.[76] Accordingly, the evidence is clear; the term "infusion rate not exceeding 10 mg/min." should be interpreted as the rate of administration based on the infused mass of nanoparticles, whether they are composed

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[74] *See Vitronics Corp.*, 90 F.3d at 1583 (claim construction excluding preferred embodiment is "rarely, if ever, correct and would require highly persuasive evidentiary support").

[75] The '025 Patent Prosecution History, Paper No. 9/B, Feb. 4, 1998 Amendment at 5 (Ex. N).

[76] John. D. Slack *et al.*, *Acute Hemodynamic Effects and Blood Pool Kinetics of Polystyrene Microspheres following Intravenous Administration*, 70 J. of Pharmaceutical Sciences, 6 (1981) (cited in the Specification of the '025 Patent, col. 1, ll. 54-55) (Ex. O).

of "crystalline organic drug substance" and "surface modifier" or "organic drug substance" and "liposome or colloidal polymeric material," as recited in claims 1 and 13, respectively.

3. "crystalline organic drug substance"

The Parties' contentions of this term as found in claim 1 of the '025 patent are found below.

Elan's Construction	*Abraxis's Construction*
a therapeutic or diagnostic agent in a nanoparticulate phase different from an amorphous phase.	"crystalline" means having a regular arrangement of atoms in a space lattice, as distinguished from amorphous. "organic drug substance" means a crystalline carbon-based therapeutic or diagnostic agent suitable for administration to a mammal.

The first and most obvious flaw in Abraxis's construction is that it is superfluous; the term "crystalline" is defined, and the term "organic drug substance" is defined in part as "crystalline." Further, Elan is unaware of any intrinsic evidence that supports this construction of "crystalline." Indeed, as discussed above with reference to the term "crystalline medicament useful in treating cancer," nanoparticles have a nanoparticulate phase, which is recognized by one skilled in the art.

The specification of the '025 Patent references the '684 Patent's description of nanoparticles.[77] Like the '363 Patent discussed above, the '684 Patent informs one skilled in the art that the nanoparticles comprise a drug substance which exists as a discrete, crystalline phase that differs from a noncrystalline or amorphous phase.[78]

[77] The '025 Patent, col. 1, ll. 24-27 (Ex. B).

[78] The '684 Patent, col. 3, ll. 32-36; col. 4, ll. 28-31 (Ex. E).

The parties agree in part that the term "organic drug substance" means a therapeutic or diagnostic agent. Because Abraxis's construction is unsupported by the intrinsic evidence and further any extrinsic evidence that it may eventually rely upon would be unsupported in the context of this patent, the term "crystalline organic drug substance" should be construed as a therapeutic or diagnostic agent in a nanoparticulate phase different from an amorphous phase.

4. "organic drug substance"

The Parties' contentions regarding the term "organic drug substance" as that term is used in claim 13 of the '025 Patent are found below.

Elan's Construction	*Abraxis's Construction*
a therapeutic or diagnostic agent	a crystalline, carbon-based therapeutic or diagnostic agent suitable for administration to a mammal.

In accordance with the plain language of claim 13, the claim is directed to a method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects. The nanoparticulate drug composition includes particles, which have two main components; the first one being an "organic drug substance"; and the second on being a "liposome or a colloidal polymeric material."[79]

The specification states that the particles "comprise a therapeutic or diagnostic agent" and that such agents "are sometimes referred to as drugs or pharmaceuticals."[80] Nothing in the specification requires that these materials be crystalline. When the patentees intend a crystalline substance, they use the word "crystalline." For example, claim 1 refers to a "crystalline organic drug substance." If "organic substances," per

[79] The '025 Patent, claim 13 (Ex. B).

[80] *Id.* col. 7, ll. 24-27.

Abraxis's construction are "crystalline," then the use of that word in this phrase would be redundant. Claims are not construed so that terms like crystalline are redundant or surplusage.[81] Abraxis improperly imports a limitation from the specification into the claim by suggesting that the "organic drug substance" must be "crystalline."[82]

Abraxis assertion that the therapeutic or diagnostic agent must be "suitable for administration to a mammal" cannot be square with the structure of the claims. In addition to the "organic drug substance" the claim recites a drug composition that includes particles and a pharmaceutically acceptable carrier thereof. It is this drug composition—not the component "organic drug substance"—that is "suitable for administration to a mammal."

5. "colloidal polymeric material"

The Parties' contentions regarding this disputed term from claim 13 of the '025 patent is represented below.

Claim 13 of the '025 Patent	
Elan's Construction	*Abraxis's Construction*
polymeric carrier or vehicle for the organic drug substance capable of forming a suspension or dispersion.	"colloid" means a stable suspension of microscopic particles, size range one nanometer to one micron, dispersed in a continuous medium. "polymeric" means of, relating to, or consisting of a polymer. "material" means of, relating to, or consisting of matter.

[81] *See K-2 Corp. v. Salomon S.A.*, 191 F.3d 1356, 1363 (Fed. Cir. 1999); *Elekta Instrument S.A. v. O.U.R. Scientific Int'l, Inc.*, 214 F.3d 1302, 1307 (Fed. Cir. 2000); *Gen. Am. Transp. Corp. v. Cryo-Trans, Inc.* 93 F.3d 766, 770 (Fed. Cir. 1996).

[82] *See Tate Access Floors, Inc. v. Interface Architectural Resources, Inc.*, 279 F.3d 1357, 1371 (Fed. Cir. 2002).

For some unknown reason, Abraxis ignores the teaching of the specification and fragments the claim term into separate, unconnected words. The claim term properly construed is "colloidal polymeric material." The specification notes that in a liposome or colloidal drug delivery system, the bioactive drug is entrapped in the liposome or colloidal particles.[83] Thus, the "colloidal polymeric material" is a carrier or vehicle for the organic drug substance capable of forming a suspension or dispersion. Indeed, the specification notes that colloids are used as carriers for an active drug and form suspensions.[84] Even Abraxis concedes that the colloidal drug delivery system forms a suspension or a dispersion (in a continuous medium).

The claim language further supports Elan's construction. The claim requires a "pharmaceutically acceptable carrier," which would constitute the "continuous medium" for the colloidal drug delivery system to form a dispersion or a suspension. The specification further supports this conclusion, *i.e.*, the organic drug substance is entrapped in the liposome or colloidal polymeric material, for the formation of a suspension or dispersion.

VI. CONCLUSION

For the foregoing reasons, Elan respectfully requests that its constructions of the claims be adopted by the Court and that Abraxis's proposed interpretations be rejected.

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[83] *Id.* col. 2, ll. 56-60.

[84] *Id.* at col. 1, ll. 45-47.

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EXHIBIT A

US005399363A

United States Patent [19]

Liversidge et al.

[11] **Patent Number: 5,399,363**

[45] **Date of Patent: Mar. 21, 1995**

[54] **SURFACE MODIFIED ANTICANCER NANOPARTICLES**

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[21] Appl. No.: **908,125**

[22] Filed: **Jul. 1, 1992**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 647,105, Jan. 25, 1991, Pat. No. 5,145,684.

[51] **Int. Cl.**[6] **A61K 9/14**

[52] **U.S. Cl.** **424/490**; 424/489

[58] **Field of Search** 424/490, 487; 514/352

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[57] **ABSTRACT**

Dispersible particles consisting essentially of a crystalline anticancer agent having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 1000 nm. Anticancer compositions comprising the particles exhibit reduced toxicity and/or enhanced efficacy, and can be administered by IV bolus injection.

17 Claims, No Drawings

SURFACE MODIFIED ANTICANCER NANOPARTICLES

CROSS REFERENCED TO RELATED APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 647,105, filed Jan. 25, 1991, now U.S. Pat. No. 5,145,684, the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention relates to anticancer agents in the form of particles, to anticancer compositions comprising the particles, and to methods employing the particles.

2. Description of the Prior Art

The therapeutic index is a measure of how selective a drug is at producing its desired effects and can be defined as the ratio of the median lethal dose to the median effective dose, i.e., (LD_{50}/ED_{50}) (see Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, Eight Edition, p. 68–69). Virtually all anticancer agents have a low therapeutic index, e.g., less than about 1.0. Increasing the therapeutic index, e.g., by reducing toxicity or enhancing efficacy would provide more latitude to physicians in their duty of administering anticancer drugs to patients in need thereof. Consequently, methods to reduce toxicity and/or enhance efficacy of anticancer drugs and thus increase the therapeutic indices of such drugs would be of great value in the treatment of cancers.

In addition, poorly water-soluble drugs, such as poorly water-soluble anticancer agents, are not readily injectable via an intravenous (IV) bolus injection. The creation of injectable forms of poorly soluble drugs represents a formidable problem. It would be highly desirable to be able to provide poorly soluble drugs, such as poorly soluble anticancer agents, in an IV bolus injectable form.

SUMMARY OF THE INVENTION

We have discovered that anticancer compositions comprising anticancer agents in the form of surface modified nanoparticles exhibit reduced toxicity and/or enhanced efficacy.

More particularly, in accordance with this invention, there are provided particles consisting essentially of a crystalline anticancer agent having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 1000 nm.

This invention further provides an anticancer composition comprising the above-described particles.

In another embodiment of the invention, there is provided a method of treating a mammal comprising administering to the mammal the above-described anticancer composition.

In yet another embodiment of the invention, there is provided a method of enhancing the efficacy and/or reducing the toxicity of an anticancer agent which includes the step of administering the agent in the form of the above-described particles.

It is an advantageous feature of this invention that anticancer compositions are provided exhibiting reduced toxicity.

It is another advantageous feature of this invention that anticancer compositions are provided exhibiting improved efficacy.

Yet another advantageous feature of this invention is that compositions are provided featuring poorly soluble anticancer agents that can be administered by IV bolus injection.

Still another advantageous feature of this invention is that compositions are provided containing poorly soluble anticancer agents exhibiting prolonged circulation in the blood pool after IV bolus injection.

Other advantageous features will become readily apparent upon reference to the following descriptions of preferred embodiments.

DESCRIPTION OF PREFERRED EMBODIMENTS

This invention is based partly on the discovery that surface modified anticancer nanoparticles exhibit reduced toxicity and/or enhanced efficacy. While the invention is described herein primarily in connection with its preferred class of drugs, i.e., anticancer agents including immunosuppressive agents, it is also useful in conjunction with poorly water soluble drugs, particularly those with low therapeutic indices, from other classes of drug substances.

The particles of this invention comprise an anticancer agent. The anticancer agent is present in one or more discrete crystalline phases. The crystalline phase differs from an amorphous, i.e., non-crystalline phase which results from conventional solvent precipitation techniques for the preparation of particles in the submicron size range, such as described in U.S. Pat. No. 4,826,689.

The invention can be practiced with a wide variety of anticancer agents. However, the anticancer agent must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble", it is meant that the drug substance has a solubility in the liquid dispersion medium, e.g., water, of less than about 10 mg/ml, and preferably, of less than 1 mg/ml at processing temperature, e.g., room temperature. The preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which the anticancer agent is dispersible including, for example, aqueous salt solutions, safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

The anticancer agent preferably is selected from alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents, such as radiosensitizers.

Examples of alkylating agents include alkylating agents having the bis-(2-chloroethyl)-amine group such as, for example, chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, and trifosfamide;

alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone and mitomycine;

alkylating agents of the alkyl sulfonate type, such as, for example, busulfan, piposulfan, and piposulfam;

alkylating N-alkyl-N-nitrosourea derivatives, such as, for example, carmustine, lomustine, semustine, or streptozotocine; and alkylating agents of the mitobronitole, dacarbazine and procarbazine type.

Examples of antimetabolites include folic acid analogs, such as, for example, methotrexate;

pyrimidine analogs such as, for example, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; and

purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine.

Examples of natural products include vinca alkaloids, such as, for example, vinblastine and vincristine;

epipodophylotoxins, such as, for example, etoposide and teniposide;

antibiotics, such as, for example, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, and mitomycin;

enzymes, such as, for example, L-asparaginase;

biological response modifiers, such as, for example, α-interferon;

camptothecin;

taxol; and

retinoids, such as retinoic acid.

Examples of hormones and antagonists include adrenocorticosteroids, such as, for example, prednisone;

progestins, such as, for example, hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate;

estrogens, such as, for example, diethylstilbestrol and ethinyl estradiol;

antiestrogens, such as, for example, tamoxifen;

androgens, such as, for example, testosterone propionate and fluoxymesterone;

antiandrogens, such as, for example, flutamide;

and gonadotropin-releasing hormone analogs, such as, for example leuprolide.

Examples of miscellaneous agents include radiosensitizers, such as, for example, 1,2,4-benzotriazin-3-amine 1,4-dioxide (SR 4889) and 1,2,4-benzotriazine-7-amine 1,4-dioxide (WIN 59075);

platinum coordination complexes such as cisplatin and carboplatin;

anthracenediones, such as, for example, mitoxantrone;

substituted ureas, such as, for example, hydroxyurea;

and adrenocortical suppressants, such as, for example, mitotane and aminoglutethimide.

In addition, the anticancer agent can be an immunosuppressive drug, such as, for example, cyclosporine, azathioprine, sulfasalazine, methoxsalen and thalidomide.

The anticancer agents useful in the practice of this invention are known compounds and/or can be prepared by techniques known in the art.

The anticancer agent can be used alone or in combination with one or more anticancer agents.

The particles of this invention contain an anticancer agent as described above having a surface modifier adsorbed on the surface thereof. Useful surface modifiers are believed to include those which physically adhere to the surface of the anticancer agent but do not chemically bond to the anticancer agent.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants. Representative examples of excipients include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, polaxomers, such as Pluronic™ F68, F108 and F127, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and poloxamines, such as Tetronic™ 908 (T908), which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine available from BASF, dextran, lecithin, Aerosol OT™ (AOT), which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol™ P, which is a sodium lauryl sulfate, available from DuPont, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 20, 40, 60 and 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Speciality Chemicals, Span 20, 40, 60 and 80, which are sorbitan esters of fatty acids, Arlacel 20, 40, 60 and 80, which are sorbitan esters of fatty acids, available from Hercules, Inc., Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, Crodesta™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodesta SL-40, which is available from Croda, Inc., hexyldecyl trimethyl ammonium chloride (CTAC), bovine serum albumin and SA90HCO, which is $C_{18}H_{37}$ CH_2 (CON (CH_3) CH_2 ($CHOH)_4CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include polyvinylpyrrolidone, Pluronic F-108, polyvinyl alcohol and gum acacia.

The surface modifier is adsorbed on the surface of the anticancer agent in an amount sufficient to maintain an effective average particle size of less than about 1000 nm. The surface modifier does not chemically react with the anticancer agent or itself. Furthermore, the individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages.

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. By "an effective average particle size of less than about 1000 nm" it is meant that at least 90% of the particles have a number average particle size of less than about 1000 nm when measured by the above-noted tech-

niques. In particularly preferred embodiments of the invention, the effective average particle size is less than about 400 nm. In some embodiments of the invention, the effective average particle size is less than about 300 nm. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size of less than the effective average, e.g., 1000 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 1000 nm.

Motoyama et al, U.S. Pat. No. 4,540,602 disclose that a solid drug can be pulverized in an aqueous solution of a water-soluble high molecular substance, and that as a result of such wet grinding, the drug is formed into finely divided particles ranging from 0.5 μm or less to 5 μm in diameter. However, there is no suggestion that particles having an average particle size of less than about 1 μm can be obtained. Attempts to reproduce the wet grinding procedures described by Motoyama et al resulted in particles having an average particle size of much greater than 1 μm.

The particles of this invention can be prepared by a method comprising the steps of dispersing an anticancer agent in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the anticancer agent to an effective average particle size of less than about 1000 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

A general procedure for preparing the particles of this invention is set forth below. The anticancer agent selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse anticancer agent selected be less than about 100 μm as determined by sieve analysis. If the coarse particle size of the anticancer agent is greater than about 100 μm, then it is preferred that the particles of the anticancer agent be reduced in size to less than 100 μm using a conventional milling method such as airjet or fragmentation milling.

The coarse anticancer agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the anticancer agent in the liquid medium can vary from about 0.1–60% and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90% and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 1000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the anticancer agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the anticancer agent conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, a planetary mill, media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. However, zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. Further, other media, such as stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 2.5 g/cm^3.

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the anticancer agent. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing pressures up to about 20 psi (1.4 kg/cm^2) are typical of media milling.

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

The relative amount of the anticancer agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular anticancer agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the surface area of the anticancer agent, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square

meter surface area of the anticancer agent. The surface modifier can be present in an amount of 0.1–90%, preferably 0.5–80% and more preferably 1–60% by weight based on the total weight of the dry particle.

A simple screening process has been developed whereby compatible surface modifiers and anticancer agents can be selected which provide stable dispersions of the desired particles. First, coarse particles of an anticancer agent are dispersed in a liquid in which the anticancer agent is essentially insoluble, e.g., water at 2% (w/v) and milled for 120 hours in a roller mill under the following milling conditions:

Grinding vessel: 8 oz. (250 ml) glass jar
Available volume of grinding vessel: 250 ml
Media volume: 120 ml
Media type: 1.0 mm pre-cleaned zirconium oxide beads (distributed by Zircoa, Inc.)
Milling time: 120 hours
Slurry volume: 60 ml
RPM: 92
Room Temperature

The slurry is separated from the milling media by conventional means, e.g., by pouring the slurry out of the vessel, or by using a pipette. The separated slurry is then divided into aliquots and surface modifiers are added at a concentration of between 2 and 50% by weight based on the total combined weight of the anticancer agent and surface modifier. The dispersions are then sonicated (1 minute, 20 kHz) or vortexed using a multitubed vortexer for one minute, to disperse agglomerates and subjected to particle size analysis, e.g., by photon correlation spectroscopy (PCS) and/or by examination under an optical microscope (1000×magnification). If a stable dispersion is observed, then the process for preparing the particular anticancer agent surface modifier combination can be optimized in accordance with the teachings above. By stable it is meant that the dispersion exhibits no flocculation or particle agglomeration visible to the naked eye and, preferably, when viewed under the optical microscope at 1000×, at least 15 minutes, and preferably, at least two days or longer after preparation. In addition, preferred particles exhibit no flocculation or agglomeration when dispersed in 0.1N HCl and/or phosphate buffered saline, pH 7.4 (PBS) or rat plasma.

The resulting dispersion is stable and consists of the liquid dispersion medium and the above-described particles. The dispersion of surface modified anticancer agent nanoparticles can be spray coated onto sugar spheres or onto a pharmaceutical excipient in a fluid-bed spray coater by techniques well known in the art.

Anticancer pharmaceutical compositions according to this invention include the particles described above and a pharmaceutically acceptable carrier therefor. Suitable pharmaceutically acceptable carriers are well known to those skilled in the art. These include non-toxic physiologically acceptable carriers, adjuvants or vehicles for parenteral injection, for oral administration in solid or liquid form, for rectal administration, nasal administration, intramuscular administration, subcutaneous administration, and the like.

A method of treating a mammal in accordance with this invention comprises the step of administering to the mammal in need of treatment an effective amount of the above-described anticancer composition. The selected dosage level of the anticancer agent for treatment is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage can be readily determined by one skilled in the art and depends upon the particular anticancer agent, the desired therapeutic effect, the route of administration, the desired duration of treatment and other factors.

It is a particularly advantageous feature that the anticancer compositions of this invention exhibit reduced toxicity and/or enhanced efficacy as illustrated in the examples that follow. Further, the particles of this invention exhibit prolonged circulation in the blood pool.

Moreover, anticancer agents which heretofore could not be administered by injection, when prepared as nanoparticles and formulated in anticancer compositions according to this invention, can be effectively administered by injection, e.g., by an intravenous bolus injection.

The following examples further illustrate the invention.

EXAMPLES 1-4 Nanoparticulate Piposulfan

EXAMPLE 1

Piposulfan (purchased from Eastman Kodak) was milled in a mixture of 0.33% polyoxyethylene sorbitan monooleate, Tween 80, (ICI Americas, Inc., Wilmington, Del.) and 0.67% sorbitan monooleate, Span 80, (ICI) using 1 mm zirconium oxide beads for about 96 hours to produce particles approximately 240 nm in diameter. The final piposulfan concentration in the suspension was 10 mg/mL. The particles were stable to flocculation/aggregation in rat plasma.

Milling Conditions: A coarse suspension of piposulfan was prepared by adding 300 mg of the drug to a 4 oz. (120 mL) amber bottle which was previously filled with 60 mL of 1 mm precleaned zirconium oxide beads (Zircoa Inc., Solon, Ohio) and 30 mL of 1% Tween 80/Span 80 (1 to 2 ratio) solution. The surfactant solution was prepared by accurately weighing 333 mg of Tween 80 and 667 mg of Span 80 in a volumetric flask followed by addition of sterile water for injection to dissolve/disperse the surfactants. Sufficient quantity of water was added to make the final volume 100 mL. Zirconium oxide beads were cleaned by first rinsing in 1N sulfuric acid followed by several rinses with deionized water. The media was dried in a vacuum oven at about 100° C. overnight.

The sealed primary container was loaded into a secondary padded aluminium containment can to ensure a tight fit. It was milled on a roller mill (US Stoneware, Mawah, N.J.) at 144 RPM for about 96 hours. At the end of the milling time the slurry was separated from the media and particle size was measured using a PCS device. Stability of these particles to rat plasma was assessed by optical microscopy at 1000×magnification. The final pH of the formulation was 6.

Control A (unmilled), a coarse suspension containing 40 mg of bulk piposulfan was dispersed in water in the presence of 3% ethanol and 1% Tween 80. This suspension could not be injected IV.

Example 1 was evaluated for efficacy studies in female mice (avg. wt. 22 g) which were implanted with early stage Mammary Adenocarcinoma #16/C on day 0. The formulation was injected starting from day 1 for several days. The antitumor activity was assessed by monitoring tumor weight and comparing it to the control animals. The results were as follows:

Treatment	Route of Adm.*	Total Dose (mg/kg)	% Wt. Loss	T/C %	Log10 Cell Kill
Control	—	—	+5.5	—	—
Example 1	IV	356	−5.5	0	2.75
(243 nm)	IV	220	−5.5	2	2.75
	IV	137	−1.8	2	2.25
	IV	85	0	18	1.0
Control A	SC	800	−10.8	0	2.1

*Administration - IV Intravenous; SC Subcutaneous

Example 1 could be injected directly as 10 mg/ml suspension. There was no acute toxicity after injection of 78 mg/kg single dose.

T/C=Tumor weight in treated animals divided by the tumor weight of the control animals, reported as percent value. Lower value indicates better efficacy, 0% suggests cures. <10% is considered highly active, 10 to 42% is moderately active formulation. >42% is considered inactive.

Log Kill=(T−C)/3.32 (Td), where T is the time in days for the median tumor to reach 1000 mg mass in treated animals, C is the time in days for the median tumor to reach 1000 mg in control animals and Td is the tumor volume doubling time in days. Cures (tumor free animals) are excluded from (T−C) calculations.

Example 1 demonstrates that a composition of this invention exhibited reduced toxicity and enhanced efficacy compared to a prior art composition and could be administered by IV bolus injection.

EXAMPLES 2-4

The milling procedure described in Example 1 was repeated except that the ratio of Tween 80 to Span 80 was 2:1. The resulting average particle size was 297 nm.

The milling procedure described in Example 1 was repeated except that the ratio of Tween 80 to Span 80 was 1:1. The resulting average particle size was 380 nm.

The milling procedure described in Example 1 was repeated except that the surface modifier was a 1:1 ratio of Tween 60 and Span 60. The resulting average particle size was 301 nm.

Stable pipsulfan nanoparticles were also prepared using bovine serum albumin as the surface modifier.

EXAMPLES 5-7 Nanoparticulate Camptothecin

EXAMPLE 5

Approximately 60 mL of precleaned zirconium oxide beads (1 mm) were placed in a 120 mL wide mouth round amber bottle. To it was added 0.35 g of Tetronic 908 (BASF) followed by 0.35 g of Camptothecin (Sigma Chemicals, 95% pure). To the above mixture, 35 mL of water for injection (Abbott) was added. The bottle was sealed and mounted on a roller mill. Milling was effected by rotating the bottle at 100 RPM for 7 days.

At the end of milling, an aliquot (100 μL) was checked for particle size using a Malvern Zetasizer. The particles were determined to have an average particle size of 240 nm.

EXAMPLE 6

Example 5 was repeated except that the Tetronic 908 was replaced by polyvinyl alcohol (MW 30 to 70K). The final particle size was 256 nm.

EXAMPLE 7

Example 5 was repeated except that Tetronic 908 was replaced by gum acacia. The final particle size was 298 nm.

Nanocamptothecin formulations were evaluated for efficacy in two murine tumor models, i.e., Mammary Adenocarcinoma #16/C and Pancreatic Ductal Adenocarcinoma #03. The antitumor activity was assessed by monitoring tumor weight from experimental and control animals.

1. Efficacy Studies in Pancreatic Ductal Adenocarcinoma #03:

Example	Route	Dose mg/kg	Wt % Loss	Drug Deaths	T/C %
Control B	SC	60	−24.1	6/6	—
	SC	40.2	−21.8	5/5	—
	SC	26.7	−18.2	5/5	—
	SC	18	−10.9	1/5	62
6	IV	83.1	−16.7	1/4	14
	IV	78.2	−14.6	1/4	55
	IV	48.6	−8.3	0/4	0
	IV	24.3	−4.2	0/4	18
PVA Control	IV	—	+6.3	0/4	100
7	IV	93.5	−16.7	1/4	7
	IV	46.8	−14.6	0/4	17
	IV	23.4	−8.3	0/4	11
Cum Acacia Control	IV	—	0.0	0/4	60

The Control B formulation consisted of 1% coarse camptothecin in 3% ethanol, and 1% Tween 20. Control B could only be administered subcutaneously and even at the lowest SC dose (18 mg/kg) was inactive. Control B was toxic to 1/5 animals tested. In contrast, doses of the nanocamptothecin formulations of this invention ranging from 24-93 mg/kg were administered intravenously (IV) and were shown to be safe and efficacious.

2. Efficacy Studies in Mammary Adenocarcinoma #16/C Murine Tumor Model

Example	Route	Dose mg/kg	% Wt Loss	Drug Deaths	T/C %
Control B	SC	60	−23.5	5/5	—
	SC	30	−20.9	5/5	—
	SC	15	−18.3	3/5	14**
5	IV	65	−17.4	0/5	23
	IV	33	−18.7	1/5	33
	IV	16	−2.2	0/5	63
T908 Control	IV	—	+4.3	0/2	100
6	IV	65	−21.7	5/5	—
	IV	33	−15.7	0/5	100
PVA Control	IV	—	+4.3	0/2	100

**% T/C for the control animals was determined from the surviving animals, N = 2.

The T908 and PVA controls consisted of 1% aqueous solutions of the respective surface modifiers. The Control B was injected subcutaneously, and it was toxic at all doses tested. Efficacious doses of the nanocamptothecin formulations of this invention were administered intravenously.

3. Blood and Tumor Clearance

To determine if the increased efficacy was related to alterations in pharmacokinetic properties, blood clear-

ance and tumor distribution were studied in the Mammary adenocarcinoma #16/C murine tumor model.

Tumor bearing mice were injected via the tail vein with 10 mg/ml of camptothecin formulated as described in Examples 5 and 6 and a control which was 5 mg/ml camptothecin solubilized by the addition of 0.1N NaOH. At various times after injection, i.e., 5 min., 30 min., 60 min., 2 hrs., 4 hrs., 8 hrs., 16 hrs., 24 hrs. and 48 hrs., animals were euthanized and a blood sample was collected and the tumor excised. Concentrations of drug samples were quantified using HPLC. Results show that the compositions of this invention affect the clearance of the drug from the circulating pool of blood and the tumor.

	T½ Blood and Tumor	
Formulation	Blood	Tumor
Example 6	18 hrs.	>48 hrs.
Example 5	13 hrs.	>48 hrs.
Control	1.6 hrs.	13.5 hrs.

When formulated in accordance with this invention, the elimination half-life and the residence time of camptothecin in the tumor were prolonged significantly. It was concluded that pharmacokinetic parameters of the nanoparticulate formulation of camptothecin are directly related to the improved performance of the drug.

EXAMPLES 8–10 Nanoparticulate Etoposide

EXAMPLE 8

Example 5 was repeated except that 1.7 g of etoposide was combined with 1.7 g of PVA and the milling time was 14 days. The final particle size was 310 nm. The particles were stable in acid and plasma.

EXAMPLE 9

Example 8 was repeated except that the PVA was replaced by Pluronic F-108 (BASF). The final particle size was 312 nm. The particles were stable in acid and plasma.

EXAMPLE 10

Etoposide (2%) was milled in sterile water for 7 days. A 1:1 mixture of the milled slurry was prepared with 2% Pluronic F127 solution. The mixture was vortexed before measuring particle size. The final size was 277 nm. The slurry was stable in simulated gastric fluid, PBS (pH 7.4) and rat plasma.

EFFICACY STUDIES

Nanoetoposide formulations were evaluated in two separate efficacy studies in pancreatic ductal adenocarcinoma #03 (PANC #03). Control C was a 2% non-aqueous etoposide solution prepared using the formula described on pages 741–743 of the 46th Edition of the Physicians' Desk Reference. As described above, antitumor activity was assessed by monitoring tumor weight from experimental and control animals. These studies demonstrate that the etoposide compositions of this invention provide a means to deliver high doses of the drug without evidence of severe toxic reaction.

1. Efficacy Studies for Nanoetoposide in PANC #03 Murine Tumor Model

Example	Route	Dose mg/kg	% Wt Loss	Drug Deaths	T/C %
Control C	IV	120	−24.0	0/5	4.0
	IV	75	−4.0	0/5	20.0
7	IV	160	−12.0	0/5	18.0
	IV	100	0.0	0/5	32.0
	IV	62	2.0	0/5	42.0
8	IV	160	−12.0	0/5	26.0
	IV	100	+2.0	0/5	35.0
	IV	62	+4.0	0/5	35.0
9	IV	170	−18.5	1/5	16
	IV	85	−2.0	0/5	35
	IV	43	+2.5	0/5	41

EXAMPLES 11–16 Nanoparticulate Taxol

EXAMPLE 11

Approximately 18 mL of precleaned zirconium oxide media (1 mm) was added to a 30 mL amber jar. To it was added 240 mg of taxol (Sigma Chemicals) and 180 mg of Tween 20. Finally, 12 mL of water for injection was added to the jar, it was sealed and mounted on a roller mill for 11 days. The final particle size was 327 nm. The formulation was stable when exposed to PBS (pH 7.4) and rat plasma.

EXAMPLE 12

Example 11 was repeated except that the Tween 20 was replaced with PVA (MW 30 to 70 k). The final particle size was 365 nm.

The above samples were evaluated in efficacy studies in mice bearing early stage mammary adenocarcinoma #16/C. The antitumor activity was assessed by comparing tumor weights of taxol treated animals with the tumor weights of untreated animals. The toxicity was assessed by dose ranging studies with death and weight loss as end points. All samples were injected IV.

Example	Dose mg/kg	% Wt Loss	Deaths	Median Tumor on Day 11	T/C %
Control D	—	+6.1	—	1539 mg	—
Example 11	108.5	−1.5	0/5	1528	13
Example 12	108.5	−3.0	0/5	201	99

A control sample of taxol (NCI) was not available. However, a single dose of taxol formulated in Cremophore EL causes immediate deaths at 25 mg/kg total dose. However, taxol formulated in compositions of this invention could be injected at a dose of 88 mg/kg with no apparent adverse effects.

A taxol suspension prepared in a manner similar to Example 11 was treated separately with several surface modifiers. After addition of the new surface modifier the mixture was vortexed and evaluated for particle size and fluid stability. All the suspensions contained 1% taxol and 0.75% Tween 20. The results are as follows.

Example/ Surface Modifier	Concentration %	Size (nm)	Fluid Stability PBS	Fluid Stability Rat Plasma
13 CTAC	0.25	364	OK	Flocculation
14 AOT	0.25	322	SA*	SA
15 F68	0.5	297	SA/OK	SA/OK

-continued

Example/ Surface Modifier	Concentration %	Size (nm)	Fluid Stability PBS	Fluid Stability Rat Plasma
16 T908	0.5	313	SA/OK	SA/OK

*SA = Slight Aggregation

EXAMPLES 17–18 Nanoparticulate WIN 59075

Approximately 60 mL of precleaned zirconium oxide (1 mm) media was transferred into a 4 oz amber jar. It was followed by addition of 1.5 g of WIN 59075 and 28.5 mL of water for injection. The jars were sealed, loaded onto a roller mill and cascaded at 95 RPM for 48 hrs. PCS analysis determined the particle size to be 322 nm, however, the presence of larger particles was suggested. Milling was continued for 5 additional days.

Studies were conducted by mixing 0.5 mL of the WIN 59075 slurry prepared above with 0.5 mL of 6% surface modifier solutions. The final concentration of the drug was 2.5% and that of the surface modifiers was 3%. The surface modifier stabilized nanosuspensions of WIN 59075 were then treated with either PBS (pH 7.0) or 0.1N HCl (pH 1). Optical microscopic observations were made to determine fluid stability. The results are as follows;

Example	Surface Modifier	Stability pH 1	Stability pH 7	Human Plasma
17	PVP (12K) (BASF)	Fine	Fine	Fine
18	Gum Acacia (Aldrich)	—	SA/OK	SA/OK

It was concluded that stable nanoparticles of WIN 59075 could be prepared.

EXAMPLES 19–22 Nanoparticulate SR 4889

7.5 mL of precleaned zirconium oxide media (1 mm) was transferred into a 15 mL amber jar along with 18.75 mg of SR 4889 and 3.75 mL of water. After 11 days of milling the nanosuspension was separated from the media. To each of the 100 μL aliquots of the suspension, 100 μL of a surfactant solution (2%) was added giving a final concentration of 0.25% drug and 1% surfactant. The mixture was vortexed and analyzed for particle size. Fluid stability was assessed microscopically by mixing 10 μL of the suspension with 90 μL of rat plasma. The results are as follows:

Example	Surface Modifier	Particle Size (nm)	Fluid Stabilty Rat Plasma
19	PVP (12K)	134	Fine
20	Gum Acacia	344	SA/OK
21	Tween 80	128	Fine
22	T908	130	Aggregates

EXAMPLE 23 Nanoparticulate Retinoic Acid

30 mL of precleaned zirconium oxide media was transferred to a 60 mL amber jar. To it was added 1 g of transretinoic acid (Sigma), 470 mg of tyloxapol and 15 mL of water. The mixture was milled on a roller mill for 15 days. The final particle size was 140 nm. The nanosuspension was stable when exposed to either rat plasma or simulated gastric fluid.

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

What is claimed:

1. Particles consisting essentially of 99.9% by weight of a crystalline medicament useful in treating cancer susceptible to treatment with said medicament, said medicament having a solubility in water of less than 10 mg/ml, and having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an average effective particle size of less than 1000 nm, wherein said medicament is selected from the group consisting of alkylating agents selected from the group consisting of alkylating agents having a bis-(2-chloroethyl)-amine group, alkylating agents having a substituted aziridine group, alkyl sulfonates, and N-alkyl-N-nitrosoureas; antimetabolites; natural products selected from the group consisting of vinca alkaloids, epipophylotoxins, adriamycine, daunomycine, doctinomycine, daunorubicin, doxorubicin, mithramycin, bleomycin, mitomycin, enzymes, biological response modifiers, camptothecin, taxol and retinoids; hormones and antagonists: radiosensitizers; platinum coordination complexes; anthracenediones; and adrenocortical suppressants.

2. The particles of claim 1 having an average effective particles size of less than 400 nm.

3. The particles of claim 1 having an average effective particle size of less than 300 nm.

4. The particles of claim 1 wherein said surface modifier is present in an amount of 1 to 75% by weight.

5. The particles of claim 1 wherein said anticancer agent is selected from the group consisting of piposulfan, piposulfam, camptothecin, etoposide, taxol, 1,2,4-benzotriazin-3-amine 1,4-dioxide, 1,2,4-benzotriazin-7-amine 1,4-dioxide and retinoic acid.

6. The particles of claim 1 wherein said surface modifier is selected from the group consisting of polyvinyl alcohol, a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, gum acacia, a block copolymer of ethylene oxide and propylene oxide, a polyoxyethylene sorbitan fatty acid ester, and a sorbitan ester of a fatty acid.

7. The particles of claim 1 wherein said anticancer agent is taxol and said surface modifier comprises a polyoxyethylene sorbitan fatty acid ester.

8. An anticancer composition comprising the particles of claim 1.

9. A method of treating a mammal comprising administering to the mammal an effective amount of the anticancer composition of claim 8.

10. In a method of treating a mammal comprising administering to the mammal an effective amount of an anticancer agent, the improvement wherein the efficacy of said anticancer agent is increased by administering said anticancer agent in the form of the particles of claim 1.

11. In a method of treating a mammal comprising administering to the mammal an effective amount of an anticancer agent, the improvement wherein the toxicity of said anticancer agent is reduced by administering said anticancer agent in the form of the particles of claim 1.

12. The particles of claim 1 wherein said surface modifier is a surfactant.

13. The particles of claim 1 wherein said surface modifier is a nonionic surfactant.

14. The particles of claim 1 wherein said surface modifier is an anionic surfactant.

15. The particles of claim 1 wherein said surface modifier is selected from the group consisting of gelatin, casein, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinyipyrrolidone, poloxomers, tyloxapol, poloxamines, dextran, a dioctyl ester of sodium sulfosuccinic acid, sodium lauryl sulfate, an alkyl aryl polyether sulfonate, a mixture of sucrose stearate and sucrose distearate, hexyldecyl trimethyl ammonium chloride, bovine serum albumin and $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$.

16. The particles of claim 1 wherein said surface modifier is present in an amount of 10 to 60% by weight based on the total weight of the dry particle.

17. The particles of claim 1 wherein said surface modifier is present in an amount of 10 to 30% by weight based on the total weight of the dry particle.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,399,363
DATED : March 21, 1995
INVENTOR(S) : Gary G. Liversidge, Elaine Liversidge and Pramod P. Sarpotdar

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14,
Line 7, delete "99.9%" and insert -- 99.9-10% --.

Signed and Sealed this

Twenty-fifth Day of April, 2006

JON W. DUDAS
Director of the United States Patent and Trademark Office

EXHIBIT B

US005834025A

United States Patent [19]

de Garavilla et al.

[11] Patent Number: 5,834,025

[45] Date of Patent: Nov. 10, 1998

[54] **REDUCTION OF INTRAVENOUSLY ADMINISTERED NANOPARTICULATE-FORMULATION-INDUCED ADVERSE PHYSIOLOGICAL REACTIONS**

[75] Inventors: **Lawrence de Garavilla**, Downingtown; **Elaine M. Liversidge**; **Gary G. Liversidge**, both of West Chester, all of Pa.

[73] Assignee: **Nanosystems L.L.C.**, King of Prussia, Pa.

[21] Appl. No.: **696,754**

[22] Filed: **Aug. 14, 1996**

Related U.S. Application Data

[60] Provisional application No. 60/004,488 Sep. 29, 1995.

[51] **Int. Cl.**[6] **A61K 9/50**; B32B 5/16; B01J 13/02

[52] **U.S. Cl.** **424/501**; 424/502; 428/402.21; 264/4.3

[58] **Field of Search** 424/501, 502; 428/402.21; 264/4.3

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,565,188 10/1996 Wong et al. 424/499

Primary Examiner—Carlos Azpuru
Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

Disclosed are methods of intravenous administration of nanoparticulate drug formulations to a mammal to avoid adverse hemodynamic effects: by reducing the rate and concentration of the nanoparticles in the formulations; or by pre-treating the subject with histamine; or by pretreating the subject with a desensitizing amount of the nanoparticulate drug formulations.

20 Claims, No Drawings

REDUCTION OF INTRAVENOUSLY ADMINISTERED NANOPARTICULATE-FORMULATION-INDUCED ADVERSE PHYSIOLOGICAL REACTIONS

BACKGROUND OF THE INVENTION

This application claims the benefit under 35 USC 119(*e*) of Provisional Application Number 60/004,488 filed Sep. 29, 1995.

Field of the Invention

The present invention is directed to nanoparticulate, liposome, emulsion and polymeric colloidal drug formulations for intravenous administration, the delivery of which to a mammal, reduces/eliminates the adverse physiological effects. More particularly, the invention relates to a method of intravenous nanoparticulate, liposome, emulsion and polymeric colloidal drug formulations to a mammal, wherein the rate of infusion and concentration of the drug is controlled.

Reported Developments

Nanoparticles are well know in the prior art, having been described, for example, in U.S. Pat. No. 5,145,684. These particles consist of a crystalline drug substance having a surface modifier adsorbed on the surface of the particles such that the average particle size is less than about 400 mm. Low solubility of solid drugs prompted the pharmaceutical industry to create nanoparticles of such drugs which then can be administered systematically to provide bioavailability. Drug substances disclosed which can be made into nanoparticles include a variety of known classes of drugs.

In order to provide nanometer-size particles, the drug substance is comminuted in the presence of a surface active agent or, alternatively, the surface active agent is allowed to be adsorbed to the nanoparticulate drug substance after the process of comminution. The surface active agent prevents flocculation or congregation of the nanoparticles. The achievements of nanoparticulate technology in the pharmaceutical industry affords the opportunity to prepare parenteral formulations of water-insoluble or poorly water-soluble drugs. These formulations by their nature are suspensions rather than solutions since the particles are dispersed/suspended in a pharmaceutically acceptable vehicle. Liposomes, emulsions and colloids when used as carriers for an active drug are also suspensions rather than solutions.

We have observed that intravascular adminstration of a suspensions to dogs causes significant cardiovascular dysfunction, such as reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility.

Other investigators have reported similar findings, for example: Slack et al., Acute Hemadynamic Effects and Blood Pool Kinetics of Polystyrene Microspheres Following Intravenous Administration, J. Pharm. Sci., 660, 1981; Faithfull et al., Cardiorespiratory Consequences of Fluro-carbon Reactions in Dogs, Bio. Art. Organs, 1, 1988; and Lorenz et al., Histamine Release in Dogs by Cremophore EL and its Derivatives, Agents and Actions, 63, 1977.

It has now been discovered that hemodynamic effects of suspensions can be eliminated or at least substantially reduced by controlling the rate of infusion and/or the concentration of the active drug in the suspensions.

The present invention will be described particularly in reference to nanoparticulate crystalline drug formulations, however, the invention encompasses the use of other carriers for active drugs that do not form a solution but rather a suspension or dispersion having nanoparticulates in the range of less than 1000 nm.

SUMMARY OF THE INVENTION

This invention provides a method of administering a nanoparticulate composition to a mammal such as a dog or other sensitive species without eliciting adverse hemodynamic effects comprising:

intravenously administering to said dog an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min comprising particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a surface modifier adsorbed on the surface thereof in an amount of 0.1–99.9% by weight and sufficient to maintain an effective average particle size of from about 50 to about 1000 nm, and a pharmaceutically acceptable carrier therefor.

In another embodiment, the present invention provides a method of administering a nanoparticulate composition to a mammal such as a dog or other sensitive species without eliciting adverse hemodynamic effects comprising:

intravenously administering to said dog an antihistamine including H1, H2 and H3 receptor antagonists in the amount of form about 5 to about 10 mg/kg of body weight, such as diphenhydramine, cimetidine or thioperamide or a combination thereof followed by intravenously administering to said dog an effective amount of a nanoparticulate drug composition comprising particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a surface modifier adsorbed on the surface thereof in an amount of 0.1–99.0% by weight and sufficient to maintain an effective average particle size of less than 1000 nm, and preferably less than about 400 nm, and a pharmaceutically acceptable carrier thereof.

In still another embodiment the present invention provides a method of administering a nanoparticulate composition to a mammal, such as a dog or other sensitive species without eliciting adverse hemodynamic effects comprising: intravenously administering to said mammal a desensitizing amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, followed by intravenous administration of an effective amount of said nanoparticulate composition comprising particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of from 50 to 1000 nm, and a pharamceutically acceptable carrier therefor.

In a liposome- or colloidal-drug delivery system, the bioactive drug is entrapped in the liposome or colloidal particles in an amount of from about 0.1 to about 99.9 to about 99.1 to about 0.1% by weight liposome or colloidal particles, such particles having a particle size of less than 1000 mm, and then administered to the patient to be treated.

As used herein, the term "desensitizing amount" means a dose low enough so as not to elicit a response but still acutely precondition the mammal so that a subsequent injection of a full dose will now be without effect.

Also, as used herein, mammal preferably means a dog and other sensitive species including human species.

DETAILED DESCRIPTION OF THE INVENTION

Nanoparticulate formulations cause a significant transient decrease in arterial blood pressure immediately following intravenous injection in anesthetized dogs. Extensive studies were conducted to evaluate the acute hemodynamic effects of intravenously administered nanoparticles. To eliminate effects which may be caused by an active drug, inert polystyrene nanoparticles were used in the studies. Parameters were monitored throughout the studies including mean arterial pressure, heart rate, mean pulmonary arterial pressure, left ventricular systolic pressure, temperature, blood chemistry and hematology. In order to distinguish and separate the effects arising from components of nanoparticulate formulations, the effects of intravenous injections of: inert polystyrene nanoparticles, surfactant-coated polystyrene nanoparticles and surfactant alone were used in separate groups of dogs in the studies.

STUDY DESIGN/METHODS

A. Animals

Mongrel dogs of either sex weighing between 13 and 16 kg were used in these studies. Dogs were acquired form Butler Farms (Clyde, N.Y.) and allowed at least a two week acclimation period following shipment and prior to conducting these studies.

B. Anesthesia and Surgical Preparation

Dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Following surgical preparation, the dogs were placed on an infusion of sodium pentobarbital (4 mg/kg/hr) to maintain an adequate, constant level of anesthesia throughout the experimental protocol. All anesthetic injections were administered via a 20 gauge percutaneous venicatheter placed in the right cephalic vein.

Following induction of anesthesia, dogs were placed in dorsal recumbency and intubated with a 7 French endotracheal tube and allowed to breathe room air. (Core temperature, as measured using a thermistor probe inserted 6 cm into the colon, was maintained between 37.5° and 38.5° C. using a thermostatically controlled heating pad (Harvard Homeothermic Heating Blanket, Harvard Instruments, Natick, Mass.).

The right carotid artery, right femoral artery, and the right and left femoral veins were surgically isolated via percutaneous incisions and blunt dissection. A high fidelity, solid state, dual sensor pressure transducer catheter (Model SPC 784A, Millar Instruments, Houston, Tex.) was advanced retrograde from the carotid artery and placed into the left ventricular chamber of the heart The distal pressure sensor, positioned in the left ventricle, was used to measure intraventricular pressure and the proximal sensor, positioned at the root of the aorta, measured intra-aortic pressure. The pulsatile left ventricular pressure (LVP) signal was electronically differentiated (dP/dt) to acquire an estimate of cardiac contractility (LV dP/dt). Heart rate (HR) was also derived from the pulsatile LVP signal. Mean arterial blood pressure (MAP) was derived form the pulsatile signal of intraaortic pressure. Another fluid-filled catheter was placed in the right femoral artery for acquisition of arterial blood samples for blood gas measurements. Arterial blood pH, PCO_2 and $_pO_2$ were measured on freshly drawn samples using an automated blood gas analyzer (Model ABL30, Radiometer Corp., Westlake, Ohio). Platinum pin electrodes were inserted subcutaneously to monitor lead II electrocardiogram. All particles were injected in the left cephalic vein via a 20 gauge percutaneous venicatheter.

Following surgical preparation and instrumentation, the dogs were allowed a 30 minute acclimation period prior to initiation of the test.

C. Data Acquisition and Analysis

The following variables were continuously recorded on a pc-based automated data acquisition system (Modular Instruments, Inc., Malvern, Pa.):

Mean arterial blood pressure (MAP; mmHg)

Heart rate (HR; min^{-1})

Mean pulmonary arterial blood pressure (PAP; mmHg)

Left ventricular systolic blood pressure (LVP_{sys}; mmHg)

Maximum left ventricular dP/dt (+LV dP/dt mmHg sec^{-1})

Maximum left ventricular dP/dt (−LV dP/dt mmHg sec^{-1})

The following variable were mathematically derived from the above variables and cardiac output (CO; L min^{-1}):

Systemic arterial vascular resistance (SVR; calculated as MAP/CO; mmHg L^{-} min)

Pulmonary vascular resistance (PVR; calculated as PAP/CO; mmHg L^{-1} min)

Stroke volume (SV; calculated as CO/HR.1000; cc).

Data were down-loaded onto a PC-based statistical package CRUNCH (Crunch Software Corporation, Oakland, Calif.). An analysis of variance followed by a Dunnett's comparison was used to test the effect of dose within each group. Between group effects (WIN 8883 vs. vehicle) were analyzed using a t-test. In all cases, data are expressed as mean±s.e.

D. Drugs and Solutions

Polystyrene Particles (Surfactant Coated)

Polystyrene particles having a diameter of 200 nm were obtained form Polysciences, Inc.

1) The Vehicle (Placebo)

A 10% solution of F108 was made by weighing out 1 g of F108 in a 50 ml plastic beaker and then adding 8 ml of water (Sterile water for injection—SWFI) to the surfactant. The beaker was placed in the refrigerator until the surfactant had gone into solution. SWIF was added to bring the final volume to 10 mls which was then filtered with a 0.1 μm syringe filter before use.

2) 5% Polystyrene Particles (PSP)+5% F108

To prepare 20 mls of 5% PSP+5% F108, 10 mls of the 200 nm particles were added to 10 mls of 10% F108 in a 50 ml centrifuge tube. The tube was placed on a mutator and the suspension was allowed to mix for 1 hour. Solutions were also prepared with 100 and 50 nm particle size polystyrene particles.

3) 5% WIN 8883+5% P108

The suspension of 5% WIN 8883+5% F108 was prepared analogously to the 5% Polystyrene+5% F108 suspension shown in #2.

4) Supernatant of 5% WIN 8883

Supernatant of WIN 8883 was prepared by centrifugation of a sample of WIN 8883 at 20,000 g for 1 hour, followed by recentrifugation of the decanted supernatant 5% WIN 8883. No WIN 8883 was found in the decanted solution.

5) Supernatant of 5% Polystyrene+5% F108

The supernatant was prepared analogously to that shown in #4. No polystyrene was found in the supernatant.

The preparations of #1, #2, #3, #4 and #5 were administered to groups of dogs subcutaneously and intravenously at various dose levels described below. Baseline hemodynamics were recorded for a 10 minute period just prior to administration of the preparations. Following administration of the preparation each dog was monitored 60 minutes.

E. Results

Results of testing are shown in the following tables.

TABLE 1

Effect of the vehicle 5% F108 and a particulate-free supernatant on the hemodynamic and hematological response in dogs when infused at a rate of 5 ml/min and a dose of 0.1 ml/kg. Values listed as means ± S.E.

Treatment	N	% Change from Baseline MAP
#5% F108	4	−1 ± 1
#5 Supernatant	3	−1 ± 1

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 2

Effect of uncoated and F108 (5%) coated 200 nM diameter polystyrene nanospheres, 5% suspension, infused at a rate of 1 ml/min and a dose of 0.1 ml/kg on the hemodynamic and hematological response in dogs. Values listed as means ± S.E.

Treatment	N	% Change from Baseline MAP
#4 Uncoated	3	−1 ± 1
#3 Coated	4	−50 ± 7

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 3

Effect of rate of infusion of 200 nM diameter polystyrene nanospheres on the hemodynamic and hematological response in dogs. Each formulation was prepared as a 1% (w:v) suspension in a solution of 5% F108 and administered intravenously at a dose of 0.1 ml/kg. Values listed as means ± S.E.

Rate (ml/min)	N	% Change from Baseline MAP
5	3	−39 ± 15
1	4	−26 ± 5
0.5	3	−5 ± 1

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 4

Effect of rate of infusion of 200 nM polystyrene nanospheres on the hemodynamic and hematological response in dogs. Each formulation was prepared as a 5% (w:v) suspension in a solution of 5% F108 and adminstered intravenously at a dose of 0.1 ml/kg. Values listed as means ± S.E.

Rate (ml/min)	N	% Change from Baseline MAP
1	4	−50 ± 7
0.1	3	−1 ± 1

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 5

Effect of pretreatment with antihistamines on the hemodynamic and hematological response in dogs following ontravenous administration of a 1% (w:v) suspension of polystyrene nanospheres, 200 nM in diameter, in a 5% solution of F108 at a dose of 0.1 ml/kg and a rate of 5 ml/min. Values listed as means ± S.E.

Antihistamine	N	% Change from Baseline MAP
None	3	−39 ± 15
Diphenhydramine 10 mg/kg Cimetidine 5 mg/kg	4	−7 ± 1
Diaphenhydramine 10/mg/kg Cimetifine 5 mg/kg Thioperamide 5 mg/kg	3	−4 ± 2

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 6

Effect of particle size on the hemodynamic and hematological response in dogs. Each formulation was prepared as a 1% (w:v) suspension in a 5% solution of F108 and administered intravenously at a dose of 0.1 ml/kg and a rate of 5 ml/min. Values listed as means ± S.E.

Diameter (nM)	N	% Change from Baseline MAP
200	3	−39 ± 15
100	4	−26 ± 14
50	3	−8 ± 5

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

The results are summarized in Table 7.

TABLE 7

% Solids (w/v)	Infusion Rate (ml/min)	Solids Dose Rate (mg/ml/min)	Response Y or N
5 (50 mg/ml)	1	50	Y
1 (10 mg/ml)	10	100	Y
1 (10 mg/ml)	5	50	Y
1 (10 mg/ml)	1	10	Y
5 (50 mg/ml)	0.1	5	N
10 ng/ml	0.5	5	N

The studies indicate that there is a critical set of parameters which control hemodynamic effects in dogs. To avoid overt deleterious hemodynamic effects the following parameters should be controlled:

1) The vehicle-surfactant administered alone does not initiate a hemodynamic response.

2) "uncoated" polystyrene nanoparticles administered at a dose of 0.1 ml/kg of body weight and a rate of 1 ml/min does not induce hemodynamic effect. However, "uncoated" nanoparticle drugs cannot be administered safely, since they coagulate/flocculate causing deleterious side effects. Accordingly, active drugs must be surfactant coated to circumvent coagulation/flocculation of the nanoparticles.

3) Hemodynamic effect is associated with the surfactant-particle combination. While the inventors would not want to be limited to a theory of mechanism in the practice of their invention, it appears that the particles act as carrier for the surfactant, delivering it to its site of action in the blood stream. Theoretically, the uncoated particle also accesses the site of action but cannot initiate the response in the absence

of the surfactant. Conversely, in the absence of particles, the surfactant also cannot access the site of action and does not initiate the response.

4) At a concentration of 1% solids, at 0.1 ml/kg and at infusion rate of 10 ml/min hemodynamic effects occur. At 5 ml/min delivery of the hemodynamic effect was about half of that of the 10 ml/min infusion.

5) At particle size of less than about 100 nm no hemodynamic effect occurs, while hemodynamic effect increases from 100 nm to 200 nm particle size and above 200 nm particle size.

6) Pretreatment with antihistamines to avoid hemodynamic effects. Plasma histamine levels appear to be elevated during the response. Thus the ability to block the effects of histamine at its receptor site can block the response or at least mitigate it.

Based on these and other studies the present invention provides methods which can be utilized to deliver effective amounts of drugs without eliciting adverse hemodynamic effects in dogs. The drug formulations and their methods of preparations will now be described.

Drugs

The particles comprise a therapeutic or diagnostic agent. (therapeutic agents are sometimes referred to as drugs or pharmaceuticals. The diagnostic agent referred to is typically a contrast agent such as an x-ray contrast agent but can also be other diagnostic materials.) The therapeutic or diagnostic agent exists as a discrete, crystalline phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as described in EPO 275,796.

The invention can be practiced with a wide variety of therapeutic or diagnostic agents. The therapeutic or diagnostic agent preferably is present in an essentially pure form. The therapeutic or diagnostic agent must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the therapeutic or diagnostic agent has a solubility in the liquid dispersion medium of less than about 10 mg/ml, and preferably of less than about 1 mg/ml. A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which a therapeutic or diagnostic agent is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

Suitable therapeutic or diagnostic agents can be selected from a variety of known classes of therapeutic or diagnostic agents including, for example, anti-inflammatory agents, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), beta-adrenoceptor blocking agents, cardiac inotropic agents, contrast media, corticosteroids, diagnostic agents, diagnostic imaging agents, dopaminergics (antiparkinsonian agents), haemostatics, immuniological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines. A description of these classes of therapeutic agents and diagnostic agents and a listing of species within each class can be found in Martindale, *The Extra Pharmacopoeia,* Twenty-ninth Edition, The Pharmaceutical Press, London, 1989. The therapeutic or diagnostic agents are commercially available and/or can be prepared by techniques known in the art.

Preferred diagnostic agents include the x-ray imaging agent WIN-8883 (ethyl 3,5-diacetamido-2,4,6triiodobenzoate) also known as the ethyl ester of diatrazoic acid (EEDA), WIN 67722, i.e., (6-ethoxy-6-oxohexyl-3,5-bis(acetamido)- 2,4,6-triiodobenzoate; ethyl-2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)butyrate (WIN 16318); ethyl diatrizoxyacetate (WIN 12901); ethyl 2-(3,5bis(acetamido)-2,4,6-triiodobenzoyloxy)propionate (WIN 16923); N-ethyl 2-(3,5-bis(acetamido)-2,4,6triiodobenzoyloxy acetamide (WIN 65312); isopropyl 2(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy) acetamide (WIN 12855); diethyl 2-(3,5-bis(acetamido)-2,4,6triiodobenzoyloxy malonate (WIN 67721); ethyl 2-(3,5bis(acetamido)-2,4,6-triiodobenzoyloxy) phenylacetate (WIN 67585); propanedioic acid, [[3,5-bis(acetylamino)2,4,5-triodobenzoyl]oxy]-,bis(1-methyl)ester (WIN 68165); and benzoic acid, 3,5-bis(acetylamino) 2,4,6triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester (WIN 68209). Suitable diagnostic agents are also disclosed in U.S. Pat. No. 5,260,478; U.S. Pat. No. 5,264,610; U.S. Pat. No. 5,322,679 and U.S. Pat. No. 5,300,739.

Preferred contrast agents include those which are expected to disintegrate relatively rapidly under physiological conditions, thus minimizing any particle associated inflammatory response.

Surface Modifiers

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and ionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronics™ F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, and polyxamines such as Tetronics™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to

ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OTs™, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanimid, Duponols™ P, which is a sodium lauryl sulfate, available from DuPont, Tritons™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohn and Haas, Tween™ 20 and Tweens™ 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Specialty Chemicals; Carbowaxs™ 3550 and 934, which are polyethylene glycols available from Union Carbide; Crodestas™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodestas™ SL-40, which is available from Croda, Inc., and SA9OHCO, which is $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4(CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include Tetronics™ 908, the Tweenss™, Pluronics™ F-68 and polyvinylpyrrolidone. Other useful surface modifiers include:

decanoyl-N-methylglucamide;

n-decyl β-D-glucopyranoside;

n-decyl β-D-maltopyranoside;

n-dodecyl β-D-glucopyranoside;

n-dodecyl β-D-maltoside;

heptanoyl-N-methylglucamide;

n-heptyl-β-D-glucopyranoside;

n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside;

nonanoyl-N-methylglucamide;

n-noyl β-D-glucopyranoside;

octanoyl-N-methylglucamide;

n-octyl-β-D-glucopyranoside;

octyl β-D-thioglucopyranoside; and the like.

Another useful surface modifier is tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type; also known as superinone or triton). This surface modifier is commercially available and/or can be prepared by techniques known in the art.

Another preferred surface modifier is p-isononylphenoxypoly(glycidol) also known as Olin-10G™ or Surfactant 10-G, is commercially available as 10G™ from Olin Chemicals, Stamford, Conn.

Non-Ionic Surface Modifiers

Preferred surface modifiers can be selected from known non-ionic surfactants, including the poloxamines such as Tetronic™908 (also known as Poloxanmine™908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, or Tetronic™ 1508 (T-1508), or a polymer of the alkyl aryl polyether alcohol type, such as tyloxapol.

The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two-or more surface modifiers can be used in combination.

Auxiliary Surface Modifiers

Particularly preferred auxiliary surface modifiers are those which impart resistance to particle aggregation during sterilization and include dioctylsulfosuccinate (DOSS), polyethylene glycol, glycerol, sodium dodecyl sulfate, dodecyl trimethyl ammonium bromide and a charged phospholipid such as dimyristoyl phophatidyl glycerol. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Block Copolvmer Surface Modifiers

One preferred surface modifier is a block copolymer linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule.

Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000–50,000, preferably 2,000–40,000 and more preferably 3,000–30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at least about 60% by weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer.

A preferred class of block copolymers useful as surface modifiers herein includes sulfated block copolymers of ethylene oxide and propylene oxide. These block copolymers in an unsulfated form are commercially available as Pluronics™. Specific examples of the unsulfated block copolymers include F68, F108 and F127.

Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics™.

Another preferred class of surface modifiers contain at least one polyethylene oxide (PEO) block as the hydrophilic portion of the molecule and at least one polybutylene oxide (PBO) block as the hydrophobic portion. Particularly preferred surface modifiers of this class are diblock, triblock, and higher block copolymers of ethylene oxide and butylene oxide, such as are represented, for example, by the following structural formula:

$$\text{-(PEO)-(PBO)-(PEO)-(PBO)-(PEO)-} \text{ and } \text{-(PEO)-(PBO)-(PEO)-(PBO)-}$$

The block copolymers useful herein are known compounds and/or can be readily prepared by techniques well known in the art.

Highly preferred surface modifiers include triblock copolymers of the -(PEO)-(PBO)-(PEO)- having molecular weights of 3800 and 5000 which are commercially available from Dow Chemical, Midland, Michigan, and are referred to as B20-3800 and B20-5000. These surface modifiers contain about 80% by weight PEO. In a preferred embodiment, the surface modifier is a triblock polymer having the structure:

$$R-Q\left(CH_2CH_2O\right)_z\left[CH_2CHO\right]_y\left(CH_2CH_2O\right)_z Q-R$$

(with C_2H_5 on the CHO unit)

Q is an anionic group

wherein R is H or a metal cation such as Na+, K+ and the like, x is 15-700, Y is 5-200 and z is 15-700.

Grinding

The described particles can be prepared in a method comprising the steps of dispersing a therapeutic or diagnostic agent in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the therapeutic or diagnostic agent to an effective average particle size of less than about 1000

nm, and preferably of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

The therapeutic or diagnostic agent selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse therapeutic or diagnostic agent selected be less than about 10 mm as determined by sieve analysis. If the coarse particle size of the therapeutic or diagnostic agent is greater than about 100 mm, then it is preferred that the particles of the therapeutic or diagnostic agent be reduced in size to less than 100 mm using a conventional milling method such as airjet or fragmentation milling.

The coarse therapeutic or diagnostic agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the therapeutic or diagnostic agent in the liquid medium can vary from about 0.1–60%, and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the therapeutic or diagnostic agent and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 1000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates-visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition. Alternatively, the therapeutic or diagnostic agnet and, optionally, the surface modifier, can be dispersed in the iquid medium using suitable agitiation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the therapeutic or diagnostic agent conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

Preparation Conditions

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the therapeutic or diagnostic agent. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Control of the temperature, e.g., by jacketing or immersion of the milling chamber in ice water are contemplated. Processing pressures from about 1 psi (0.07 kg/cm2) up to about 50 psi (3.5 kg/cm2) are contemplated. Processing pressures from about 10 psi (0.7 kg/cm2) to about 20 psi 1.4 kg/cm2)

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

After attrition is completed, the grinding media is separated from the milled particulate product (in either a dry or liquid dispersion form) using conventional separation techniques, such as by filtration, sieving through a mesh screen, and the like.

Grinding Media

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. We have found that zirconium oxide, such as 95% ZrO2 stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO2 stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 3 g/cm3.

Polymeric Grinding Media

The grinding media can comprise particles, preferably substantially spherical in shape, e.g., beads, consisting essentially of polymeric resin. Alternatively, the grinding media can comprise particles comprising a core having a coating of the polymeric resin adhered thereon.

In general, polymeric resins suitable for use herein are chemically and physically inert, substantially free of metals, solvent and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene, styrene copolymers, polycarbonates, polyacetals, such as Delrin™, vinyl chloride polymers and copolymers, polyurethanes, polyamides, poly (tetrafluoroethylenes), e.g., Teflon™, and other fluoropolymers, high density polyethylenes,

polypropylenes, cellulose ethers and esters such as cellulose acetate, polyhydroxymethacrylate, polyhydroxyethyl acrylate, silicone containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable polymers include poly(lactides), poly (glycolide) copolymers of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacylate), poly (imino carbonates), poly(N-acylhydroxyproline)esters, poly (N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymers, poly(orthoesters), poly(caprolactones), and poly(phosphazenes). In the case of biodegradable polymers, contamination from the media itself advantageously can metabolize in vivo into biologically acceptable products which can be eliminated from the body.

The polymeric resin can have a density from 0.8 to 3.0 g/cm3. Higher density resins are preferred inasmuch as it is believed that these provide more efficient particle size reduction.

The media can range in size from about 0.1 to 3 mm. For fine grinding, the particles preferably are from 0.2 to 2 mm, more preferably, 0.25 to 1 mm in size.

In a particularly preferred method, a therapeutic or diagnostic agent is prepared in the form of submicron particles by grinding the agent in the presence of a grinding media having a mean particle size of less than about 75 microns.

The core material of the grinding media preferably can be selected from materials known to be useful as grinding media when fabricated as spheres or particles. Suitable core materials include zirconium oxides (such as 95% zirconium oxide stabilized with magnesia or yttrium), zirconium silicate, glass, stainless steel, titania, alumina, ferrite and the like. Preferred core materials have a density greater than about 2.5 g/cm^3. The selection of high density core materials is believed to facilitate efficient particle size reduction.

Useful thicknesses of the polymer coating on the core are believed to range from about 1 to about 500 microns, although other thicknesses outside this range may be useful in some applications. The thickness of the polymer coating preferably is less than the diameter of the core.

The cores can be coated with the polymeric resin by techniques known in the art. Suitable techniques include spray coating, fluidized bed coating, and melt coating. Adhesion promoting or tie layers can optionally be provided to improve the adhesion between the core material and the resin coating. The adhesion of the polymer coating to the core material can be enhanced by treating the core material to adhesion promoting procedures, such as roughening of the core surface, corona discharge treatment, and the like.

Continuous Grinding

In a preferred grinding process, the particles are made continuously rather than in a batch mode. The continuous method comprises the steps of continuously introducing the therapeutic or diagnostic agent and rigid grinding media into a milling chamber, contacting the agent with the grinding media while in the chamber to reduce the particle size of the agent, continuously removing the agent and the grinding media from the milling chamber, and thereafter separating the agent from the grinding media.

The therapeutic or diagnostic agent and the grinding media are continuously removed from the milling chamber. Thereafter, the grinding media is separated from the milled particulate agent (in either a dry or liquid dispersion form) using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed.

In a preferred embodiment, the agent and grinding media are recirculated through the milling chamber. Examples of suitable means to effect such recirculation include conventional pumps such as peristaltic pumps, diaphragm pumps, piston pumps, centrifugal pumps and other positive displacement pumps which do not use sufficiently close tolerances to damage the grinding media. Peristaltic pumps are generally preferred.

Another variation of the continuous process includes the use of mixed media sizes. For example, larger media may be employed in a conventional manner where such media is restricted to the milling chamber. Smaller grinding media may be continuously recirculated through the system and permitted to pass through the agitated bed of larger grinding media. In this embodiment, the smaller media is preferably between about 1 and 300 mm in mean particle size and the larger grinding media is between about 300 and 1000 mm in mean particle size.

Precipitation Method

Another method of forming the desired nanoparticle dispersion is by microprecipitation. This is a method of preparing stable dispersions of therapeutic and diagnostic agents in the presence of a surface modifying and colloid stability enhancing surface active agent free of trace of any toxic solvents or solubilized heavy metal inpurities by the following procedural steps:

1. Dissolving the therapeutic or diagnostic agent in aqueous base with stirring,

2. Adding above #1 formulation with stirring to a surface active surfactant (or surface modifiers) solution to form a clear solution, and

3. Neutralizing above formulation #2 with stirring with an appropriate acid solution. The procedure can be followed by:

4. Removal of formed salt by dialysis or diafiltration and

5. Concentration of dispersion by conventional means.

This microprecipitation process produces dispersion of therapeutic or diagnostic agents with Z-average particle diameter less than 400 nm (as measured by photon correlation spectroscopy) that are stable in particle size upon keeping under room temperature or refrigerated conditions. Such dispersions also demonstrate limited particle size growth upon autoclave-decontamination conditions used for standard blood-pool pharmaceutical agents.

Step 3 can be carried out in semicontinuous, continuous batch, or continuous methods at constant flow rates of the reacting components in computercontrolled reactors or in tubular reactors where reaction pH can be kept constant using pH-stat systems. Advantages of such modifications are that they provide cheaper manufacturing procedures for large-scale production of nanoparticulate dispersion systems.

Additional surface modifier may be added to the dispersion after precipitation. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonicationstep, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

In a preferred embodiment, the above procedure is followed with step 4 which comprises removing the formed salts by diafiltration or dialysis. This is done in the case of dialysis by standard dialysis equipment and by diafiltration using standard diafiltration equipment known in the art. Preferably, the final step is concentration to a desired con-

centration of the agent dispersion. This is done either by diafiltration or evaporation using standard equipment known in this art

An advantage of microprecipitation is that unlike milled dispersion, the final product is free of heavy metal contaminants arising from the milling media that must be removed due to their toxicity before product is formulated.

A further advantage of the microprecipitation method is that unlike solvent precipitation, the final product is free of any trace of trace solvents that may be toxic and must be removed by expensive treatments prior to final product formulation.

In another preferred embodiment of the microprecipitation process, a crystal growth modifier is used. A crystal growth modifier is defined as a compound that in the co-precipitation process incorporates into the crystal structure of the microprecipitated crystals of the pharmaceutical agent, thereby hindering growth or enlargement of the microcrystalline precipitate, by the so called Ostwald ripening process. A crystal growth modifier (or a CGM) is a chemical that is at least 75% identical in chemical structure to the pharmaceuticl agent. By "identical" is meant that the structures are identical atom for atom and their connectivity. Structural identity is charactarized as having 75% of the chemical structure, on a molecular weight basis, identical to the therapeutic or diagnostic agent. The remaining 25% of the structure may be absent or replaced by different chemical structure in the CGM. The crystal growth modifier is dissolved in step #1 with the therapeutic or diagnostic agent.

Particle Size

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. When photon correlation spectroscopy (PCS) is used as the method of particle sizing the average particle diameter is the Z-average particle diameter known to those skilled in the art. By "an effective average particle size of less than about 1000 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 1000 nm when measured by the above-noted techniques. In preferred embodiments, the effective average particle size is less than about 400 nm and more preferrably less than about 300 nm. In some embodiments, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 1000 nm. In particularly preferred embodiments essentially all of the particles have a size less than 1000 nm. In some embodiments, essentially all of the particles have a size less than 400 nm.

Ratios

The relative amount of therapeutic or diagnostic agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular therapeutic or diagnostic agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square meter surface area of the therapeutic or diagnostic agent. The surface modifier can be present in an amount of 0.1–90%, preferably 20–60% by weight based on the total weight of the dry particle.

Diagnosis

A method for diagnostic imaging for use in medical procedures in accordance with this invention comprises intravenously administering to the body of a test subject in need of a diagnostic image an effective contrast producing amount of the diagnostic image contrast composition. Thereafter, at least a portion of the body containing the administered contrast agent is exposed to x-rays or a magnetic field to produce an x-ray or magnetic resonance image pattern corresponding to the presence of the contrast agent. The image pattern can then be visualized.

Any x-ray visualization technique, preferably, a high contrast technique such as computed tomography, can be applied in a conventional manner. Alternatively, the image pattern can be observed directly on an x-ray sensitive phosphor screen-silver halide photographic film combination or by use of a storage phosphor screen.

Visualization with a magnetic resonance imaging system can be accomplished with commercially available magnetic imaging systems such as a General Electric 1.5 T Sigma imaging system [1H resonant frequency 63.9 megahertz (MHz)]. Commercially available magnetic resonance imaging systems are typically characterized by the magnetic field strength used, with a field strength of 2.0 Tesla as the current maximum and 0.2 Tesla as the current minimum. For a given field strength, each detected nucleus has a characteristic frequency. For example, at a field strength of 1.0 Tesla, the resonance frequency for hydrogen is 42.57 10 MHz; for phosphorus-31 it is 17.24 MHz; and for sodium23 it is 11.26 Mhz.

A contrast effective amount of the diagnostic agent containing composition is that amount necessary to provide tissue visualization with, for example, magnetic resonance imaging or x-ray imaging. Means for determining a contrast effective amount in a particular subject will depend, as is well known in the art, on the nature of the magnetically reactive material used, the mass of the subject being imaged, the sensitivity of the magnetic resonance or x-ray imaging system and the like.

After administration of the compositions, the subject is maintained for a time period sufficient for the administered compositions to be distributed throughout the subject and enter the tissues of the mammal. Typically, a sufficient time period is from about 20 minutes to about 90 minutes and, preferably from about 20 minutes to about 60 minutes.

The invention has been described with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

What is claimed is:

1. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising intravenously administering to said mammal an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, wherein said drug composition comprises:

(a) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml;

(b) a surface modifier adsorbed on the surface of the drug substance in an amount of from about 0.1 to about

99.9% by weight and sufficient to maintain an effective average particle size of from about 50 nm to about 1000 nm; and

(c) a pharmaceutically acceptable carrier therefor.

2. The method of claim 1 wherein the effective average particle size of said drug is from about 50 to about 400 nm.

3. The method of claim 1 wherein said drug is an organic therapeutic substance.

4. The method of claim 1 wherein said drug is a diagnostic agent.

5. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal an antihistamine in the amount of from about 5 to about 10 mg/kg of body weight; and

(b) subsequently intravenously administering to said mammal an effective amount of a nanoparticulate drug composition comprising: (1) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml; and (2) a surface modifier adsorbed on the surface of the drug substance in an amount of from about 99.9 to about 0.1% by weight and sufficient to maintain an effective average particle size of less than about 1000 nm.

6. The method of claim 5 wherein the effective average particle size of said drug is of from about 50 to about 400 nm.

7. The method of claim 5 wherein said drug is an organic therapeutic substance.

8. The method of claim 5 wherein said drug is a diagnostic agent.

9. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal a desensitizing amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min; and

(b) intravenously administering an effective amount of said nanoparticulate composition comprising: (1) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml; (2) a surface modifier adsorbed on the surface of the drug substance in an amount sufficient to maintain an effective average particle size of from about 100 to about 1000 nm; and (3) a pharmaceutically acceptable carrier therefor.

10. The method of claim 9 wherein said drug is an organic therapeutic substance.

11. The method of claim 9 wherein said drug is a diagnostic agent.

12. The method of claim 9 wherein the effective average particle size of said drug is of from about 100 to about 400 nm.

13. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising intravenously administering to said mammal an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, wherein said drug composition comprises:

(a) particles having an effective average particle size of from about 50 to about 1000 nm and consisting essentially of from about 0.1 to about 99.9% by weight of an organic drug substance entrapped in from about 99.9 to about 0.1% by weight of liposome or a colloidal polymeric material; and

(b) a pharmaceutically acceptable carrier therefor.

14. The method of claim 13 wherein the effective average particle size of said drug is of from about 50 to about 400 nm.

15. The method of claim 13 wherein said drug is an organic therapeutic substance.

16. The method of claim 13 wherein said drug is a diagnostic agent.

17. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal an antihistamine in the amount of from about 5 to about 10 mg/kg of body weight; and

(b) subsequently intravenously administering to said mammal an effective amount of a nanoparticulate drug composition comprising: (1) particles having an effective average particle size of from about 50 to bout 1000 nm and consisting essentially of from about 0.1 to about 99.9% by weight of an organic drug substance entrapped in from about 99.9 to about 0.1% by weight of liposome or a colloidal polymeric material; and (2) a pharmaceutically acceptable carrier therefor.

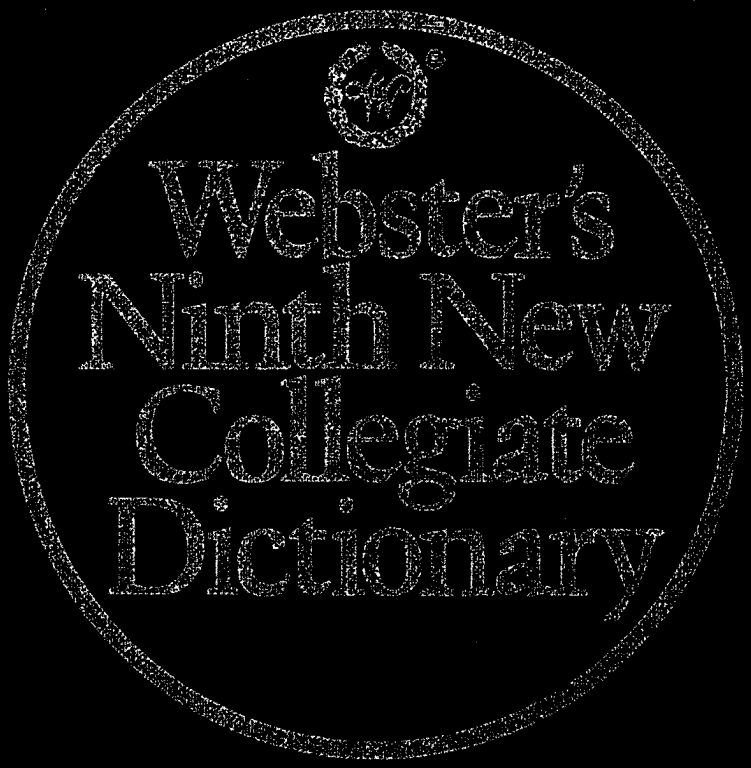
18. The method of claim 17 wherein the effective average particle size of said drug is of from about 50 to about 400 nm.

19. The method of claim 17 wherein said drug is an organic therapeutic substance.

20. The method of claim 17 wherein said drug is a diagnostic agent.

* * * * *

EXHIBIT C



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esp. in speech : PRUDENT, *esp* : capable of preserving prudent silence 2 : UNPRETENTIOUS, MODEST ⟨the warmth and ~ elegance of a civilized home —Joseph Wechsberg⟩ — **dis-creet-ly** *adv* — **dis-creet-ness** *n*

dis-crep-an-cy \dis-'krep-ən-sē\ *n, pl* **-cies** (ca. 1623) 1 : the quality or state of being discrepant : DIFFERENCE 2 : an instance of being discrepant

dis-crep-ant \-ənt\ *adj* [L *discrepant-, discrepans*, prp. of *discrepare* to sound discordantly, fr. *dis-* + *crepare* to rattle, creak — more at RAVEN] (15c) : being at variance : DISAGREEING ⟨widely ~ conclusions⟩ — **dis-crep-ant-ly** *adv*

dis-crete \dis-'krēt, 'dis-,\ *adj* [ME, fr. L *discretus*] (14c) 1 : constituting a separate entity : individually distinct 2 **a** : consisting of distinct or unconnected elements : NONCONTINUOUS **b** : taking on or having a finite or countably infinite number of values : not mathematically continuous ⟨a ~ random variable⟩ *syn* see DISTINCT — **dis-crete-ly** *adv* — **dis-crete-ness** *n*

dis-cre-tion \dis-'kresh-ən\ *n* (14c) 1 : the quality of being discreet : CIRCUMSPECTION; *esp* : cautious reserve in speech 2 : ability to make responsible decisions 3 **a** : individual choice or judgment ⟨left the decision to his ~⟩ **b** : power of free decision or latitude of choice within certain legal bounds ⟨reached the age of ~⟩ 4 : the result of separating or distinguishing ⟨breaking down every operation into discrete parts, and then making verbal the ~s that are made —Elinor Langer⟩

dis-cre-tion-ary \-'kresh-ə-,ner-ē\ *adj* (1698) 1 : left to discretion : exercised at one's own discretion 2 : available for discretionary use ⟨~ purchasing power⟩

discretionary account *n* (ca. 1920) : a security or commodity market account in which an agent (as a broker) is given power of attorney allowing him to make independent decisions and buy and sell for the account of his principal

dis-crim-i-na-bil-i-ty \-,krim-(ə-)nə-'bil-ət-ē\ *n, pl* **-ties** (ca. 1901) 1 : the quality of being discriminable ⟨the ~ of the various senses of a word⟩ 2 : the ability to discriminate

dis-crim-i-na-ble \dis-'krim-(ə-)nə-bəl\ *adj* (1730) : capable of being discriminated — **dis-crim-i-na-bly** \-blē\ *adv*

dis-crim-i-nant \-'krim-(ə-)nənt\ *n* (ca. 1948) : a mathematical expression providing a criterion for the behavior of another more complicated expression, relation, or set of relations

discriminant function *n* (ca. 1936) : a function of a set of variables (as measurements of taxonomic specimens) that is evaluated for samples of events or objects and used as an aid in discriminating between or classifying them

dis-crim-i-nate \dis-'krim-ə-,nāt\ *vb* **-nat-ed; -nat-ing** [L *discriminatus*, pp. of *discriminare*, fr. *discrimin-, discrimen* distinction, fr. *discernere* to distinguish between — more at DISCERN] *vt* (1628) 1 **a** : to mark or perceive the distinguishing or peculiar features of **b** : DISTINGUISH, DIFFERENTIATE ⟨~ hundreds of colors⟩ 2 : to distinguish by discerning or exposing differences; *esp* : to distinguish from another like object ⟨~ *vi* 1 **a** : to make a distinction ⟨~ among the methods which should be used⟩ **b** : to use good judgment 2 : to make a difference in treatment or favor on a basis other than individual merit ⟨~ in favor of your friends⟩ ⟨~ against a certain nationality⟩

dis-crim-i-nat-ing *adj* (1647) 1 : making a distinction : DISTINGUISHING 2 : marked by discrimination: **a** : DISCERNING, JUDICIOUS **b** : DISCRIMINATORY — **dis-crim-i-nat-ing-ly** \-,nāt-iŋ-lē\ *adv*

dis-crim-i-na-tion \dis-,krim-ə-'nā-shən\ *n* (1648) 1 **a** : the act of discriminating **b** : the process by which two stimuli differing in some aspect are responded to differently : DIFFERENTIATION 2 : the quality or power of finely distinguishing 3 **a** : the act, practice, or an instance of discriminating categorically rather than individually **b** : prejudiced or prejudicial outlook, action, or treatment ⟨racial ~⟩ *syn* see DISCERNMENT — **dis-crim-i-na-tion-al** \-shnəl, -shən-ᵊl\ *adj*

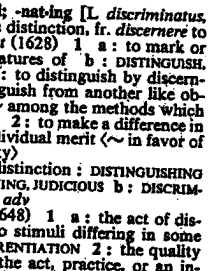
dis-crim-i-na-tive \dis-'krim-ə-,nāt-iv, -'krim-(ə-)nət-\ *adj* (1677) 1 : making distinctions 2 : DISCRIMINATORY 2

dis-crim-i-na-tor \dis-'krim-ə-,nāt-ər\ *n* (1828) : one that discriminates; *specif* : a circuit that can be adjusted to accept or reject signals of different characteristics (as amplitude or frequency)

dis-crim-i-na-to-ry \dis-'krim-(ə-)nə-,tōr-ē, -,tȯr-\ *adj* (1828) 1 : DISCRIMINATIVE 1 2 : applying or favoring discrimination in treatment — **dis-crim-i-na-to-ri-ly** \-,krim-(ə-)nə-'tōr-ə-lē, -'tȯr-\ *adv*

dis-cur-sive \dis-'kər-siv\ *adj* [ML *discursivus*, fr. L *discursus*, pp. of *discurrere* to run about — more at DISCOURSE] (1598) 1 **a** : moving from topic to topic without order : RAMBLING **b** : proceeding coherently from topic to topic 2 : marked by analytical reasoning — **dis-cur-sive-ly** *adv* — **dis-cur-sive-ness** *n*

dis-cus \'dis-kəs\ *n, pl* **dis-cus-es** [L — more at DISH] (1656) 1 : a disk (as of wood or plastic) that is thicker in the center than at the perimeter and that is hurled for distance as a track-and-field event; *also* : the event 2 : DISK 2, 3



discus 1

dis-cuss \dis-'kəs\ *vt* [ME *discussen*, fr. L *discussus*, pp. of *discutere* to disperse, fr. *dis-* apart + *quatere* to shake — more at DISQUASH] (14c) 1 *obs* : DISPEL 2 **a** : to investigate by reasoning or argument **b** : to present in detail for examination or consideration ⟨~*ed* plans for the party⟩ **c** : to talk about 3 *obs* : DECLARE — **dis-cuss-able** *or* **dis-cuss-ible** \-ə-bəl\ *adj* — **dis-cuss-er** *n*

syn DISCUSS, ARGUE, DEBATE, DISPUTE mean to discourse about in order to reach conclusions or to convince. DISCUSS implies a sifting of possibilities esp. by presenting considerations pro and con; ARGUE implies the offering of reasons or evidence in support of convictions already held; DEBATE suggests formal or public argument between opposing parties; it may also apply to deliberation with oneself; DISPUTE implies contentious or heated argument.

dis-cus-sant \dis-'kəs-ənt\ *n* (1926) : one who takes part in a formal discussion or symposium

dis-cus-sion \dis-'kəsh-ən\ *n* (14c) 1 : consideration of a question in open and usu. informal debate 2 : a formal treatment of a topic in

[1]dis-dain \dis-'dān\ *n* [ME *desdeyne*, fr. OF *desdeign*, fr. *desdeignier*] (13c) : a feeling of contempt for what is beneath one : SCORN

[2]disdain *vt* [ME *desdeynen*, fr. MF *desdeignier*, fr. OF; fr. (assumed) VL *disdignare*, fr. L *dis-* + *dignare* to deign — more at DEIGN] (14c) 1 : to look with scorn on 2 : to refuse or abstain from because of disdain 3 : to treat disdainfully *syn* see DESPISE

dis-dain-ful \-fəl\ *adj* (1542) : full of or expressing disdain *syn* see PROUD — **dis-dain-ful-ly** \-fə-lē\ *adv* — **dis-dain-ful-ness** *n*

dis-ease \diz-'ēz\ *n* [ME *disese*, fr. MF *desaise*, fr. *des-* dis- + *aise* ease] (14c) 1 *obs* : TROUBLE 2 : a condition of the living animal or plant body or of one of its parts that impairs the performance of a vital function : SICKNESS, MALADY 3 : a harmful development (as in a social institution) — **dis-eased** \-'ēzd\ *adj*

dis-econ-o-my \,dis-i-'kän-ə-mē\ *n* (1937) 1 : a lack of economy 2 : a factor responsible for an increase in cost

dis-em-bark \,dis-əm-'bärk\ *vb* [MF *desembarquer*, fr. *des-* + *embarquer* to embark] *vt* (1582) : to remove to shore from a ship ~ *vi* 1 : to go ashore out of a ship 2 : to get out of a vehicle or craft — **dis-em-bar-ka-tion** \(,)dis-,em-,bär-'kā-shən, -bər-\ *n*

dis-em-bar-rass \,dis-əm-'bar-əs\ *vt* (1726) : to free from something troublesome or superfluous *syn* see EXTRICATE

dis-em-body \,dis-əm-'bäd-ē\ *vt* (1714) : to divest of a body, of corporeal existence, or of reality

dis-em-bogue \,dis-əm-'bōg\ *vb* **-bogued; -bogu-ing** [modif. of Sp *desembocar*, fr. *des-* dis- (fr. L *dis-*) + *embocar* to put into the mouth, fr. *en* in (fr. L *in*) + *boca* mouth, fr. L *bucca* — more at POCK] *vi* (1595) : to flow or come forth from or as if from a channel ~ *vt* : to pour out : EMPTY

dis-em-bow-el \,dis-əm-'baù(-ə)l\ *vt* (1613) 1 : to take out the bowels of : EVISCERATE 2 : to remove the substance of — **dis-em-bow-el-ment** \-mənt\ *n*

dis-en-chant \,dis-ᵊn-'chant\ *vt* [MF *desenchanter*, fr. *des-* + *enchanter* to enchant] (1586) : to free from illusion — **dis-en-chant-er** *n* — **dis-en-chant-ing** *adj* — **dis-en-chant-ing-ly** \-iŋ-lē\ *adv* — **dis-en-chant-ment** \-mənt\ *n*

dis-en-cum-ber \,dis-ᵊn-'kəm-bər\ *vt* [MF *desencombrer*, fr. *des-* + *encombrer* to encumber] (1598) : to free from encumbrance : DISBURDEN *syn* see EXTRICATE

dis-en-dow \,dis-ᵊn-'daù\ *vt* (1861) : to strip of endowment — **dis-en-dow-er** \-'daù(-ə)r\ *n* — **dis-en-dow-ment** \-'daù-mənt\ *n*

dis-en-fran-chise \,dis-ᵊn-'fran-,chīz\ *vt* (1664) : DISFRANCHISE — **dis-en-fran-chise-ment** \-,chīz-mənt, -chəz-\ *n*

dis-en-gage \,dis-ᵊn-'gāj\ *vb* [F *désengager*, fr. MF, fr. *des-* + *engager* to engage] *vt* (1611) : to release from something that engages ~ *vi* : to release or detach oneself : WITHDRAW — **dis-en-gage-ment** \-mənt\ *n*

dis-en-tail \,dis-ᵊn-'tā(ə)l\ *vt* (1641) : to free from entail

dis-en-tan-gle \,dis-ᵊn-'taŋ-gəl\ *vt* (1598) : to free from entanglement : UNRAVEL ~ *vi* : to become disentangled *syn* see EXTRICATE — **dis-en-tan-gle-ment** \-mənt\ *n*

dis-en-thrall *also* **dis-en-thral** \,dis-ᵊn-'thrȯl\ *vt* (1643) : to free from bondage : LIBERATE

dis-equil-i-brate \,dis-i-'kwil-ə-,brāt\ *vt* (1891) : to put out of balance — **dis-equil-i-bra-tion** \-,kwil-ə-'brā-shən\ *n*

dis-equi-lib-ri-um \(,)dis-,ē-kwə-'lib-rē-əm, -,ek-wə-\ *n* (1840) : loss or lack of equilibrium

dis-es-tab-lish \,dis-ə-'stab-lish\ *vt* (1598) : to deprive of an established status; *esp* : to deprive of the status and privileges of an established church — **dis-es-tab-lish-ment** \-mənt\ *n*

dis-es-tab-lish-men-tar-i-an \-,stab-lish-,men-'ter-ē-ən, -mən-\ *n, often cap* [*disestablishment*] (1885) : one who opposes an established order — **disestablishmentarian** *adj, often cap*

[1]dis-es-teem \,dis-ə-'stēm\ *vt* (1594) : to regard with disfavor

[2]disesteem *n* (1603) : DISFAVOR, DISREPUTE

di-sesuse \dē-'zə(r)z, -'züz\ *n, pl* **di-seuses** \-'zə(r)z(-əz), -'züz(-əz)\ [F, fem. of *diseur*, fr. OF, fr. *dire* to say, fr. L *dicere* — more at DICTION] (1895) : a skilled and usu. professional woman reciter

[1]dis-fa-vor \(ᵊ)dis-'fā-vər\ *n* [prob. fr. MF *desfaveur*, fr. *des-* dis- + *faveur* favor, fr. OF *favor*] (1533) 1 : DISAPPROVAL, DISLIKE ⟨practices looked upon with ~⟩ 2 : the state or fact of being no longer favored ⟨fell into ~⟩ 3 : DISADVANTAGE

[2]disfavor *vt* (1570) : to withhold or withdraw favor from

dis-fea-ture \(ᵊ)dis-'fē-chər\ *vt* (1659) : to mar the features of — **dis-fea-ture-ment** \-mənt\ *n*

dis-fig-ure \dis-'fig-yər, *esp Brit* -'fig-ər\ *vt* [ME *disfiguren*, fr. MF *defigurer*, fr. *des-* + *figure* figure] (14c) 1 : to impair (as in beauty) by deep and persistent injuries ⟨a face *disfigured* by smallpox⟩ 2 *obs* : DISGUISE — **dis-fig-ure-ment** \-mənt\ *n*

dis-fran-chise \(ᵊ)dis-'fran-,chīz\ *vt* (15c) : to deprive of a franchise, of a legal right, or of some privilege or immunity; *esp* : to deprive of the right to vote — **dis-fran-chise-ment** \-,chīz-mənt, -chəz-\ *n*

dis-frock \(ᵊ)dis-'frik\ *vt* (1837) : UNFROCK

dis-func-tion *var of* DYSFUNCTION

dis-fur-nish \(ᵊ)dis-'fər-nish\ *vt* [MF *desfourniss-*, stem of *desfournir*, fr. *des-* + *fournir* to furnish] (1531) : to make destitute of possessions : DIVEST — **dis-fur-nish-ment** \-mənt\ *n*

dis-gorge \(ᵊ)dis-'gȯ(ə)rj\ *vb* [ME, fr. MF *desgorger*, fr. *des-* + *gorge* to gouge] *vt* (15c) 1 **a** : to discharge by the throat and mouth : VOMIT **b** : to discharge violently, confusedly, or as a result of force **c** : to give up on request or under pressure ⟨refused to ~ his ill-gotten gains⟩ 2 : to discharge the contents of (as the stomach) ~ *vi* : to discharge contents ⟨where the river ~s into the sea⟩

[1]dis-grace \dis-'grās\ *vt* (1580) 1 *archaic* : to humiliate by a superior showing 2 : to be a source of shame to ⟨your actions *disgraced* the family⟩ 3 : to cause to lose favor or standing ⟨was *disgraced* by the hint of scandal⟩ — **dis-grac-er** *n*

[2]disgrace *n* [MF, fr. OIt *disgrazia*, fr. *dis-* (fr. L) + *grazia* grace, fr. L *gratia* — more at GRACE] (1586) 1 **a** : the condition of one fallen from grace or honor **b** : loss of grace, favor, or honor 2 : something that disgraces ⟨your manners are a ~⟩

syn DISGRACE, DISHONOR, DISREPUTE, INFAMY, IGNOMINY mean the loss or condition of suffering loss of esteem and of enduring repu... DISGRACE often implies complete...

the loss of self-esteem; DISREPUTE stresses loss ... the acquiring of a bad reputation; INFAMY us... well as exceeding shame; IGNOMINY stresses t... contemptibility or despicableness of the disgrac...

dis-grace-ful \dis-'grās-fəl\ *adj* (1597) : bringing ... — **dis-grace-ful-ly** \-fə-lē\ *adv* — **dis-grace-ful-ne...**

dis-grun-tle \dis-'grənt-ᵊl\ *vt* **dis-grun-tled; dis-grun-tling** \-liŋ\ [*dis-* + *gruntle* (to grumble), fr. ME *grunt*] (1682) : to make ill-humored or discont... *disgruntled* with their wages⟩ — **dis-grun-tle-men...**

dis-guise \dis-'gīz\ *vt* **dis-guised; dis-guis-ing** ... *desguiser*, fr. OF, fr. *des-* + *guise* guise] (14c) ... customary dress or appearance of **b** : to furni... ance or an assumed identity 2 *obs* : DISFIGU... existence or true state or character of : CO... \-'gīz(-ə)d-lē\ *adv* — **dis-guise-ment** \-'gīz-mənt\ ... *syn* DISGUISE, CLOAK, MASK, DISSEMBLE mean to ... pearance so as to conceal the identity or true ... a change in appearance or behavior that mi... different apparent identity; CLOAK suggests a m... ment or an intention completely; MASK sugge... means of preventing recognition and need n... pretense; DISSEMBLE stresses simulation for the ...

disguise *n* (14c) 1 : apparel assumed to conceal ... terfeit another's 2 : the act of disguising 3 **a** ... the true nature of something ⟨blessings in ~⟩ ... : PRETENSE ⟨threw off all ~⟩

dis-gust \dis-'gəst\ *n* (1598) : marked aversion ... highly distasteful : REPUGNANCE

disgust *vb* [MF *desgouster*, fr. *des-* + *goust* taste, *gustare* to taste — more at CHOOSE] *vt* (1650) 1 ... ing, repugnance, or aversion : be offensive to ... lose an interest or intention ⟨his failures ~*ed* h... stopped trying⟩ ~ *vi* : to cause disgust — **dis-ed-ly** *adv*

dis-gust-ful \-'gəst-fəl\ *adj* (1616) 1 : provoking ... accompanied by disgust — **dis-gust-ful-ly** \-fə-lē\ ...

dis-gust-ing *adj* (1754) : exciting disgust — **dis-... ly** *adv*

dish \'dish\ *n* [ME, fr. OE *disc* plate, fr. L *disc* ... Gk *diskos*, fr. *dikein* to throw] (bef. 12c) 1 **a** : ... vessel from which food is served **b** : the cont... strawberries⟩ 2 : food prepared in a particula... of various shallow concave vessels; *broadly* ... concave (2) : a directional microwave antenna ... parabolic reflector **b** : the state of being co... concavity 4 **a** : something that is favored ⟨en... his ~⟩ **b** : an attractive woman

dish *vt* (14c) 1 : to put (as food for serving) in... with *up* 2 : PRESENT — usu. used with *up* 3 : ... dish

dis-ha-bille \,dis-ə-'bē(ə)l, -'bil, -'bē\ *n* [F *déshabiller* to undress, fr. *dés-* dis- + *habiller* to ... TMENT] (1673) 1 *a archaic* : NEGLIGEE **b** : th... in a casual or careless style 2 : a deliberately ... ner

dis-har-mon-ic \,dis-(,)här-'män-ik\ *adj* (1887) ... tion of bodily characters that results in an unus... 2: exhibiting or marked by allometry

dis-har-mo-ni-ous \-'mō-nē-əs\ *adj* (1659) 1 : ... : DISHARMONIC

dis-har-mo-nize \(ᵊ)dis-'här-mə-,nīz\ *vt* (1801) : t... **dis-har-mo-ny** \-nē\ *n* (1602) : lack of harmony ...

dish-cloth \'dish-,klȯth\ *n* (ca. 1828) : a cloth for ...

dish-clout \'dish-,klaùt\ *n; Brit* (ca. 1530) : DISHC...

dis-heart-en \(ᵊ)dis-'härt-ᵊn\ *vt* (1599) : to cause ... — **dis-heart-en-ing-ly** \-'härt-niŋ-lē, -ᵊn-iŋ-\ *a...* \-'härt-ᵊn-mənt\ *n*

dished \'disht\ *adj* (1737) 1 : CONCAVE 2 *of* ... ; nearer together at the bottom than at the top

dish-ev-el \'dish-'ev-əl\ *vt* **dish-evel-ed** *or* **dish-el-ed; dish-evel-ing** \-'ev-(ə-)liŋ\ [back-formation fr. ... throw into disorder or disarray

dish-evel-ed *or* **dish-elled** *adj* [ME *dischevele*, ... *chevelé*, fr. pp. of *descheveler* to disarrange the ... hair, fr. L *capillus*] (1583) : marked by disorder

dis-hon-est \(ᵊ)dis-'än-əst\ *adj* [ME, fr. MF *deshonest*] (14c) 1 *obs* : SHAMEFUL, UNCHASTE 2 ... of truth, honesty, or trustworthiness : UNFAIR ... est-ly *adv*

syn DISHONEST, DECEITFUL, MENDACIOUS, LYI... unworthy of trust or belief. DISHONEST implie... truth in order to deceive, cheat, or defraud; DE... intent to mislead and commonly suggests a fa... ble-dealing; MENDACIOUS is less forthright th... bland or even harmlessly mischievous deceit, ... suggests a habit of telling untruths; LYING h... instance rather than a habit or tendency; UNT... term than LYING and in application to account ... a discrepancy between what is said and fact ... intent to deceive

dis-hon-es-ty \-ə-stē\ *n* (1599) 1 : lack of hones... tion to defraud or deceive 2 : a dishonest act : ...

dis-hon-or \(ᵊ)dis-'än-ər\ *vt* (13c) 1 **a** : to trea... **b** : to bring shame on 2 : to refuse to accept ... check, or note)

dishonor *n* [ME *dishonour*, fr. MF *deshonor*, f... (14c] 1 : lack or loss of honor or reputation ... has lost honor or prestige : SHAME 3 : a cau... nonpayment or nonacceptance of commercial ... whom it is drawn *syn* see DISGRACE — **dis-hon...**

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el·e·vat·ed \-,vāt-əd\ *adj* (1553) 1 **a** : raised esp. above the ground or other surface ⟨an ~ highway⟩ **b** : increased esp. abnormally (as in degree or amount) ⟨~ blood pressure⟩ 2 **a** : being morally or intellectually on a high plane ⟨an ~ mind⟩ **b** : FORMAL, DIGNIFIED ⟨~ diction⟩ 3 : exhilarated in mood or feeling

elevated railroad *n* (1868) : an urban or interurban railroad operating chiefly on an elevated structure — called also *elevated railway*

el·e·va·tion \,el-ə-'vā-shən\ *n* (14c) 1 : the height to which something is elevated: as **a** : the angular distance of a celestial object above the horizon **b** : the degree to which a gun is aimed above the horizon **c** : the height above the level of the sea : ALTITUDE 2 : a ballet dancer's or a skater's leap and seeming suspension in the air; *also* : the ability to achieve an elevation 3 : an act or instance of elevating 4 : something that is elevated: as **a** : an elevated place **b** : a swelling esp. on the skin 5 : the quality or state of being elevated 6 : a geometrical projection (as of a building) on a vertical plane *syn* see HEIGHT

el·e·va·tor \'el-ə-,vāt-ər\ *n* (15c) 1 : one that raises or lifts something up: as **a** : an endless belt or chain conveyor with cleats, scoops, or buckets for raising material **b** : a cage or platform and its hoisting machinery for conveying something to different levels **c** : a building for elevating, storing, discharging, and sometimes processing grain 2 : a movable auxiliary airfoil usu. attached to the tail plane of an airplane for producing motion up or down — see AIRPLANE illustration

elev·en \i-'lev-ən\ *n* [ME *enleven*, fr. *enleven*, adj., fr. OE *endleofan*, fr. *end-* (akin to OE *ān* one) + *-leofan*; perh. akin to OE *lēon* to lend — more at ONE, LOAN] (bef. 12c) 1 — see NUMBER table 2 : the 11th in a set or series 3 : something having 11 units or members; *esp* : a football team — **eleven** *adj or pron* — **elev·enth** \-ən(t)th\ *adj or n*

eleven-plus \i-,lev-ən-'pləs\ *n, Brit* (1937) : an examination taken between the ages of 11 and 12 that determines the type of secondary education to which a student is assigned

elev·ens·es \-ən-zəz\ *n pl but sometimes sing in constr* [irreg. pl. of *eleven* (o'clock)] *Brit* (ca. 1819) : light refreshment (as a snack) taken in the middle of the morning

eleventh hour *n* (1826) : the latest possible time ⟨won his reprieve at the *eleventh hour*⟩

el·e·von \'el-ə-,vän\ *n* [*elevator* + *aileron*] (1944) : an airplane control surface that combines the functions of elevator and aileron

elf \'elf\ *n, pl* **elves** \'elvz\ [ME, fr. OE *ælf*; akin to ON *alfr* elf & prob. to L *albus* white — more at ALB] (bef. 12c) 1 : a small often mischievous fairy 2 **a** : a small lively creature; *esp* : a mischievous child **b** : a usu. lively mischievous or malicious person — **elf·ish** \'el-fish\ *adj* — **elf·ish·ly** *adv*

elf·in \'el-fən\ *adj* [irreg. fr. *elf*] (1596) 1 **a** : of, relating to, or produced by an elf **b** : resembling an elf 2 : having an otherworldly or magical quality or charm

elf·lock \'el-,fläk\ *n* (1592) : hair matted as if by elves — usu. used in pl.

el·hi \('),el-'hī\ *adj* [*elementary* (school) + *high* (school)] (1948) : of, relating to, or designed for use in grades 1 to 12

Eli \'ē-,lī\ *n* [Heb *Ēlī*] : a judge and priest of Israel who according to the account in I Samuel was entrusted with the care of the boy Samuel

Eli·as \i-'lī-əs\ *n* [LL, fr. Gk *Ēlias*, fr. Heb *Ēlīyāh*] : ELIJAH

elic·it \i-'lis-ət\ *vt* [L *elicitus*, pp. of *elicere*, fr. *e-* + *lacere* to allure — more at DELIGHT] (1605) 1 **a** : to draw forth or bring out (something latent or potential) **b** : to derive (as a truth) by logical processes 2 : to call forth or draw out (a response or reaction) *syn* see EDUCE — **elic·i·ta·tion** \i-,lis-ə-'tā-shən, ē-\ *n* — **elic·i·tor** \i-'lis-ət-ər\ *n*

elide \i-'līd\ *vt* **elid·ed; elid·ing** [L *elidere* to strike out, fr. *e-* + *laedere* to injure by striking] (1796) 1 **a** : to suppress or alter (as a vowel or syllable) by elision **b** : to strike out (as a written word or passage) 2 **a** : to leave out of consideration : OMIT **b** : CURTAIL, ABRIDGE

el·i·gi·ble \'el-ə-jə-bəl\ *adj* [ME, fr. MF & LL; MF, fr. LL *eligibilis*, fr. L *eligere* to choose — more at ELECT] (15c) 1 **a** : qualified to be chosen : ENTITLED ⟨~ for sophomore standing⟩ ⟨~ to retire⟩ **b** : permitted under football rules to catch a forward pass ⟨an ~ receiver⟩ 2 : worthy of being chosen : DESIRABLE ⟨an ~ young bachelor⟩ — **el·i·gi·bil·i·ty** \,el-ə-jə-'bil-ət-ē\ *n* — **eligible** *n* — **el·i·gi·bly** \'el-ə-jə-blē\ *adv*

Eli·jah \i-'lī-jə\ *n* [Heb *Ēlīyāh*] : a Hebrew prophet of the 9th century B.C. who according to the account in I Kings championed the worship of Jehovah as against Baal

elim·i·nate \i-'lim-ə-,nāt\ *vt* **-nat·ed; -nat·ing** [L *eliminatus*, pp. of *eliminare*, fr. *e-* + *limin-*, *limen* threshold — more at LIMB] (1568) 1 **a** : to cast out or get rid of : REMOVE, ERADICATE ⟨the need to ~ poverty⟩ **b** : to set aside as unimportant : IGNORE 2 : to expel (as waste) from the living body 3 : to cause to disappear by combining two or more equations — **elim·i·na·tion** \-,lim-ə-'nā-shən\ *n* — **elim·i·na·tive** \-'lim-ə-,nāt-iv\ *adj* — **elim·i·na·tor** \-,nāt-ər\ *n*

Eli·sha \i-'lī-shə\ *n* [Heb *Ēlīshā'*] : a Hebrew prophet and disciple and successor of Elijah

eli·sion \i-'lizh-ən\ *n* [LL *elision-*, *elisio*, fr. L *elisus*, pp. of *elidere*] (1581) 1 **a** : the use of a speech form that lacks a final or initial sound which a variant speech form has ⟨the use of 's instead of *is* in *there's* is an example of ~⟩ **b** : the omission of an unstressed vowel or syllable in a verse to achieve a uniform metrical pattern 2 : the act or an instance of omitting something : OMISSION

elite \ā-'lēt, i-\ *n* [F *élite*, fr. OF *eslite*, fr. fem. of *eslit*, pp. of *eslire* to choose, fr. L *eligere*] (1823) 1 **a** : the choice part or segment; *esp* : a socially superior group **b** : a powerful minority group ⟨a power ~ inside the government⟩ 2 : a typewriter type providing 12 characters to the linear inch — **elite** *adj*

elit·ism \-'lēt-,iz-əm\ *n* (1947) 1 **a** : leadership or rule by an elite **b** : belief in or advocacy of such elitism 2 : consciousness of being or belonging to an elite — **elit·ist** \-'lēt-əst\ *n or adj*

elix·ir \i-'lik-sər\ *n* [ME, fr. ML, fr. Ar *al-iksīr* the elixir, fr. *al* the + *iksīr* elixir, prob. fr. Gk *xērion* desiccative powder, fr. *xēros* dry — more at SERENE] (14c) 1 **a** : a substance held capable of changing base metals into gold : PHILOSOPHERS' STONE **b** (1) : a substance held capable of prolonging life indefinitely (2) : CURE-ALL (3) : a sweetened liquid usu. containing alcohol that is used as a vehicle for medicinal agents 2 : the essential principle

Eliz·a·be·than \i-,liz-ə-'bē-thən\ *adj* (1817) : of, relating to, or characteristic of Elizabeth I of England or her age — **Elizabethan** *n*

elk \'elk\ *n, pl* **elks** [ME, prob. fr. OE *eolh*; akin to OHG *elaho* elk, Gk *elaphos* deer] (bef. 12c) 1 *pl usu* **elk** **a** : the largest existing deer (*Alces alces*) of Europe and Asia resembling but not so large as the moose of No. America **b** : a No. American deer (*Cervus canadensis*) similar to the red deer of Europe and related forms — called also *wapiti* **c** : any of various large Asian deer 2 : soft tanned rugged leather 3 *cap* [Benevolent and Protective Order of *Elks*] : a member of a major benevolent and fraternal order

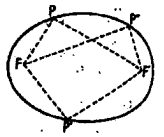
elk·hound \'elk-,haund, 'el-,kaund\ *n* (1835) : NORWEGIAN ELKHOUND

¹ell \'el\ *n* [ME *eln*, fr. OE; akin to OHG *elina* ell, L *ulna* elbow, arm, Gk *ōlenē* elbow, Skt *āni* linchpin, thigh] (bef. 12c) 1 : a former English unit of length (as for cloth) equal to 45 inches 2 : any of various units of length similar in use to the English ell

²ell *n* [alter. of *'el*] (1773) 1 : an extension at right angles to the length of a building 2 : an elbow in a pipe or conduit

el·lag·ic acid \,el-,laj-ik-, e-\ *n* [F *ellagique*, fr. *ellag*, anagram of *galle* gall] (1810) : a crystalline phenolic compound $C_{14}H_6O_8$ with two lactone groupings that is obtained esp. from oak galls and some tannins and is used medicinally as a hemostatic

el·lipse \i-'lips, e-\ *n* [Gk *elleipsis*] (1753) 1 **a** : OVAL **b** : a closed plane curve generated by a point moving in such a way that the sums of its distances from two fixed points is a constant : a plane section of a right circular cone that is a closed curve 2 : ELLIPSIS



ellipse 1b: *F, F'* foci; *P, P', P''* any point on the curve; $FP + PF' = FP'' + P''F' = FP' + P'F$

el·lip·sis \i-'lip-səs, e-\ *n, pl* **el·lip·ses** \-,sēz\ [L, fr. Gk *elleipsis* ellipsis, ellipse, fr. *elleipein* to leave out, fall short, fr. *en* in + *leipein* to leave — more at IN, LOAN] (1540) 1 **a** : the omission of one or more words that are obviously understood but that must be supplied to make a construction grammatically complete ⟨"the man that he sees" may be changed by ~ to "the man he sees"⟩ **b** : a leap or sudden passage without logical connectives from one topic to another 2 : marks or a mark (as . . . or *** or —) indicating an omission (as of words) or a pause

el·lip·soid \i-'lip-,sȯid, e-\ *n* (1721) : a surface all plane sections of which are ellipses or circles — **ellipsoid** *or* **el·lip·soi·dal** \i-,lip-'sȯid-ᵊl, (,)e-\ *adj*

el·lip·tic \i-'lip-tik, e-\ *or* **el·lip·ti·cal** \-ti-kəl\ *adj* [Gk *elleiptikos* defective, marked by ellipsis, fr. *elleipein*] (1656) 1 : of, relating to, or shaped like an ellipse 2 **a** : of, relating to, or marked by ellipsis or an ellipsis **b** (1) : of, relating to, or marked by extreme economy of speech or writing (2) : of or relating to deliberate obscurity (as of literary or conversational style) — **el·lip·ti·cal·ly** \-ti-k(ə-)lē\ *adv*

el·lip·tic·i·ty \i-,lip-'tis-ət-ē, (,)e-\ *n* (1753) : deviation of an ellipse or a spheroid from the form of a circle or a sphere

elm \'elm\ *n* [ME, fr. OE; akin to OHG *elme* elm, L *ulmus*] (bef. 12c) 1 : any of a genus (*Ulmus* of the family Ulmaceae, the elm family) comprising large graceful trees with alternate stipulate leaves and small apetalous flowers 2 : the wood of an elm

elm bark beetle *n* (1909) : either of two beetles that are vectors for the fungus causing Dutch elm disease: **a** : a beetle (*Hylurgopinus rufipes*) native to eastern No. America **b** : a European beetle (*Scolytus multistriatus*) that is established in eastern No. America

elm leaf beetle *n* (1881) : a small orange-yellow black-striped Old World chrysomelid beetle (*Pyrrhalta luteola*) that is a leaf-eating pest of elms in eastern No. America as a larva and as an adult

El Ni·ño \el-'nēn-yō\ *n* [Sp, lit., the child] (1925) : an irregularly occurring flow of unusually warm surface water along the western coast of South America that is accompanied by abnormally high rainfall in usu. arid areas and that prevents upwelling of nutrient-rich cold deep water causing a decline in the regional fish population

el·o·cu·tion \,el-ə-'kyü-shən\ *n* [ME *elocucioun*, fr. L *elocution-*, *elocutio*, fr. *elocutus*, pp. of *eloqui*] (15c) 1 : the art of effective public speaking 2 : a style of speaking esp. in public — **el·o·cu·tion·ar·y** \-shə-,ner-ē\ *adj* — **el·o·cu·tion·ist** \-sh(ə-)nəst\ *n*

elo·dea \i-'lōd-ē-ə\ *n* [NL, fr. Gk *helodēs* marshy, fr. *helos* marsh; akin to Skt *saras* pond] (ca. 1868) : any of a small American genus (*Elodea*) of submerged aquatic monocotyledonous herbs

eloign \i-'lȯin\ *vt* [ME *eloynen*, fr. MF *esloigner*, fr. OF, fr. *es-* ex- (fr. L *ex-*) + *loing* (adv.) far, fr. L *longe*, fr. *longus* long] (1500) 1 *archaic* : to take (oneself) far away 2 *archaic* : to remove to a distant or unknown place : CONCEAL

¹elon·gate \i-'lȯŋ-gāt\ *vb* **-gat·ed; -gat·ing** [LL *elongatus*, pp. of *elongare*, to withdraw, fr. L *e-* + *longus*] *vt* (1578) : to extend the length of ~ *vi* : to grow in length

²elongate *or* **elon·gat·ed** *adj* (1828) 1 : stretched out 2 : SLENDER

elon·ga·tion \(,)ē-,lȯŋ-'gā-shən\ *n* (14c) 1 **a** : the angular distance of a celestial body from another around which it revolves or from a particular point in the sky **b** : the daily extreme east or west position of a star with reference to the north celestial pole 2 **a** : the state of being elongated or lengthened; *also* : the process of growing or increasing in length **b** : something that is elongated

elope \i-'lōp\ *vi* **eloped; elop·ing** [AF *aloper*] (1628) 1 **a** : to run away from one's husband with a lover **b** : to run away secretly with the intention of getting married usu. without parental consent 2 : to slip away : ESCAPE — **elope·ment** \-'lōp-mənt\ *n* — **elop·er** *n*

el·o·quence \'el-ə-kwən(t)s\ *n* (14c) 1 : discourse marked by force and persuasiveness; *also* : the art or power of using such discourse 2 : the quality of forceful or persuasive expressiveness

el·o·quent \-kwənt\ *adj* [ME, fr. MF, fr. L *eloquent-*, *eloquens*, fr. prp. of *eloqui* to speak out, fr. *e-* + *loqui* to speak] (14c) 1 : marked by forceful and fluent expression ⟨an ~ preacher⟩ 2 : vividly or movingly expressive or revealing ⟨an ~ monument⟩ — **el·o·quent·ly** *adv*

¹else \'el(t)s\ *adv* [ME *elles*, fr. OE; akin to L *alius* other, *alter* other of two, Gk *allos* other] (bef. 12c) 1 **a** : in a different manner or place or at a different time ⟨how ~ could he have acted⟩ ⟨here and nowhere ~⟩ **b** : in an additional manner or place or at an additional time ⟨where ~ is gold found⟩ 2 : if not : OTHERWISE ⟨leave or ~ you'll be sorry⟩ — used absolutely to express a threat ⟨do what I tell you or ~⟩

²else *adj* (bef. 12c) : OTHER: **a** : being different in identity ⟨it must have been somebody ~⟩ **b** : being in addition ⟨what ~ did he say⟩

else·where \-,(h)we(ə)r, -,(h)wa(ə)r\ *adv* [ME *elleswher*, fr. OE *ellshwær*] (bef. 12c) : in or to another place ⟨took his business ~⟩

el·u·ant *or* **el·u·ent** \'el-yə-wənt\ *n* [L *eluent-*] (1941) : a solvent used in eluting

el·u·ate \'el-yə-wət, -,wāt\ *n* [L *eluere* + E *-ate*] : a solution obtained by eluting

el·u·ci·date \i-'lü-sə-,dāt\ *vb* **-dat·ed; -dat·ing** [L *elucidare*, fr. L *e-* + *lucidus* lucid] *vt* (1568) : to make clear the meaning of : explain ⟨~ a theory⟩ ⟨~ a passage⟩ ⟨~ a problem⟩ ⟨~ the structure of a molecule⟩ ⟨~ a point of grammar⟩ ⟨~ a point of view⟩ ⟨~ the nature or analysis ~ *vi* : to give a clarifying explanation — **el·u·ci·da·tion** \-,lü-sə-'dā-shən\ *n* — **el·u·ci·da·tive** \-'lü-sə-,dāt-iv\ *adj* — **el·u·ci·da·tor** \-,dāt-ər\ *n*

el·u·cu·brate \i-'lü-k(y)ə-,brāt\ *vt* **-brat·ed; -brat·ing** [L *elucubrare* to compose by lamplight, fr. *e-* + *lucubrare* to work by lamplight — more at LUCUBRATION] (ca. 1623) : to produce by studious effort — **el·u·cu·bra·tion** \-,lü-k(y)ə-'brā-shən\ *n*

elude \ē-'lüd\ *vt* **elud·ed; elud·ing** [L *eludere*, fr. *e-* + *ludere* to play — more at LUDICROUS] (1612) 1 : to avoid adroitly or escape by artful means ⟨~ the traps⟩ ⟨managed to ~ capture⟩ 2 : to escape the understanding, or grasp of ⟨subtlety ~s continued to ~ us⟩ 3 : DEFY 4 ⟨it ~s explanation⟩

Elul \e-'lül\ *n* [Heb *Ēlūl*] (1535) : the 12th month of the civil year and the 6th month of the ecclesiastical year in the Hebrew calendar — see MONTH table

elu·sion \ē-'lü-zhən\ *n* [ML *elusion-*, *elusio*, fr. L *elusus*, pp. of *eludere*] (1624) : an act of eluding or escaping : EVASION

elu·sive \ē-'lü-siv, -'lü-ziv\ *adj* (1719) : tending to elude grasp or pursuit ⟨an eligible though elusive bachelor⟩ ⟨~ to comprehend or define ⟨an ~ concept that many people⟩ **c** : hard to isolate or identify ⟨a hard-to-find and ~ bug⟩ — **elu·sive·ly** *adv* — **elu·sive·ness** *n*

elute \ē-'lüt\ *vt* **elut·ed; elut·ing** [L *elutus*, pp. of *eluere* + *lavere* to wash — more at LYE] (1731) : ELUVIATE (adsorbed material) from an adsorbent by means of a solvent — **elution** \-'lü-shən\ *n*

elu·tri·ate \ē-'lü-trē-,āt\ *vt* **-at·ed; -at·ing** [L *elutriatus*, pp. of *elutriare*, irreg. fr. *elutus*] (ca. 1727) : to purify, separate, or wash by suspending in a fluid and decanting — **elu·tri·a·tor** \-,āt-ər\ *n*

elu·vi·al \ē-'lü-vē-əl\ *adj* (1862) 1 : of, relating to, or resembling eluvium 2 : of or relating to eluviation or areas

elu·vi·ate \-vē-,āt\ *vi* **-at·ed; -at·ing** (1926) : to undergo eluviation

elu·vi·a·tion \(,)ē-,lü-vē-'ā-shən\ *n* (1899) : the process of dissolved or suspended material within the soil being carried when rainfall exceeds evaporation

elu·vi·um \ē-'lü-vē-əm\ *n* [NL, fr. L *eluere*] (1) : material produced by the weathering and disintegration of rock in place (2) : soil or sand deposited by wind

el·ver \'el-vər\ *n* [alter. of *eelfare* (migration of young eel

elves *pl of* ELF

el·vish \'el-vish\ *adj* (13c) 1 : of or relating to elves 2 : BLISSFUL, DELIGHTFUL

elysian fields *n pl, often cap E* (1579) : ELYSIUM 2: PARADISE 2

Ely·si·um \i-'liz(h)-ē-əm\ *n, pl* **-si·ums** *or* **-si·a** (1590) 1 : the abode of the blessed after death 2: PARADISE 2

elytr- *or* **elytri-** *or* **elytro-** *comb form* [NL *elytr-* (*elytriferous*)

el·y·tron \'el-ə-,trän\ *also* **el·y·trum** \-trəm\ *n* [NL] : the sheath, wing cover, fr. *ellyein* to roll, wrap] : one of the anterior wings in beetles and some other insects that serve to protect the posterior pair of functional wings

em \'em\ *n* (13c) 1 : the letter *m* 2 : the square about as wide as it is tall used as a unit of measure in printing

em- — see EN-

'em \əm; *after* p,b,f, *or* v *often* ᵊm\ *pron* [M dat. pl. of *he* he] (bef. 12c) : THEM

ema·ci·ate \i-'mā-shē-,āt\ *vb* **-at·ed; at·ing** [L *emaciatus*, fr. *e-* + *macies* leanness, fr. *macer* lean — more at MEAGER] *vt* 1 : to cause to lose flesh so as to become very lean 2 : to waste away physically — **ema·ci·a·tion** \-,mā-shē-'ā-shən\ *n*

emalangeni *pl of* LILANGENI

em·a·nate \'em-ə-,nāt\ *vb* **-nat·ed; -nat·ing** [L *emanatus*, fr. *e-* + *manare* to flow] *vi* (1756) : to come forth : EMIT *syn* see SPRING

em·a·na·tion \,em-ə-'nā-shən\ *n* (1570) 1 : the origination of the world by a series of successive radiations from the Godhead through intermediaries **b** : a heavy gaseous element produced by the disintegration of radium ⟨radium ~⟩ — **em·a·na·tion·al** \-shnol, -shən-ᵊl\ *adj*

em·a·nate \'em-ə-,nāt-iv\ *adj*

eman·ci·pate \i-'man(t)-sə-,pāt\ *vt* **-pat·ed; -pat·ing** [L *emancipatus*, pp. of *emancipare*, fr. *e-* + *mancipare* to transfer, fr. *manceps* purchaser, fr. *manus* hand + *capere* to take] : to free from restraint, control, or the influence of another; *esp* : to free from bondage 2 : to release from parental authority and responsibility and make sui juris 3 : to free from restraint, influence (as traditional mores or beliefs)

eman·ci·pa·tor \-,pāt-ər\ *n*

eman·ci·pa·tion \i-,man(t)-sə-'pā-shən\ *n* (1)

emancipating — **eman·ci·pa·tion·ist** \-sh(ə-)nəst\ *n or adj*

emar·gin·ate \(')mär-jə-nət, -,nāt\ *adj* [L *emarginare* to deprive of a margin, fr. *e-* + *margin-*, *margo* margin] : having the margin notched — **emar·gi·nate** \-,nāt\ *vt* — **emar·gi·na·tion** \-,mär-jə-'nā-shən\ *n*

emas·cu·late \i-'mas-kyə-,lāt\ *vt* **-lat·ed; -lat·ing** [L *emasculare*, fr. *e-* + *masculus* male — more at MASCULINE] : to deprive of virile or procreative power : CASTRATE 2 : to deprive of strength, vigor, or spirit : WEAKEN 3 : to remove the stamens from (a flower) in the process of artificial cross-pollination — **emas·cu·late** \-lət\ *adj* — **emas·cu·la·tion** \-,mas-kyə-'lā-shən\ *n* — **emas·cu·la·tor** \-'mas-kyə-,lāt-ər\ *n*

na·ga·na \nə-'gän-ə\ *n* [Zulu *u-nakane, ulu-nakane*] (1895) : trypanosomiasis (esp. when caused by *Trypanosoma brucei*) of domestic animals

nah \'na, 'nä, 'ná\ *var of* NO

Na·huatl \'nä-,wät-ᵊl\ *n, pl* **Nahuatl** *or* **Nahuatls** [Sp *nahuatle*, fr. Nahuatl *Nahuatl*] (1822) 1 : a group of Indian peoples of southern Mexico and Central America 2 : the Uto-Aztecan language of the Nahuatl peoples — **Na·huat·lan** \nä-'wät-lən\ *adj or n*

Na·hum \'nā-(h)əm\ *n* [Heb *Naḥūm*] 1 : a Hebrew prophet of the 7th century B.C. 2 : a prophetic book of canonical Jewish and Christian Scripture — see BIBLE table

na·iad \'nā-əd, 'nī-, -,ad\ *n, pl* **na·iads** *or* **na·ia·des** \-ə-,dēz\ [ME, fr. MF or L; MF *naïade*, fr. L *naiad-, naias*, fr. Gk, fr. *nan* to flow — more at NOURISH] (14c) 1 : any of the nymphs in classical mythology living in and giving life to lakes, rivers, springs, and fountains 2 : any of the aquatic young of a mayfly, dragonfly, damselfly, or stone fly — compare NYMPH 3 3 : any of a genus (*Naias*) of submerged aquatic plants

na·if *or* **na·ïf** \nä-'ēf\ *adj* [F] (1598) : NAIVE — **naïf** *n*

¹nail \'nā(ə)l\ *n* [ME, fr. OE *nægl*; akin to OHG *nagal* nail, fingernail, L *unguis* fingernail, toenail, claw, Gk *onyx*] (bef. 12c) 1 a : a horny sheath protecting the upper end of each finger and toe of man and most other primates b : a structure (as a claw) that terminates a digit and corresponds to a nail 2 : a slender usu. pointed and headed fastener designed to be pounded in 3 : an English unit of length equal to 1/16 yard

²nail *vt* (bef. 12c) 1 : to fasten with or as if with a nail 2 : to fix in steady attention ⟨~*ed* his eye on the crack⟩ 3 : CATCH, TRAP; *esp* : to detect and expose usu. so as to discredit 4 a : STRIKE, HIT b : to put out (a runner) in baseball — **nail·er** *n*

nail·brush \'nā(ə)l-,brəsh\ *n* (1802) : a small firm-bristled brush for cleaning the hands and esp. the fingernails

nail down *vt* (1947) 1 : to settle or establish clearly and unmistakably 2 : to gain or win decisively ⟨*nail down* his consent⟩

nail file *n* (1875) : a small narrow instrument (as of metal or cardboard) with a rough or emery surface that is used for shaping fingernails

nain·sook \'nān-,sůk\ *n* [Hindi *nainsukh*, fr. *nain* eye + *sukh* delight] (1790) : a soft lightweight muslin

nai·ra \'nī-rə\ *n* [alter. of *Nigeria*] (1972) — see MONEY table

na·ive *or* **na·ïve** \nä-'ēv\ *adj* **na·ïv·er; -est** [F *naïve*, fem. of *naïf*, fr. OF, inborn, natural, fr. L *nativus* native] (1650) 1 : marked by unaffected simplicity : ARTLESS, INGENUOUS 2 a : deficient in worldly wisdom or informed judgment; *esp* : CREDULOUS b : not previously subjected to experimentation or a particular experimental situation ⟨made the test with ~ rats⟩; *also* : not having previously used a particular drug (as marijuana) 3 : PRIMITIVE 3d *syn* see NATURAL — **na·ive·ly** *adv* — **na·ive·ness** *n*

na·ïve·té *also* **na·ive·té** *or* **na·ive·te** \(,)nä-,ēv(-ə)-'tā, nä-'ēv(-ə)-,tā\ *n* [F *naïveté*, fr. OF, inborn character, fr. *naïf*] (1673) 1 : a naive remark or action 2 : the quality or state of being naive

na·ive·ty *also* **na·ïve·ty** \nä-'ē-vət-ē, -'ēv-tē\ *n, pl* **-ties** *chiefly Brit* (1708) : NAÏVETÉ

na·ked \'nā-kəd, *esp Southern* 'nek-əd\ *adj* [ME, fr. OE *nacod*; akin to OHG *nackot* naked, L *nudus*, Gk *gymnos*] (bef. 12c) 1 : not covered by clothing : NUDE 2 : devoid of customary or natural covering : BARE: as a : not enclosed in a sheath or scabbard b : not provided with a shade c *of a plant or one of its parts* : lacking pubescence or enveloping or subtending parts d : lacking foliage or vegetation e *of an animal or one of its parts* : lacking an external covering (as of hair, feathers, or shell) 3 a : scantily supplied or furnished b : lacking embellishment : UNADORNED 4 : UNARMED, DEFENSELESS 5 : lacking confirmation or support 6 : devoid of concealment or disguise 7 : unaided by any optical device or instrument ⟨visible to the ~ eye⟩ 8 : not backed by the writer's ownership of the commodity contract or security *syn* see BARE — **na·ked·ly** *adv* — **na·ked·ness** *n*

na·led \'nā-,led\ *n* [origin unknown] (ca. 1962) : a short-lived insecticide of relatively low toxicity to warm-blooded animals that is used esp. to control crop pests and mosquitoes

na·li·dix·ic acid \nā-lə-,dik-sik-\ *n* [perh. fr. *naphthyridine* ($C_8H_6N_2$ — fr. *naphth-* + *pyridine*) + carboxy*lic acid*] (1964) : an antibacterial agent $C_{12}H_{12}N_2O_3$ that is used esp. in the treatment of genitourinary infections

na·lor·phine \nal-'ōr-,fēn\ *n* [*N-allyl* + *morphine*] (ca. 1953) : a white crystalline compound $C_{19}H_{21}NO_3$ that is derived from morphine and is used in the form of its hydrochloride as a respiratory stimulant to counteract poisoning by morphine and similar narcotic drugs

nal·ox·one \nal-'äk-,sōn\ *n* [*N-allyl* + *hydroxy-* + *-one*] (1964) : a potent antagonist $C_{19}H_{21}NO_4$ of narcotic drugs and esp. morphine that is administered esp. as the hydrochloride

nal·trex·one \nal-'trek-,sōn\ *n* [*nal-* (designation of nonmorphine narcotic antagonist) + *trex-* (as in *methotrexate*) + *-one*] (1973) : a narcotic antagonist $C_{20}H_{23}NO_4$

nam·by–pam·by \,nam-bē-'pam-bē\ *adj* [*Namby Pamby*, nickname given to Ambrose Philips] (1726) 1 : lacking in character or substance : INSIPID 2 : WEAK, INDECISIVE — **namby–pamby** *n*

¹name \'nām\ *n* [ME, fr. OE *nama*; akin to OHG *namo* name, L *nomen*, Gk *onoma, onyma*] (bef. 12c) 1 a : a word or phrase that constitutes the distinctive designation of a person or thing b : a word or symbol used in logic to designate an entity 2 : a descriptive often disparaging epithet ⟨called him ~s⟩ 3 a : REPUTATION ⟨gave the town a bad ~⟩ b : an illustrious record : FAME ⟨made a ~ for himself in golf⟩ c : a person or thing with a reputation 4 : FAMILY, CLAN 5 : appearance as opposed to reality ⟨a friend in ~ only⟩ 6 : one referred to by a name ⟨praise his holy ~⟩ — **in the name of** 1 : by authority of ⟨open *in the name of* the law⟩ 2 : for the reason of : using the excuse of ⟨called for reforms *in the name of* progress⟩

²name *vt* **named; nam·ing** (bef. 12c) 1 : to give a name to : CALL 2 a : to mention or identify by name b : to accuse by name 3 : to nominate for office : APPOINT 4 : to decide on : CHOOSE ⟨~ the day for the wedding⟩ 5 : to mention explicitly : SPECIFY ⟨unwilling to ~ a price⟩ — **nam·er** *n*

³name *adj* (1610) 1 : of, relating to, or bearing a name ⟨~ tags⟩ 2 : appearing in the name of a literary or theatrical production 3 a : having an established reputation b : featuring celebrities

name·able *also* **nam·able** \'nā-mə-bəl\ *adj* (1780) 1 : worthy of being named : MEMORABLE 2 : capable of being named : IDENTIFIABLE

name–call·ing \'nām-,kȯ-liŋ\ *n* (1853) : the use of offensive names esp. to win an argument or to induce rejection or condemnation (as of a person or project) without objective consideration of the facts

name day *n* (1721) : the church feast day of the saint after whom one is named

name–drop·ping \-,dräp-iŋ\ *n* (1950) : the practice of seeking to impress others by studied but apparently casual mention of prominent persons as associates — **name–drop·per** \-ər\ *n*

name·less \'nām-ləs\ *adj* (14c) 1 : OBSCURE, UNDISTINGUISHED 2 : not known by name : ANONYMOUS 3 : having no legal right to a name : ILLEGITIMATE 4 : not having been given a name : UNNAMED 5 : not marked with a name ⟨a ~ grave⟩ 6 a : incapable of precise description : INDEFINABLE b : too repulsive or distressing to describe — **name·less·ly** *adv* — **name·less·ness** *n*

name·ly \'nām-lē\ *adv* (14c) : that is to say : TO WIT

name of the game (1966) 1 : the essential quality or matter ⟨patience is the *name of the game* in coastal duck hunting —Dick Beals⟩ 2 : the fundamental goal of an activity

name·plate \-,plāt\ *n* (ca. 1864) : something (as a plate or plaque) bearing a name (as of a resident or manufacturer)

name·sake \-,sāk\ *n* [prob. fr. *name's sake*] (1646) : one that has the same name as another; *esp* : one named after another

nana \'nan-ə\ *n* [baby-talk for *grandma*] (1844) : GRANDMOTHER

nance \'nan(t)s\ *n* [short for *nancy*, fr. the name *Nancy*] (1920) 1 : an effeminate male — often used disparagingly 2 : HOMOSEXUAL — often used disparagingly

NAND \'nand\ *n* [*not AND*] (1958) : a computer logic circuit that produces an output which is the inverse of that of an AND circuit

nan·keen \(ᵊ)nan-'kēn\ *n* [*Nanking*, China] (1755) 1 : a durable brownish yellow cotton fabric orig. loomed by hand in China 2 *pl* : trousers made of nankeen

Nan·kin \'nan-'kin, 'nän-\ *or* **Nan·king** \-'kiŋ\ *n* [*Nanking*, China] (1781) : Chinese porcelain decorated in blue on a white ground

nan·no·plank·ton \,nan-ō-'plaŋ(k)-tən, -,tän\ *n* [NL, fr. Gk *nanos* dwarf + NL *plankton* plankton] (1912) : the smallest plankton that consists of those organisms (as bacteria) passing through nets of very fine mesh silk cloth

nan·ny *also* **nan·nie** \'nan-ē\ *n, pl* **nannies** [prob. of baby-talk origin] *chiefly Brit* (1795) : a child's nurse : NURSEMAID

nan·ny goat \'nan-ē-\ *n* [*Nanny*, nickname for *Anne*] (1788) : a female domestic goat

nano- \'nan-(,)ō, -ə\ *comb form* [ISV, fr. Gk *nanos* dwarf] : one billionth (10^{-9}) part of ⟨*nano*second⟩

nano·gram \'nan-ə-,gram\ *n* [ISV] (1951) : one billionth of a gram

nano·me·ter \'nan-ə-,mēt-ər\ *n* [ISV] (1963) : one billionth of a meter

nano·sec·ond \-,sek-ənd, -ən(t)\ *n* [ISV] (1959) : one billionth of a second

Nan·tua sauce \nän(n)-,twä-\ *n* [*Nantua*, France] (ca. 1961) : a cream sauce flavored with shellfish (as lobster)

Na·o·mi \nā-'ō-mē\ *n* [Heb *Nā'ōmī*] : the mother-in-law of the Old Testament heroine Ruth

¹nap \'nap\ *vi* **napped; nap·ping** [ME *nappen*, fr. OE *hnappian*; akin to OHG *hnaffezen* to doze] (bef. 12c) 1 : to sleep briefly esp. during the day : DOZE 2 : to be off guard

²nap *n* (14c) : a short sleep esp. during the day : SNOOZE

³nap *n* [ME *noppe*, fr. MD, flock of wool, nap; akin to OE *hnoppian* to pluck, Gk *konis* ashes — more at INCINERATE] (15c) : a hairy or downy surface (as on a woven fabric) — **nap·less** \-ləs\ *adj* — **napped** \'napt\ *adj*

⁴nap *vt* **napped; nap·ping** (1620) : to raise a nap on (fabric or leather)

⁵nap *n* [fr. to go *nap* (to make all the points in the card game Napoleon)] *Brit* (1895) : a pick or recommendation as a good bet to win a contest (as a horse race); *also* : one named in a nap

⁶nap *vt* **napped; nap·ping** *Brit* (1927) : to pick or single out (as a racehorse) in a nap

¹na·palm \'nā-,päm *also* 'na-, -,pälm; nə-'pä(l)m\ *n* [*naphthene* + *palmitate*] (1942) 1 : a thickener consisting of a mixture of aluminum soaps used in jelling gasoline (as for incendiary bombs) 2 : fuel jelled with napalm

²napalm *vt* (1950) : to assault with napalm

nape \'nāp, 'nap\ *n* [ME] (14c) : the back of the neck

na·pery \'nā-p(ə-)rē\ *n* [ME, fr. MF *naperie*, fr. *nappe, nape* tablecloth — more at NAPKIN] (14c) : household linen; *esp* : TABLE LINEN

Naph·ta·li \'naf-tə-,lī\ *n* [Heb *Naphtālī*] : a son of Jacob and the traditional eponymous ancestor of one of the tribes of Israel

naphth- *or* **naphtho-** *comb form* [ISV, fr. *naphtha* & *naphthalene*] 1 : naphtha ⟨*naphth*ene⟩ 2 : naphthalene ⟨*naphtho*quinone⟩

naph·tha \'naf-thə, 'nap-\ *n* [L, fr. Gk, of Iranian origin; akin to Pers *naft* naphtha] (1572) 1 : any of various volatile often flammable liquid hydrocarbon mixtures used chiefly as solvents and diluents 2 : PETROLEUM

naph·tha·lene \-,lēn\ *n* [alter. of earlier *naphthaline*, irreg. fr. *naphtha*] (1821) : a crystalline aromatic hydrocarbon $C_{10}H_8$ usu. obtained by distillation of coal tar and used esp. in organic synthesis — **naph·tha·len·ic** \,naf-thə-'lēn-ik, ,nap-, -'len-\ *adj*

naph·thol \'naf-,thōl, 'nap-, -,thōl\ *n* [ISV] (1849) 1 : either of two isomeric derivatives $C_{10}H_8O$ of naphthalene found in coal tar or made synthetically and used as antiseptics and in manufacture of dyes 2 : any of various hydroxy derivatives of naphthalene that resemble the simpler phenols

naph·thyl·amine \naf-'thil-ə-,mēn, nap-\ *n* [ISV] (1857) : either of two isomeric crystalline bases $C_{10}H_9N$ used esp. as dye intermediates

na·pi·er grass \'nā-pē-ər-\ *n* [*Napier*, town in So. Africa] (1914) : a tall stout perennial grass (*Pennisetum purpureum*) that resembles sugarcane and is widely grown for forage — called also *elephant grass*

Na·pier·ian logarithm \nə-,pir-ē-ən-, nā-\ *n* [John *Napier*] (1816) : NATURAL LOGARITHM

Na·pi·er's bones \,nā-pē-ərz-\ *n* [fr. the short rods by which it was operated] (1658) : a set of graduated rods (as of wood or bone) invented by John Napier and used for multiplication and division based on the principles of logarithms

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publicly defending one unjustly attacked or t
is own cause.
the act or process of supporting : the condition
: one that supports
one that supports or acts as a support: as
\N b : one of two figures (as of men or animal
ide of an escutcheon and exterior to it c : GART
ORTER
: elastic stockings
) : a price level on a declining market at which
er decline due to increased attractiveness to trad
ed also *support area*
zə-bəl\ *adj* (1643) : capable of being suppo
p-pos-ably \-blē\ *adv*
n (14c) 1 : the act or process of supposing
ed : HYPOTHESIS, SUPPOSITION
renest eight "*I*" 'spōz\ *vb* sup-posed; sup-pos-i
F supposer, fr. ML *supponere* (perf. indic. *suppos*
substitute, fr. *sub-* + *ponere* to put — more at P
: to lay down tentatively as a hypothesis, assu
- a fire broke out) ⟨~ you bring the salad⟩ b
iion : BELIEVE ⟨they *supposed* they were early⟩
or in keeping with the facts ⟨seems reasonable to
t) 2 a : CONCEIVE, IMAGINE b : to have a sus
OSE ~ *vi* : CONJECTURE, OPINE
Ib & 2a usu -'pō-zəd, *3 & 4 often* -'pōst\ *adj* (15
:welve hours are ~ to elapse between Acts I and
) : ALLEGED ⟨trusted my ~ friends⟩ 2 a : held
ED; *also* : mistakenly believed : IMAGINED ⟨the sig
ror true —Shak.⟩ b : considered probable or c
was not ~ that everybody could master the tech
Murray⟩ c : UNDERSTOOD ⟨you will be ~ to refer
. B. Shaw⟩ 3 : made or fashioned by intent
button ~ to do⟩ ⟨pills that are ~ to kill pain⟩
s if by authority ⟨soldiers are ~ to obey their co
b : given permission : PERMITTED ⟨was not ~
-pos-ed-ly \-'pō-zəd-lē *also* -'pōz-dlē\ *adv*
g\ *conj* (ca. 1843) : if by way of hypothesis : on

-'zish-ən\ *n* [ME, fr. LL *supposition-*, *suppositio*
eneath, fr. *suppositus*, pp. of *supponere*] (15c)
supposed : HYPOTHESIS 2 : the act of supposing
ish-nəl, -ən-'ī\ *adj*
-əs\ *adj* [by contr.] (1624) : SUPPOSITITIOUS
-,päz-ə-'tish-əs\ *adj* [L *suppositicius*, fr. *suppositi*
substitute] (1611) 1 a : fraudulently substitu
child (1) : falsely presented as a genuine heir
influenced in meaning by *supposition*] a : IMA
ure of or based on a supposition : HYPOTHETICAL
adv
äz-ə-,tōr-ē, -,tòr-\ *n, pl* -ries [ML *suppositorium*,
itorius placed beneath, fr. L *suppositus*, pp. of
] (15c) : a solid but readily meltable cone or cy
ed material for insertion into a bodily passage
n)
vt [ME *suppressen*, fr. L *suppressus*, pp. of
ɔremere to press — more at PRESS] (14c) 1 : to p
or force : SUBDUE 2 : to keep from public kno
p secret b : to stop or prohibit the publicatio
: to exclude from consciousness b : to keep fr
CK 4 *obs* : to press down 5 a : to restrain fro
ɔn : ARREST ⟨~ a cough⟩ b : to inhibit the gro
STUNT 6 : to inhibit the genetic expression of
ress-ibil-i-ty \-,pres-ə-'bil-ət-ē\ *n* — sup-press-
- sup-pres-sive \-'pres-iv\ *adj* — sup-pres-sive-

s-'nt\ *n* (1942) : an agent (as a drug) that tend
in intensity rather than eliminate something

sh-ən\ *n* (15c) 1 : an act or instance of suppr
ng suppressed 2 : the conscious intentional ex
ness of a thought or feeling
r\ *n* (1560) : one that suppresses; *esp* : a gene
ression of another nonallelic gene when both

,rāt\ *vi* -rat-ed; -rat-ing [L *suppuratus*, pp. of
ur-, *pus* pus — more at FOUL] (1656) : to form
ıp-pu-ra-tion \,səp-yə-'rā-shən\ *n* — sup-pu-ra-
; 'səp-rət-iv\ *adj*
, *adv* [L] (1592) : earlier in this writing : ABOVE
upra above, beyond, earlier; akin to L *super* over
1 : SUPER- 2a ⟨*supra*orbital⟩ 2 : transcend

rə-'lim-ən-'ī, -,prā-\ *adj* [*supra-* + L *limin-*, *li*
: LIMB] (1892) 1 : existing above the threshol
adequate to evoke a response or induce a sensa

mə-'lek-yə-lər\ *adj* (ca. 1909) : more complex
mposed of many molecules
ash-nəl, -ən-'ī\ *adj* (1908) : transcending natio
ty, or interests ⟨a ~ authority, regulating oc
ɔy⟩ ⟨taking a ~ view of economic problems⟩ —
,iz-əm\ *n* — su-pra-na-tion-al-ist \-ə-st\ *n* — sup
ı-ə-'nal-ət-ē\ *n*
:\ *adj* (1948) : situated above the optic chias
nucleus of closely packed neurons overlying
timately connected with the neurohypophysis
bət-'ī\ *adj* [NL *supraorbitalis*, fr. L *supra-* +
situated or occurring above the orbit of the ey
-,test\ *n* [modif. of It *sopra protesto* upon pro
ɔtance or payment of a bill by a third person fo
after protest for nonacceptance or nonpayment

su-pra-ra-tio-nal \-'rash-nəl, -ən-'ī\ *adj* (1894) : transcending the ratio-
nal : based on or involving factors not to be comprehended by reason
alone ⟨the stars inspire ~ dreams —R. J. Dubos⟩
su-pra-re-nal \-'rēn-'ī\ *adj* [NL *suprarenalis*, fr. L *supra-* + *renes* kid-
neys] (1828) : situated above or anterior to the kidneys; *specif* : ADRE-
NAL
suprarenal *n* (1841) : a suprarenal part; *esp* : ADRENAL GLAND
suprarenal gland *n* (1876) : ADRENAL GLAND
su-pra-seg-men-tal \,sü-prə-,seg-'ment-'ī, -,prā-\ *adj* (1941) : of or relat-
ing to significant features (as stress, pitch, or juncture) that occur si-
multaneously with vowels and consonants in an utterance
su-pra-vi-tal \-'vīt-'ī\ *adj* [ISV] (ca. 1919) : constituting or relating to
the staining of living tissues or cells surviving after removal from a
living body by dyes that penetrate living substance but induce more or
less rapid degenerative changes — compare INTRAVITAL 2 — su-pra-vi-
tal-ly \-'ī-ē\ *adv*
su-prem-a-cist \sə-'prem-ə-səst, sü-\ *n* (1949) 1 : an advocate or adher-
ent of group supremacy 2 : WHITE SUPREMACIST
su-prem-a-cy \sə-'prem-ə-sē, sü- *also* -'prēm-\ *n, pl* -cies [*supreme* + *-acy*
(as in *primacy*)] (1547) : the quality or state of being supreme; *also*
: supreme authority or power
su-preme \sə-'prēm, sü-; *in rapid speech also* 'sprēm; *attrib also* 'sü-
,prēm\ *adj* [L *supremus*, superl. of *superus* upper — more at SUPERIOR]
(1520) 1 : highest in rank or authority ⟨the ~ commander⟩ 2 : high-
est in degree or quality ⟨~ endurance in war and in labour —R. W.
Emerson⟩ 3 : ULTIMATE, FINAL ⟨the ~ sacrifice⟩ — su-preme-ly *adv* —
su-preme-ness *n*
Supreme Being *n* (1699) : GOD 1
supreme court *n* (1709) 1 : the highest judicial tribunal in a political
unit (as a nation or state) 2 : a court of original jurisdiction in New
York state that is subordinate to a final court of appeals
Supreme Soviet *n* (1943) : the highest legislative body of the Soviet
Union consisting of two chambers one of which represents the overall
population and the other the constituent republics
su-pre-mo \sə-'prē-(,)mō, sü-\ *n, pl* -mos [Sp & It, fr. *supremo*, adj., su-
preme, fr. L *supremus*] *Brit* (1937) : one who is highest in rank or au-
thority
sur- *prefix* [ME, fr. OF, fr. L *super-*] 1 : over : SUPER- ⟨*sur*print⟩ ⟨*sur-*
tax⟩ 2 : above : up ⟨*sur*base⟩
surah \'sůr-ə\ *n* [Ar *sūrah*, lit., row] (1661) : a chapter of the Koran
surah \'sůr-ə\ *n* [prob. alter. of *surat* (a cotton produced in Surat, In-
dia)] (1881) : a soft twilled fabric of silk or rayon
sur-base \'sər-,bās\ *n* (1678) : a molding just above the base of a wall,
pedestal, or podium
sur-cease \(,)sər-'sēs, 'sər-,\ *vb* sur-ceased; sur-ceas-ing [ME *sursesen*,
surcesen, fr. MF *sursis*, pp. of *surseoir*, fr. L *supersedēre* — more at SU-
PERSEDE] *vi* (15c) : to desist from action; *also* : to come to an end
: CEASE ~ *vt* : to put an end to : DISCONTINUE
sur-cease \'sər-,sēs, (,)sər-'\ *n* (1586) : CESSATION; *esp* : a temporary
respite or end
sur-charge \'sər-,chärj\ *vt* [ME *surchargen*, fr. MF *surcharger*, fr. *sur-*
+ *charger* to charge, fr. OF — more at CHARGE] (15c) 1 a : OVER-
CHARGE b : to charge an extra fee c : to show an omission in (an
account) for which credit ought to have been given 2 *Brit* : OVER-
STOCK 3 : to fill or load to excess ⟨the atmosphere . . . was *surcharged*
with war hysteria —H. A. Chippendale⟩ 4 a : to mark a new de-
nomination figure or a surcharge on (a stamp) b : OVERPRINT ⟨~ a
banknote⟩
surcharge *n* (1601) 1 a : an additional tax, cost, or impost b : an
extra fare (a sleeping car ~) c : an instance of surcharging an account
2 : an excessive load or burden 3 : the action of surcharging : the
state of being surcharged 4 a (1) : an overprint on a stamp; *specif*
: one that alters the denomination (2) : a stamp bearing such an
overprint b : an overprint on a currency note
sur-cin-gle \'sər-,siŋ-gəl\ *n* [ME *sursengle*, fr. MF *surcengle*, fr. *sur-* +
cengle girdle, fr. L *cingulum* — more at CINGULUM] (15c) 1 : a belt,
band, or girth passing around the body of a horse to bind a saddle or
pack fast to the horse's back 2 *archaic* : the cincture of a cassock
sur-coat \'sər-,kōt\ *n* [ME *surcote*, fr. MF, fr. *sur-* + *cote* coat] (14c)
: an outer coat or cloak; *specif* : a tunic worn over armor
surd \'sərd\ *adj* [L *surdus* deaf, silent, stupid; akin to L *susurrus* hum
— more at SWARM] (1551) 1 : lacking sense : IRRATIONAL ⟨the ~
mystery and the strange forces of existence —D. C. Williams⟩ 2
: VOICELESS — used of speech sounds
surd *n* (1557) 1 a : an irrational root (as $\sqrt{3}$) b : IRRATIONAL NUM-
BER 2 : a surd speech sound
sure \'shů(ə)r, *esp Southern* 'shō(ə)r\ *adj* sur-er; sur-est [ME, fr. MF
seur, fr. L *securus* secure] (14c) 1 *obs* : safe from danger or harm 2
: firmly established : STEADFAST ⟨a ~ hold⟩ 3 : RELIABLE, TRUSTWOR-
THY 4 : marked by or given to feelings of confident certainty ⟨he was
~ he was right⟩ 5 : admitting of no doubt : INDISPUTABLE ⟨spoke
from ~ knowledge⟩ 6 a : bound to happen : INEVITABLE ⟨~ disas-
ter⟩ b : BOUND, DESTINED ⟨he is ~ to win⟩ 7 : careful to remember,
attend to, or find out something ⟨be ~ to lock the door⟩ ⟨make ~ you
give them our regards⟩ — sure-ness *n*
syn SURE, CERTAIN, POSITIVE, COCKSURE mean having no doubt or uncer-
tainty. SURE usu. stresses the subjective or intuitive feeling of assur-
ance; CERTAIN may apply to a basing of a conclusion or conviction on
definite grounds or indubitable evidence; POSITIVE intensifies sureness
or certainty and may imply opinionated conviction or forceful expres-
sion of it; COCKSURE implies presumptuous or careless positiveness.
for sure : without doubt or question : CERTAINLY — to be sure : it
must be acknowledged : ADMITTEDLY
sure *adv* (15c) : SURELY
usage Most commentators consider the adverb *sure* to be something
less than completely standard; *surely* is usu. recommended as a substi-
tute. Our current evidence shows, however, that *sure* and *surely* have
become differentiated in use. *Sure* is used in much more informal con-
texts than *surely*. It is used as a simple intensive ⟨I can never know
how much I bored her, but, be certain, she *sure* amused me —Norman
Mailer⟩ and, because it connotes strong affirmation, it is used when
the speaker or writer expects to be agreed with ⟨it's a moot point
whether politicians are less venal than in Twain's day. But they're *sure*
as the devil more intrusive —Alan Abelson⟩ ⟨he *sure* gets them to play
—D.S. Looney⟩ *Surely*, like *sure*, is used as a simple intensive ⟨I *surely*
don't want to leave the impression that I had an unhappy childhood
—E.C. Welsh⟩ but it occurs in more formal contexts than *sure*. Unlike
sure it may be used neutrally — the reader or hearer may or may not
agree ⟨it would *surely* be possible, within a few years, to program a
computer to construct a grammar —Noam Chomsky⟩ and it is often
used when the writer is trying to persuade ⟨*surely* a book on the avant-
garde cannot be so conventional —Karl Shapiro⟩
sure-enough \,shůr-ə-,nəf\ *adj* (1846) : ACTUAL, GENUINE, REAL
sure enough *adv* (1545) : as one might confidently expect : CERTAINLY
sure-fire \,shůr-,fī(ə)r\ *adj* (1917) : certain to get successful or expected
results ⟨a ~ recipe⟩
sure-foot-ed \'shů(ə)r-'fůt-əd\ *adj* (1633) : not liable to stumble, fall, or
err — sure-foot-ed-ly *adv* — sure-foot-ed-ness *n*
sure-hand-ed \'shůr-'han-dəd\ *adj* (1946) : proficient and confident in per-
formance esp. using the hands — sure-hand-ed-ness *n*
sure-ly \'shů(ə)r-lē, *esp Southern* 'shō(ə)r-\ *adv* (14c) 1 : in a sure man-
ner: a *archaic* : without danger or risk of injury or loss : SAFELY b
(1) : with assurance : CONFIDENTLY ⟨answered quickly and ~⟩ (2)
: without doubt : CERTAINLY ⟨they will ~ be heard from in the future
—R. J. Lifton⟩ 2 : INDEED, REALLY — often used as an intensive ⟨you
~ don't believe that⟩ *usage* see ²SURE
sure thing *n* (1836) : one that is certain to succeed : a sure bet
sure-ty \'shůr-ət-ē, 'shů(ə)rt-ē\ *n, pl* -ties [ME *surte*, fr. MF *surté*, fr. L
securitat-, *securitas* security, fr. *securus*] (14c) 1 : the state of being
sure: as a : sure knowledge : CERTAINTY b : confidence in manner or
behavior : ASSURANCE 2 a : a formal engagement (as a pledge) given
for the fulfillment of an undertaking : GUARANTEE b : ground of confi-
dence or security 3 : one who has become legally liable for the debt,
default, or failure in duty (as appearance in court) of another — sure-
ty-ship \-,ship\ *n*
surety bond *n* (1911) : a bond guaranteeing performance of a contract
or obligation
¹surf \'sərf\ *n* [origin unknown] (1685) 1 : the swell of the sea that
breaks upon the shore 2 : the foam, splash, and sound of breaking
waves
²surf *vi* (1926) : to ride the surf (as on a surfboard) — surf-er *n*
³sur-face \'sər-fəs\ *n* [F, fr. *sur-* + *face* face, fr. OF — more at FACE] (ca.
1611) 1 : the exterior or upper boundary of an object or body 2 : a
plane or curved two-dimensional locus of points (as the boundary of a
three-dimensional region) ⟨plane ~⟩ ⟨~ of a sphere⟩ 3 a : the exter-
nal or superficial aspect of something b : an external part or layer
⟨sand down the damaged ~⟩ — on the surface : to all outward ap-
pearances
²surface *adj* (1664) 1 a : of, located on, or designed for use at the
surface of something b : situated, transported, or employed on the
surface of the earth ⟨~ mail⟩ ⟨~ vehicles⟩ 2 : appearing on the sur-
face only : SUPERFICIAL ⟨~ friendships⟩
³surface *vb* sur-faced; sur-fac-ing *vt* (1778) 1 : to give a surface to: as a
: to plane or make smooth b : to apply the surface layer to ⟨~ a
highway⟩ 2 : to bring to the surface ~ *vi* 1 : to work on or at the
surface 2 : to come to the surface 3 : to come into public view
: SHOW UP — sur-fac-er *n*
surface-active *adj* (1920) : altering the properties and esp. lowering the
tension at the surface of contact between phases ⟨soaps and wetting
agents are typical ~ substances⟩
surface feeder *n* (1907) : DABBLER b
surface of revolution (1840) : a surface formed by the revolution of a
plane curve about a line in its plane
surface-rip-ened \'sər-fəs-,rī-pənd, -,rīp-'nd\ *adj, of cheese* (1945) : rip-
ened by the action of microorganisms (as molds or bacteria) on the
surface
surface structure *n* (1964) : a formal representation of the phonetic
form of a sentence; *also* : the structure which such a representation
describes
surface tension *n* (1876) : the attractive force exerted upon the surface
molecules of a liquid by the molecules beneath that tends to draw the
surface molecules into the bulk of the liquid and makes the liquid as-
sume the shape having the least surface area
surface-to-air missile *n* (1949) : a usu. guided missile launched from
the ground against a target in the air
sur-fac-ing *n* (1882) : material forming or used to form a surface
sur-fac-tant \(,)sər-'fak-tənt, 'sər-,\ *n* [*surface-active* + *-ant*] (1950) : a
surface-active substance (as a detergent) — surfactant *adj*
surf-bird \'sərf-,bərd\ *n* (1839) : a shorebird (*Aphriza virgata*) of the
Pacific coasts of America that is related to the turnstones and has the
tail blackish at the tip and white at the base
surf-board \-,bō(ə)rd, -,bò(ə)rd\ *n* (1826) : a long narrow buoyant
board (as of lightweight wood or fiberglass-covered foam) used in the
sport of surfing — surfboard *vi* — surf-board-er *n*
surf-boat \-,bōt\ *n* (1856) : a boat for use in heavy surf
surf casting *n* (1935) : a method of fishing in which artificial or natural
bait is cast into the open ocean or in a bay where waves break on a
beach — surf caster *n*
surf clam *n* (ca. 1883) : any of various typically rather large surf-
dwelling edible clams (family Mactridae)
¹sur-feit \'sər-fət\ *n* [ME *surfait*, fr. MF, fr. *surfaire* to overdo, fr. *sur-* +
faire to do, fr. L *facere* — more at DO] (14c) 1 : an overabundant
supply : EXCESS 2 : an intemperate or immoderate indulgence in
something (as food or drink) 3 : disgust caused by excess
²surfeit *vt* (14c) : to feed, supply, or give to surfeit ~ *vi, archaic* : to
indulge to satiety in a gratification (as indulgence of the appetite or
senses) *syn* see SATIATE — sur-feit-er *n*
surf fish *n* (ca. 1882) : SURFPERCH
sur-fi-cial \,sər-'fish-əl\ *adj* [*surface* + *-icial* (as in *superficial*)] (1892)
: of or relating to a surface ⟨~ geologic processes⟩

EXHIBIT D

The Pharmacological Basis of Therapeutics

SEVENTH EDITION

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Louis S. Goodman
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Case 1:06-cv-00438-GMS Document 104-2 Filed 05/11/2007 Page 31 of 42

1000:1 if such a system came to a steady state. For a weak base with a pK_a of 4.4 ($BH^+ \rightleftharpoons B + H^+$), the ratio would be reversed. These considerations have obvious implications for the absorption and excretion of drugs, as will be discussed more specifically below. The establishment of concentration gradients of weak electrolytes across membranes with a pH gradient is a purely physical process and does not require an active transport system. All that is necessary is a membrane preferentially permeable to one form of the weak electrolyte and a pH gradient across the membrane. The establishment of the pH gradient is, however, an active process.

Bulk flow through *intercellular pores* is the major mechanism of passage of drugs across most capillary endothelial membranes, with the important exception of the central nervous system (CNS) (*see* below). These intercellular gaps are sufficiently large that diffusion across most capillaries is limited by blood flow and not by the lipid solubility of drugs or pH gradients. This is an important factor in absorption of drugs after parenteral administration and in filtration across glomerular membranes in the kidney (*see* below). *Tight junctions* are characteristic of capillaries of the CNS and a variety of epithelia. Intercellular diffusion is consequently limited. *Pinocytosis*, the formation and movement of vesicles across cell membranes, has been implicated in drug absorption. However, the quantitative significance of pinocytosis is questionable.

Carrier-Mediated Membrane Transport. While passive diffusion through the bilayer is dominant in the absorption and distribution of most drugs, more active and selective mechanisms can play important roles. *Active transport* of some drugs occurs across neuronal membranes, the choroid plexus, renal tubular cells, and hepatocytes. The characteristics of active transport—selectivity, competitive inhibition by congeners, a requirement for energy, saturability, and movement against an electrochemical gradient—may be important in the mechanism of action of drugs that are subject to active transport or that interfere with the active transport of natural metabolites or neurotransmitters. The term *facilitated diffusion* describes a carrier-mediated transport process to which there is no input of energy, and movement of the substance in question thus cannot occur against an electrochemical gradient. Such mechanisms, which may also be highly selective for specific conformational structures of drugs, are necessary for the transport of endogenous compounds whose rate of movement across biological membranes by simple diffusion would otherwise be too slow.

DRUG ABSORPTION, BIOAVAILABILITY, AND ROUTES OF ADMINISTRATION

Absorption may be described as the rate at which a drug leaves its site of administration and the extent to which this occurs. However, the clinician is primarily concerned with a parameter designated as *bioavailability*, rather than absorption. Bioavailability is a term used to indicate the extent to which a drug reaches its site of action *or* a biological fluid from which the drug has access to its site of action. For example, a drug that is absorbed from the stomach and intestine must first pass through the liver before it reaches the systemic circulation. If the drug is metabolized in the liver or excreted in the bile, some of the active drug will be inactivated before it can reach the general circulation and be distributed to its sites of action. If the metabolic or excretory capacity of the liver for the agent in question is great, bioavailability will be substantially decreased (the so-called first-pass effect). This decrease in availability is a function of the anatomical site from which absorption takes place; other anatomical, physiological, and pathological factors can influence bioavailability (*see* below), and the choice of the route of drug administration must be based on an understanding of these conditions. Moreover, factors that modify the absorption of a drug can change its bioavailability.

Factors That Modify Absorption. Many variables, in addition to the physicochemical factors that affect transport across membranes, influence the absorption of drugs. Absorption, regardless of the site, is dependent upon drug *solubility*. Drugs given in aqueous solution are more rapidly absorbed than those given in oily solution, suspension, or solid form because they mix more readily with the aqueous phase at the absorptive site. For those given in solid form, the rate of *dissolution* may be the limiting factor in their absorption. Local conditions at the site of absorption alter solubility, particularly in the gastrointestinal tract. Aspirin, which is relatively insoluble in acidic gastric contents, is a common example of such a drug. The *concentration* of a drug influences its rate of absorption. Drugs ingested or injected in solutions of high concentration are absorbed more rapidly than are drugs in solutions of low concentration. The *circulation to the site of absorption* also affects drug absorption. Increased blood flow, brought about by

EXHIBIT E

US005145684A

United States Patent [19]

Liversidge et al.

[11] Patent Number: 5,145,684

[45] Date of Patent: Sep. 8, 1992

[54] SURFACE MODIFIED DRUG NANOPARTICLES

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[22] Filed: Jan. 25, 1991

[51] Int. Cl.[5] A61K 9/14

[52] U.S. Cl. 424/489; 424/495; 424/499

[58] Field of Search 424/495, 489, 499

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[57] ABSTRACT

Dispersible particles consisting essentially of a crystalline drug substance having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 400 nm, methods for the preparation of such particles and dispersions containing the particles. Pharmaceutical compositions containing the particles exhibit unexpected bioavailability and are useful in methods of treating mammals.

20 Claims, No Drawings

SURFACE MODIFIED DRUG NANOPARTICLES

FIELD OF THE INVENTION

This invention relates to drug particles, methods for the preparation thereof and dispersions containing the particles. This invention further relates to the use of such particles in pharmaceutical compositions and methods of treating mammals.

BACKGROUND OF THE INVENTION

Bioavailability is the degree to which a drug becomes available to the target tissue after administration. Many factors can affect bioavailability including the dosage form and various properties, e.g., dissolution rate of the drug. Poor bioavailability is a significant problem encountered in the development of pharmaceutical compositions, particularly those containing an active ingredient that is poorly soluble in water. Poorly water soluble drugs, i.e., those having a solubility less than about 10 mg/ml, tend to be eliminated from the gastrointestinal tract before being absorbed into the circulation. Moreover, poorly water soluble drugs tend to be unsafe for intravenous administration techniques, which are used primarily in conjunction with fully soluble drug substances.

It is known that the rate of dissolution of a particulate drug can increase with increasing surface area, i.e., decreasing particle size. Consequently, methods of making finely divided drugs have been studied and efforts have been made to control the size and size range of drug particles in pharmaceutical compositions. For example, dry milling techniques have been used to reduce particle size and hence influence drug absorption. However, in conventional dry milling, as discussed by Lachman, et al., *The Theory and Practice of Industrial Pharmacy*, Chapter 2, "Milling", p. 45, (1986), the limit of fineness is reached in the region of 100 microns (100,000 nm) when material cakes on the milling chamber. Lachman, et al. note that wet grinding is beneficial in further reducing particle size, but that flocculation restricts the lower particle size limit to approximately 10 microns (10,000 nm). However, there tends to be a bias in the pharmaceutical art against wet milling due to concerns associated with contamination. Commercial airjet milling techniques have provided particles ranging in average particle size from as low as about 1 to 50 μm (1,000–50,000 nm).

Other techniques for preparing pharmaceutical compositions include loading drugs into liposomes or polymers, e.g., during emulsion polymerization. However, such techniques have problems and limitations. For example, a lipid soluble drug is often required in preparing suitable liposomes. Further, unacceptably large amounts of the liposome or polymer are often required to prepare unit drug doses. Further still, techniques for preparing such pharmaceutical compositions tend to be complex. A principal technical difficulty encountered with emulsion polymerization is the removal of contaminants, such as unreacted monomer or initiator, which can be toxic, at the end of the manufacturing process.

U.S. Pat. No. 4,540,602 (Motoyama et al.) discloses a solid drug pulverized in an aqueous solution of a water-soluble high molecular substance using a wet grinding machine. However, Motoyama et al. teach that as a result of such wet grinding, the drug is formed into

finely divided particles ranging from 0.5 μm (500 nm) or less to 5 μm (5,000 nm) in diameter.

EPO 275,796 describes the production of colloidally dispersible systems comprising a substance in the form of spherical particles smaller than 500 nm. However, the method involves a precipitation effected by mixing a solution of the substance and a miscible non-solvent for the substance and results in the formation of non-crystalline nanoparticle. Furthermore, precipitation techniques for preparing particles tend to provide particles contaminated with solvents. Such solvents are often toxic and can be very difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels to be practical.

U.S. Pat. No. 4,107,288 describes particles in the size range from 10 to 1,000 nm containing a biologically or pharmacodynamically active material. However, the particles comprise a crosslinked matrix of macromolecules having the active material supported on or incorporated into the matrix.

It would be desirable to provide stable dispersible drug particles in the submicron size range which can be readily prepared and which do not appreciably flocculate or agglomerate due to interparticle attractive forces and do not require the presence of a crosslinked matrix. Moreover, it would be highly desirable to provide pharmaceutical compositions having enhanced bioavailability.

SUMMARY OF THE INVENTION

We have discovered stable, dispersible drug nanoparticles and a method for preparing such particles by wet milling in the presence of grinding media in conjunction with a surface modifier. The particles can be formulated into pharmaceutical compositions exhibiting remarkably high bioavailability.

More specifically, in accordance with this invention, there are provided particles consisting essentially of a crystalline drug substance having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 400 nm.

This invention also provides a stable dispersion consisting essentially of a liquid dispersion medium and the above-described particles dispersed therein.

In another embodiment of the invention, there is provided a method of preparing the above-described particles comprising the steps of dispersing a drug substance in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the drug substance to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

In a particularly valuable and important embodiment of the invention, there is provided a pharmaceutical composition comprising the above-described particles and a pharmaceutically acceptable carrier therefor. Such pharmaceutical composition is useful in a method of treating mammals.

It is an advantageous feature that a wide variety of surface modified drug nanoparticles free of unacceptable contamination can be prepared in accordance with this invention.

It is another advantageous feature of this invention that there is provided a simple and convenient method

for preparing drug nanoparticles by wet milling in conjunction with a surface modifier.

Another particularly advantageous feature of this invention is that pharmaceutical compositions are provided exhibiting unexpectedly high bioavailability.

Still another advantageous feature of this invention is that pharmaceutical compositions containing poorly water soluble drug substances are provided which are suitable for intravenous administration techniques.

Other advantageous features will become readily apparent upon reference to the following Description of Preferred Embodiments.

DESCRIPTION OF PREFERRED EMBODIMENTS

This invention is based partly on the discovery that drug particles having an extremely small effective average particle size can be prepared by wet milling in the presence of grinding media in conjunction with a surface modifier, and that such particles are stable and do not appreciably flocculate or agglomerate due to interparticle attractive forces and can be formulated into pharmaceutical compositions exhibiting unexpectedly high bioavailability. While the invention is described herein primarily in connection with its preferred utility, i.e., with respect to nanoparticulate drug substances for use in pharmaceutical compositions, it is also believed to be useful in other applications such as the formulation of particulate cosmetic compositions and the preparation of particulate dispersions for use in image and magnetic recording elements.

The particles of this invention comprise a drug substance. The drug substance exists as a discrete, crystalline phase. The crystalline phase differs from a noncrystalline or amorphous phase which results from precipitation techniques, such as described in EPO 275,796 cited above.

The invention can be practiced with a wide variety of drug substances. The drug substance preferably is present in an essentially pure form. The drug substance must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the drug substance has a solubility in the liquid dispersion medium of less than about 10 mg/ml, and preferably of less than about 1 mg/ml. A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which a drug substance is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

Suitable drug substances can be selected from a variety of known classes of drugs including, for example, analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio-pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines. Preferred drug substances include those intended for oral administration and intravenous administration. A description of these classes of drugs and a listing of species within each class can be found in Martindale, The Extra Pharmacopoeia, Twenty-ninth Edition, The Pharmaceutical Press, London, 1989, the disclosure of which is hereby incorporated by reference in its entirety. The drug substances are commercially available and/or can be prepared by techniques known in the art.

Representative illustrative species of drug substances useful in the practice of this invention include:

17-α-pregno-2,4-dien-20-yno-[2,3-d]-isoxazol-17-ol (Danazol);

5α,17α,-1'-(methylsulfonyl)-1'H-pregn-20-yno[3,2-c]-pyrazol-17-ol (Steroid A);

piposulfam;

piposulfan;

camptothecin; and

ethyl-3,5-diacetoamido-2,4,6-triiodobenzoate

In particularly preferred embodiments of the invention, the drug substance is a steriod such as danazol or Steroid A or an antiviral agent.

The particles of this invention contain a discrete phase of a drug substance as described above having a surface modifier adsorbed on the surface thereof. Useful surface modifiers are believed to include those which physically adhere to the surface of the drug substance but do not chemically bond to the drug.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants. Representative examples of excipients include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986, the disclosure of which is hereby incorporated by reference in its entirety. The surface modifiers are commercially available and/or can be prepared by techniques known in the art.

Particularly preferred surface modifiers include polyvinyl pyrrolidone, Pluronic F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, Tetronic 908, which is a tetrafunctional block copolymer derived from sequential addition of ethylene

oxide and propylene oxide to ethylenediamine, dextran, lecithin, Aerosol OT, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol P, which is a sodium lauryl sulfate, available from DuPont, Triton X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 80, which is a polyoxyethylene sorbitan fatty acid ester, available from ICI Specialty Chemicals, and Carbowax 3350 and 934, which are polyethylene glycols available from Union Carbide. Surface modifiers which have found to be particularly useful include polyvinylpyrrolidone, Pluronic F-68, and lecithin.

The surface modifier is adsorbed on the surface of the drug substance in an amount sufficient to maintain an effective average particle size of less than about 400 nm. The surface modifier does not chemically react with the drug substance or itself. Furthermore, the individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages.

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. By "an effective average particle size of less than about 400 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 400 nm when measured by the above-noted techniques. In preferred embodiments of the invention, the effective average particle size is less than about 250 nm. In some embodiments of the invention, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 400 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 400 nm. In some embodiments, essentially all of the particles have a size less than 250 nm.

The particles of this invention can be prepared in a method comprising the steps of dispersing a drug substance in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the drug substance to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

A general procedure for preparing the particles of this invention is set forth below. The drug substance selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse drug substance selected be less than about 100 μm as determined by sieve analysis. If the coarse particle size of the drug substance is greater than about 100 μm, then it is preferred that the particles of the drug substance be reduced in size to less than 100 μm using a conventional milling method such as airjet or fragmentation milling.

The coarse drug substance selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the drug substance in the liquid medium can vary from about 0.1–60%, and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 400 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the drug substance and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the drug substance conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. We have found that zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 3 g/cm^3.

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the drug substance. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing pressures up to about 20 psi (1.4 kg/cm^2) are typical of media milling.

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

The relative amount of drug substance and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular drug substance and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square meter surface area of the drug substance. The surface modifier can be present in an amount of 0.1–90%, preferably 20–60% by weight based on the total weight of the dry particle.

As indicated by the following examples, not every combination of surface modifier and drug substance provides the desired results. Consequently, the applicants have developed a simple screening process whereby compatible surface modifiers and drug substances can be selected which provide stable dispersions of the desired particles. First, coarse particles of a selected drug substance of interest are dispersed in a liquid in which the drug is essentially insoluble, e.g., water at 5% (w/w) and milled for 60 minutes in a DYNO-MILL under the standard milling conditions which are set forth in Example 1 which follows. The milled material is then divided into aliquots and surface modifiers are added at concentrations of 2, 10 and 50% by weight based on the total combined weight of the drug substance and surface modifier. The dispersions are then sonicated (1 minute, 20 kHz) to disperse agglomerates and subjected to particle size analysis by examination under an optical microscope (1000×magnification). If a stable dispersion is observed, then the process for preparing the particular drug substance surface modifier combination can be optimized in accordance with the teachings above. By stable it is meant that the dispersion exhibits no flocculation or particle agglomeration visible to the naked eye at least 15 minutes, and preferably, at least two days or longer after preparation.

The resulting dispersion of this invention is stable and consists of the liquid dispersion medium and the above-described particles. The dispersion of surface modified drug nanoparticles can be spray coated onto sugar spheres or onto a pharmaceutical excipient in a fluid-bed spray coater by techniques well known in the art.

Pharmaceutical compositions according to this invention include the particles described above and a pharmaceutically acceptable carrier therefor. Suitable pharmaceutically acceptable carriers are well known to those skilled in the art. These include non-toxic physiologically acceptable carriers, adjuvants or vehicles for parenteral injection, for oral administration in solid or liquid form, for rectal administration, and the like. A method of treating a mammal in accordance with this invention comprises the step of administering to the mammal in need of treatment an effective amount of the above-described pharmaceutical composition. The selected dosage level of the drug substance for treatment is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore, depends upon the particular drug substance, the desired therapeutic effect, on the route of administration, on the desired duration of treatment and other factors. As noted, it is a particularly advantageous feature that the pharmaceutical compositions of this invention exhibit unexpectedly high bioavailability as illustrated in the examples which follow. Furthermore, it is contemplated that the drug particles of this invention provide more rapid onset of drug action and decreased gastrointestinal irritancy.

It is contemplated that the pharmaceutical compositions of this invention will be particularly useful in oral and parenteral, including intravenous, administration applications. It is expected that poorly water soluble drug substances, which prior to this invention, could not have been administered intravenously, may be administered safely in accordance with this invention. Additionally, drug substances which could not have been administered orally due to poor bioavailability may be effectively administered in accordance with this invention.

While applicants do not wish to be bound by theoretical mechanisms, it is believed that the surface modifier hinders the flocculation and/or agglomeration of the particles by functioning as a mechanical or steric barrier between the particles, minimizing the close, interparticle approach necessary for agglomeration and flocculation. Alternatively, if the surface modifier has ionic groups, stabilization by electrostatic repulsion may result. It was surprising that stable drug particles of such a small effective average particle size and free of unacceptable contamination could be prepared by the method of this invention.

The following examples further illustrate the invention.

EXAMPLE 1

PVP Modified Danazol Particles Prepared in a Ball Mill

A nanoparticulate dispersion of Danazol was prepared using a DYNO-MILL (Model KDL, manufactured by Willy A. Bachoffen AG Maschinenfabrik). The following ingredients were added to a glass vessel and agitated on a roller for 24 hours to dissolve the polyvinylpyrrolidone-surface modifier.

Polyvinylpyrrolidone K-15 (made by GAF)—98 g
High purity water—664 g

Subsequently, 327 grams of dry powdered Danazol was added to the above solution and rolled for one week. This step aided in evenly dispersing the Danazol in the surface modifier solution, thereby reducing the treatment time required in the media mill. The Danazol was purchased in a micronized form (average particle size of about 10 microns) from Sterling Drug Inc. The particles had been prepared by a conventional airjet milling technique. This premix was added to a holding vessel and agitated with a conventional propeller mixer at low speed to maintain a homogeneous mixture for the media milling event. The media mill was prepared accordingly for the media milling process. The mill grinding chamber was partially filled with silica glass spheres and the premix was continuously recirculated through the media mill operating at the following conditions:

Grinding vessel: water jacketed stainless steel chamber
Premix flow rate: 250 ml per minute
Available volume of grinding vessel: 555 ml
Media volume: 472 ml of glass beads

Media type: size range of 0.5–0.75 mm silica glass beads, unleaded (distributed by Glen Mills, Inc.)
Recirculation time: 240 min
Residence time: 60 min
Impeller speed: 3000 RPM, tangential speed 1952 ft/min (595 m/min)
Grinding vessel coolant: water
Coolant temperature: 50° F. (10° C.)

After recirculating the slurry for 240 minutes, a sample of the dispersion was removed and evaluated for particle size distribution using a sedimentation field flow fractionator (made by DuPont). The particles were determined to have a number average diameter of 77.5 nm and a weight average diameter of 139.6 nm. The particle size of the dispersion ranged in size from 3–320 nm.

EXAMPLE 2

PVP Modified Danazol Particles Prepared in a Ball Mill at Low Solids.

A nanoparticulate dispersion of Danazol was prepared using a ball mill process. A 600 ml cylindrical glass vessel (inside diameter=3.0 inches (7.6 cm)) was filled approximately halfway with the following grinding media:

Grinding media: zirconium oxide grinding spheres (made by Zircoa, Inc.)
Media size: 0.85–1.18 mm diameter
Media volume: 300 ml

The following dry ingredients were added directly to this glass vessel:

Danazol (micronized): 10.8 g
Polyvinylpyrrolidone K-15: 3.24 g
High purity water: 201.96 g

Danazol was purchased in the micronized form (average particle size 10 microns) from Sterling Drug Inc. and the polyvinylpyrrolidone was K-15 grade produced by GAF. The cylindrical vessel was rotated horizontally about its axis at 57% of the "critical speed". The critical speed is defined as the rotational speed of the grinding vessel when centrifuging of the grinding media occurs. At this speed the centrifugal force acting on the grinding spheres presses and holds them firmly against the inner wall of the vessel. Conditions that lead to unwanted centrifuging can be computed from simple physical principles.

After 5 days of ball milling, the slurry was separated from the grinding media through a screen and evaluated for particle size with the sedimentation field flow fractionator. The number average particle diameter measured was 84.9 nm and the weight average particle diameter was 169.1 nm. The particles varied in size from 26 to 340 nm. The amount and type of surface modifier was sufficient to provide colloidal stability to agglomeration and to maintain a homogeneous blend of ingredients assuring precise material delivery during subsequent processing steps.

BIOAVAILABILITY TESTING

Bioavailability of Danazol from the nanoparticulate dispersion described above was compared to that from a suspension of unmilled Danazol in fasted male beagle dogs. The unmilled material was prepared as a suspension in the same manner as the dispersion, with the exception of the ball milling process. Both formulations were administered to each of five dogs by oral gavage and plasma obtained via a cannula in the cephalic vein. Plasma Danazol levels were monitored over 24 hours. The relative bioavailability of Danazol from the nanoparticulate dispersion was 15.9 fold higher than from the Danazol suspension containing Danazol particles having an average particle size of about 10 microns prepared by conventional airjet milling. Comparison of oral plasma levels with dose corrected plasma levels following intravenous administration of Danazol gave a mean absolute bioavailability (±SEM) of 82.3±10.1% for the nanoparticulate dispersion and 5.1±1.9% for the unmilled material.

EXAMPLE 3

PVP Modified Danazol Particles Prepared in a Ball Mill at High Solids

A nanoparticle dispersion of Danazol was prepared using 1 mm diameter glass grinding media (0.85–1.18 mm from Potters Industries). A cylindrical glass vessel having a diameter of 2.75 inches (7.0 cm) with a volume of 400 ml was charged with 212 ml of unleaded glass grinding media. The following ingredients were added to this vessel:

30.4 g of micronized Danazol
9.12 g of Polyvinylpyrrolidone K-15
112.48 g of high purity water

This vessel was rotated horizontally on its axis at a controlled rotational speed of 80.4 revolutions per minute (50% of critical speed) for 5 days. The slurry was immediately separated from the grinding media and evaluated for particle size and grinding media attrition using inductively coupled plasma emissions (ICP). The particle size measured with a sedimentation field flow fractionator yielded a number average diameter of 112.7 nm and a weight average diameter of 179.3 nm. The extent of media attrition was measured to establish the purity of the final dispersion using an inductively coupled plasma-atomic emission spectroscopy method. The level of silicon in the final dispersion was less than 10 parts of elemental silicon per million parts of the slurry.

EXAMPLE 4

PVP Modified Danazol Particles

A nanoparticle dispersion of Danazol was prepared for clinical evaluation using a ball milling dispersion method. This dispersion was prepared by milling with glass grinding media. The grinding media used was:

Media type: 0.85–1.18 mm unleaded glass spheres
Media quantity: 6100 ml

The media was added to a 3 gallon porcelain jar. The following ingredients were then added to the jar:

1000 g Danazol (micronized)
300 g Polyvinylpyrrolidone K-15
3700 g high purity water

The vessel was rolled 5 days at a rotational speed of 39.5 revolutions per minute (50% critical speed). The liquid slurry was separated from the grinding media with a screen and used to prepare solid oral doses for clinical studies. The dispersion was assessed for particle size using the sedimentation field flow fractionator and was measured to have a number average diameter of 134.9 nm and a weight average diameter of 222.2 nm. The level of contamination from the grinding media was measured (by ICP) to be 36 parts of silicon per million parts of dispersion. Less than 5 ppm of aluminum was detected. X-ray powder diffraction data of the starting powder was compared with the dispersed Danazol and showed the crystal structure morphology

of the solid dispersed particles was unchanged by the dispersion process.

EXAMPLE 5

PVP Modified Danazol Particles

A nanoparticulate dispersion of Danazol was prepared using a laboratory media mill and glass grinding media. The media mill was equipped with a 50 ml grinding chamber and the mill was a "Mini" Motormill manufactured by Eiger Machinery Inc.

The media mill was operated at the following process conditions:

Bead charge: 42.5 ml glass spheres

Rotor speed: 5000 RPM (2617 feet per minute (798 m/min) tangential speed)

Grinding media: 0.75–1.0 mm unleaded glass beads (distributed by Glens Mills)

The dispersion formula was prepared by dissolving 27 g of polyvinylpyrrolidone in 183 g of water and agitated in a steel vessel with a 50 mm "Cowles" type blade until the solution was clear and free of undissolved PVP polymer. The rotational speed of the mixer was maintained at 5000 RPM. 90 g of micronized Danazol was slowly added to this blend with the same mixing for 30 min. 200 cc of the premix was added to the holding tank of the mill and recirculated for 5 hours and 51 minutes. The final residence time in the grinding zone was 40 minutes.

The final average particle size was measured and determined to have a number average diameter of 79.9 nm and a weight average diameter of 161.2 nm. The particles varied in size from 30–415 nm. The level of attrition from erosion of the grinding media and grinding vessel were measured (by ICP) to be 170 ppm of iron and 71 ppm silicon. The crystal structure was determined by X-ray diffraction to be unchanged by the dispersion process.

EXAMPLE 6

Lecithin Modified Steroid A Particles

A nanoparticulate dispersion of Steroid A was prepared by ball milling with zirconium oxide grinding beads. The dispersion was prepared in the absence of a surface modifier and a post addition of Lecithin and a sonication step were required to stablize the dispersed phase of Steroid A and prevent agglomeration and rapid sedimentation. A fine particle dispersion of Steroid A was prepared by ball milling the following ingredients:

5 g Steroid A

95 g high purity water

Steroid A was in the form of unmilled coarse grains having a particle size of about 100 μm and ranging in size up to about 400 μm.

The following process conditions were used:

Media: 135 ml

Vessel volume: 240 ml

Media type: 0.85–1.18 mm Zirbeads (manufactured by Zircoa Inc.)

Milling time: 4 days

Milling speed: 86 RPM (50% critical speed)

After four days of ball milling the slurry was separated from the grinding media through a screen. One gram of this unstabilized slurry was added to 10 g of an aqueous solution of Lecithin (1% Centrolex "P" by weight in high purity water, Lecithin manufactured by Central Soya Company, Inc.) and mixed by vigorous shaking, followed by a sonication step for 20 seconds using an ultrasonic horn (Model 350 Branson Ultrasonic Power Supply, Horn Diameter=0.5 inch (1.27 cm), Power setting=2). The slurry was sized under a microscope. An Olympus BH-2 optical microscope equipped with phase contrast illumination was used to observe the size and condition of the dispersion.

A drop of the above dilute slurry was placed between a microscope slide and glass cover slip and observed microscopically at high magnification (1,000 times) and compared to the slurry similarly diluted with water only (no surface modifier). The unmodified dispersion exhibited extensive particle agglomeration. The particle size of the unmodified dispersion was more than 10 microns and the unmodified dispersion exhibited no Brownian Motion. Brownian motion is the oscillatory or jiggling motion exhibited by particles in a liquid that fall in the size range of less than about 1 micron. The Lecithin modified particles exhibited rapid Brownian motion. The thus observed dispersion had the characteristics and appearance consistent with a number average particle size of less than 400 nm. Furthermore, it is expected that additional milling would lead to further particle size reduction.

EXAMPLE 7

Alkyl Aryl Polyether Sulfonate Modified Steroid A

Example 6 was repeated except that the Lecithin was replaced with Triton X-200 (manufactured by Rohm and Haas). Similar results were observed.

EXAMPLE 8

Gum Acacia Modified Steroid A

Example 6 was repeated except that the Lecithin was replaced with gum acacia (available from Eastman Kodak Co.) Similar results were observed.

EXAMPLE 9

Sodium Lauryl Sulfate Modified Steroid A

Example 6 was repeated except that the Lecithin was replaced with sodium lauryl sulfate (available as Duponol ME from DuPont, Inc.). Similar results were observed.

EXAMPLE 10

Steroid A Modified with a Dioctylester of Sodium Sulfosuccinic Acid

Example 6 was repeated except that the Lecithin was replaced with Aerosol OT (available from American Cyanamid Chemical Products, Inc.). Similar results were observed.

EXAMPLE 11

Steroid A Modified with a Block Copolymer of Ethylene Oxide and Propylene Oxide

Example 6 was repeated except that the Lecithin was replaced with Pluronic F68 (available from BASF Corp.). Similar results were observed.

EXAMPLE 12

Steroid A Modified with a Block Copolymer of Ethylene Oxide and Propylene Oxide

A nanoparticulate dispersion of Steroid A was prepared by ball milling with zirconium oxide grinding

media for 5 days. 70 cc of grinding media were added to a 115 cc vessel followed by:

2.5 g Steroid A

0.75 g of Pluronic F68

46.75 g high purity water

The resulting mixture was ball milled for 5 days at 50% of the critical rotational speed. The final dispersion was separated from the grinding media and microscopically evaluated for particle size as in Example 6. The dispersion exhibited rapid Brownian Motion and no particles were larger than 1 micron. Most particles were less than 400 nm.

EXAMPLE 13

Lecithin Modified Steroid A Particles

Example 12 was repeated except that the Pluronic F68 was replaced with Centrolex P. No particles larger than 1 micron were observed microscopically and most were less than 400 nm.

EXAMPLE 14

Steroid A Particles Modified with a Block Copolymer of Ethylene Oxide and Propylene Oxide

A nanoparticulate dispersion of Steroid A was prepared by a ball milling process. The following ingredients were added to a cylindrical 0.95 l vessel. The vessel was filled approximately halfway with the following grinding media:

Grinding media: 0.85–1.18 mm diameter zirconium oxide spheres (made by Zircoa)

The following dispersion ingredients were added directly to the glass vessel:

18 g Steroid A

4.5 g Pluronic F68 (purchased from BASF Corp.)

336.6 g high purity water

Steroid A was purchased from Sterling Drug Inc. in the form of unmilled tabular crystals having an average particle size of approximately 100 μm.

The vessel was rotated concentrically on its axis at 50% critical speed for 5 days. After this time 4.45 g of Pluronic F68 was added to the slurry and rolled for 5 more days at the same conditions. The slurry was then discharged and separated from the grinding media and evaluated for particle size using the sedimentation field flow fractionator. The number average particle size measured was 204.6 nm and the weight average particle size was 310.6 nm. The particle size distribution ranged from approximately 68–520 nm. The dispersion was examined with an optical microscope. It exhibited excellent particle integrity, free flocculation and agglomeration. The dispersion particles exhibited rapid Brownian motion.

BIOAVAILABILITY TESTING

Bioavailability of Steroid A from the nanoparticulate dispersion described above was compared to that from a suspension of unmilled Steroid A (having an average particle size of about 100 μm) in male beagle dogs. The unmilled material was prepared as a suspension in the same manner as the dispersion, with the exception of the ball milling process. Both formulations were administered to each of five dogs by oral gavage and plasma obtained via a cannula in the cephalic vein. Plasma Steroid A levels were monitored over 24 hours. The relative bioavailability of Steroid A from the nanoparticulate dispersion was 7.1 fold higher than from the unmilled Steroid A suspension. Comparison of oral plasma levels with dose corrected plasma levels following intravenous administration of Steroid A gave a mean absolute bioavailability (±SEM) of 14.8±3.5% for the nanoparticulate dipersion and 2.1±1.0% for the unmilled material.

COMPARATIVE EXAMPLE A

A dispersion of Steroid A was prepared using a ball milling process with zirconium oxide grinding beads. The dispersion was prepared in the absence of a surface modifier and a post-sonication step was used to minimize flocculation and reaggregation.

A fine particle dispersion was prepared by ball milling the following ingredients:

5 g Steroid A

95 g high purity water

The following process conditions were used:

Grinding media: 135 ml

Vessel volume: 240 ml

Grinding media: 0.85–1.18 mm Zirbeads XR

Milling time: 4 days

Milling speed: 86 RPM (50% critical speed)

After four days of ball milling, the slurry was separated from the grinding media through a screen. One gram of the unstablized slurry was blended with 10 grams of high purity water and mixed by vigorous shaking, followed by a sonication step for 20 seconds using an ultrasonic horn (Model 350 Branson Ultrasonic Power Supply, Horn diameter=0.5 inch, Power setting=2). The slurry was sized under a microscope. An optical microscope equipped with phase contrast illumination was used to observe the condition of the dispersion.

A drop of the dilute slurry was placed between a microscope slide and a glass cover slip and observed at high magnification (400×). The dispersion exhibited severe particle aggregation. The aggregate size was greater than 10 microns and exhibited no Brownian particle movement.

COMPARATIVE EXAMPLE B

Comparative Example A was repeated except that 1 gram of the slurry was added to 10 grams of a dilute solution (1% by weight) of PVP K-15. The resultant dispersion after 6 days was aggregated. The aggregate size was at least 5 microns. After 6 days of holding, the dispersion settled completely leaving a clear supernatant and a layer of Steroid A sediment.

COMPARATIVE EXAMPLE C

Comparative Example B was repeated except that the 1% PVP solution was replaced with a 1% solution of purified sodium methyl oleoyl taurate (available from GAF as Igepon T and subsequently purified at Eastman Kodak). After 6 days the dilute dispersion was partially flocculated and had many aggregates larger than 1 micron.

COMPARATIVE EXAMPLE D

Comparative Example B was repeated except that 1 gram of slurry was added to 10 grams of a 1% aqueous solution of polyethylene glycol (available from Union Carbide as PEG 3350). The dispersion after sonification was flocculated with particles larger than 35 microns.

COMPARATIVE EXAMPLE E

Comparative Example B was repeated except that the dispersion was diluted with an aqueous 1% solution of

gum tragacanth (available from Eastman Kodak). This freshly prepared dispersion exhibited flocculation with particles 10 microns and larger.

COMPARATIVE EXAMPLE F

A dispersion of Danazol was prepared by ball milling with zirconium oxide grinding spheres. A cylindrical glass vessel was filled about halfway with the following ingredients:

Grinding media: 135 ml 0.85–1.18 mm Zirbeads XR
5 g Danazol
1 g PVP K-15
94 g high purity water

This vessel was rotated horizontally on its axis at a controlled speed of 50% critical speed (85 RPM) for 4 days. The slurry was discharged and separated from the grinding media through a screen and examined for particle size with an optical microscope. The slurry was examined for particle size after holding it for 4 days at room temperature (23° C.). A drop of undiluted slurry was placed between a glass microscope slide and a glass cover slip and observed optically at high magnification (400X). The slurry was partially aggregated with particles up to 10 microns in diameter. Unlike Examples 1–5, the amount of PVP present was insufficient to hinder particle agglomeration.

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

What is claimed is:

1. Particles consisting essentially of 99.9–10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm.

2. The particles of claim 1 having an effective average particle size of less than 250 nm.

3. The particles of claim 1 having an effective average particle size of less than 100 nm.

4. The particles of claim 1 wherein said drug substance is selected from analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives, astringents, beta-adrenoceptor blocking agents, contrast media, corticosteroids, cough suppressants, diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics, haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin, prostaglandins, radio-pharmaceuticals, sex hormones, anti-allergic agents, stimulants, sympathomimetics, thyroid agents, vasodilators and xanthines.

5. The particles of claim 1 wherein said drug substance is a steroid.

6. Particles consisting essentially of 99.9–10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier absorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm, wherein said drug substance is selected from the group consisting of Danazol, 5α,17α,-1'-(methylsulfonyl)-1'H-pregn-20-yno-[3,2-c]-pyrazol-17-ol, piposulfam, piposulfan, camptothecin, and ethyl-3,5-diacetamido-2,4,6-triiodobenzoate.

7. The particles of claim 1 wherein said surface modifier is selected from the group consisting of gelatin, casein, lecithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene caster oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone.

8. The particles of claim 1 wherein said surface modifier is selected from the group consisting of polyvinylpyrrolidone, an ethylene oxide-propylene oxide block copolymer, lecithin, an alkyl aryl polyether sulfonate, gum acacia, sodium dodecylsulfate, and a dioctylester of sodium sulfosuccinic acid.

9. Particles consisitng essentially of 80–40% by weight of crystalline danazol having polyvinyl pyrroldone adsorbed on the surface thereof in an amount of 20–60% by weight and sufficient to maintain an effective average particle size of less than about 100 nm.

10. Particles consisting essentially of 99.9–10% by weight of crystalline 5α, 17α,-1'-(methylsulfonyl)-1'H-pregn-20-yno-pyrazol-17-ol having an ethylene oxide propylene-oxide block copolymer adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm.

11. A stable dispersion consisting essentially of a liquid dispersion medium and the particles of claim 1.

12. The dispersion of claim 11 wherein said dispersion medium is water.

13. The dispersion of claim 11 wherein said dispersion medium is selected from the group consisting of safflower oil, ethanol, t-butanol, hexane and glycol.

14. A pharmaceutical composition comprising the particles of claim 1 and a pharmaceutically acceptable carrier therefor.

15. A method of treating a mammal comprising the step of administering to the mammal an effective amount of the pharmaceutical composition of claim 14.

16. A method of preparing the particles of claim 1 comprising the steps of dispersing a drug substance in a liquid dispersion medium and wet grinding said drug substance in the presence of rigid grinding media having an average particle size of less than 3 mm and a surface modifier to reduce the particle size of said drug substance to an effective average particle size of less than about 400 nm.

17. A method of preparing the particles of claim 1 comprising the steps of dispersing a drug substance in a liquid dispersion medium, wet grinding said drug substance in the presence of rigid grinding media having an average particle size of less than 3 mm, thereafter contacting said drug substance with a surface modifier by mixing said surface modifier with said dispersion me-

dium to form particles having an effective average particle size of less than about 400 nm.

18. The method of claim 17 further including the step of subjecting the dispersion medium containing said drug substance and said surface modifier to ultrasonic energy.

19. The method of claim 16 wherein said grinding media have an average particle size of less than 1 mm.

20. The method of claim 16 wherein said grinding media have a density greater than 3 g/cm^3.

* * * * *

EXHIBIT F

10a 11/8/91 Dellw

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Gary G. Liversidge, et al	:	Group Art Unit 152
	:	
Title: Surface Modified Drug Nanoparticles	:	Examiner W. Benston, Jr.
	:	
Serial No. 647,105	:	Malvern, PA 19355
	:	
Filed January 25, 1991	:	
	:	

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NOV 18 1991
GROUP 150

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

AMENDMENT

In response to the Office Action mailed May 21, 1991, the period for response having been extended by 3 months to expire on November 21, 1991 by the petition and fee authorization enclosed herewith, please amend this application, without prejudice, as follows:

In the Claims

Please rewrite claims 1 and 6 as follows:

1. (Amended) Particles consisting essentially of 99.9-10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm.

6. (Amended) [The particles of claim 1] Particles consisting essentially of 99.9-10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm, wherein said drug substance is selected from the group consisting of Danazol, 5α,17α,-1'-(methylsulfonyl)-1'H-pregn-20-yno-[3,2-c]-pyrazol-17-ol, piposulfam, piposulfan, camptothecin, and ethyl-3,5-diacetamido-2,4,6-triiodo-benzoate.

In claim 8, line 3, correct the spelling of "propylene".

Please cancel claim 9 as being redundant in view of the amendment to claim 1.

Please rewrite claims 10, 11, and 17-19 as follows:

16.9 (Amended) Particles consisting essentially of 80-40% by weight of crystalline [discrete phase of] danazol having polyvinyl pyrrolidone adsorbed on the surface thereof in an amount of 20-60% by weight and sufficient to maintain an effective average particle size of less than about 100 nm.

11.10 (Amended) Particles consisting essentially of 99.9-10% by weight of crystalline [a discrete phase of] 5α, 17α,-1'-(methylsulfonyl)-1'H-pregn-20-yno-[3,2-c]-pyrazol-17-ol having an ethylene oxide propylene-oxide block copolymer adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm.

16 17. (Amended) A method of preparing the particles of claim 1 comprising the steps of dispersing a drug substance in a liquid dispersion medium [and applying mechanical means] and wet grinding said drug substance in the presence of rigid grinding media having an average particle size of less than 3 mm and a surface modifier to reduce the particle size of [the] said drug substance to an effective average particle size of less than about 400 nm.

18.17 (Amended) A method of preparing the particles of claim 1 comprising the steps of dispersing a drug substance in a liquid dispersion medium, [applying mechanical means] wet grinding said drug substance in the presence of rigid grinding media having an average particle size of less than 3 mm, and thereafter contacting said drug substance with

a surface modifier by mixing said surface modifier with said dispersion medium to form particles having [to reduce the size of the drug substance to] an effective average particle size of less than about 400 nm [,and, thereafter, contacting the particles with a surface modifier].

~~19.~~ 18 (Amended). The method of claim ~~18~~ 17 further including the step of subjecting the dispersion medium containing said drug substance and [particles in contact with] said surface modifier to ultrasonic energy.

[Please add claims 20-21 as follows:]

~~20.~~ 19 The method of claim ~~17~~ 16 wherein said grinding media have an average particle size of less than 1 mm.

~~21.~~ 20 The method of claim ~~17~~ 16 wherein said grinding media have a density greater than 3 g/cm^3.

In the Specification

On page 3, line 30, correct the spelling of "nanoparticle"

REMARKS

Applicants acknowledge with appreciation the courteous and helpful interview conducted by Examiner William Benston and Supervisory Primary Examiner Thurman Page with Dr. Gary Liversidge, Martin Katz and applicants' undersigned attorney on November 12, 1991. The substance of such interview is incorporated in this response and in the interview summary record. As discussed during the interview

and indicated in the interview summary record, the submission of the instant Amendment and Declarations is believed to place this application in condition for allowance.

Claims 1-8 and 10-21 are pending in this application. Applicants acknowledge with appreciation the Examiner's indication in the Office Action that claims 6, 10 and 11 are considered allowable. Reconsideration of this application in its amended form and withdrawal of the rejection is respectfully requested for the reasons which follow. Before considering the specifics of the rejection it will be helpful to briefly consider the nature and significance of applicants' claimed invention.

Poor bioavailability is a significant problem encountered in the development of pharmaceutical compositions, particularly with respect to those compositions containing a drug substance that is poorly soluble in water. Bioavailability is the degree to which a drug becomes available to the target tissue after administration. There currently exist a wide variety of approaches for preparing pharmaceutical compositions containing poorly soluble drug substances and for improving the bioavailability thereof. However, none of these approaches is entirely satisfactory. For example, poorly soluble drug substances have been loaded into polymers and liposomes. Polymerization techniques require the arduous removal of contaminants, such as unreacted monomers and/or initiator, which can be toxic. A

lipid soluble drug is often required in preparing suitable liposomes, and unacceptably large amounts of the liposomes or polymer are often required to prepare unit drug doses.

Another approach, exemplified by the teachings of U. S. Patent 4,826,689 and EPO 275,796, involves solvent precipitation techniques wherein a solution of the drug substance is mixed with a miscible non-solvent for the substance. Such techniques have provided non-crystalline, e.g., amorphous particles smaller than 500 nm. However, solvent precipitation techniques provide amorphous particles which are unstable and contaminated with solvents. Such solvents are often toxic and can be very difficult, it not impossible, to adequately remove to pharmaceutically acceptable levels to be practical.

Dry milling techniques have been used to prepare pharmaceutical formulations containing particles of several μm or greater in diameter. However, conventional dry milling techniques often cause unacceptable levels of dust. Moreover, it is difficult to obtain fine particles by conventional dry milling techniques, e.g., jet milling. As stated in EPO 411,629, "preparation of submicron particles of less than 1 μm in diameter is almost impossible." The reason for this is the inherent tendency of such particles to agglomerate, aggregate, solidify, and/or adhere as the particle size decreases.

Notwithstanding such teaching in the art to the contrary, the applicants have discovered a method for preparing stable drug nanoparticles having an average particle size of less than about 400 nm (0.4 µm). The particles are prepared by wet grinding coarse crystals of a poorly soluble drug substance in the presence of grinding media and in conjunction with a surface modifier. The particles are free of unacceptable solvent contamination and consist essentially of the crystalline drug substance and the surface modifier adsorbed on the surface thereof. The particles can be formulated into pharmaceutical compositions exhibiting remarkably high bioavailability and other advantageous properties, including, for example, improved dose proportionality, decreased fed-fasted variability and more rapid onset of action. In addition, certain particles are extraordinarily useful as contrast agents in x-ray contrast compositions.

Applicants' claims are directed to particles consisting essentially of a poorly soluble crystalline drug substance having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 400 nm. Applicants' claims are further directed to methods of making such particles, and to dispersions, pharmaceutical compositions, and methods of treatment using the particles. Pharmaceutical compositions containing the particles of this invention are

particularly useful in methods of treating mammals and exhibit unexpected bioavailability and other advantageous properties including improved dose proportionality, decreased fed-fasted variability, and more rapid onset of action.

Turning to the Office Action, the rejection of claims 1-19 under 35 U.S.C.§ 112 is respectfully traversed and/or obviated by the present amendment. Applicants have amended the claims and the specification in response to the Office Action and to clarify and more accurately point out the subject matter which the applicants regard as their invention.

More particularly, responsive to the Office Action, claim 1 has been amended to specify that the "crystalline drug" is poorly soluble, i.e., it has a solubility in water of less than 10 mg/ml. Support for such amendment can be found beginning on page 6, line 8 of the instant specification. Applicants' invention can be practiced with virtually all poorly soluble drug substances as is supported by numerous working examples of poorly soluble drug substances that have been prepared in the form of nanoparticles according to the teaching of applicants' specification. Moreover, Table 1 in the Rule 132 Declaration of Eugene Cooper, Ph.D., submitted herewith, illustrates that eleven poorly soluble drug substances of radically different chemical structure and from a wide variety of therapeutic classes have been prepared in the form of nanoparticles.

This evidence demonstrates conclusively that the invention can be practiced with an extremely wide variety of poorly soluble drug substances. Applicants' specification teaches that the particles of the invention contain the poorly soluble drug substance in a crystalline phase. The crystalline phase differs from non-crystalline and amorphous phases. The crystalline particles of this invention exhibit improved stability compared to particles containing a drug substance in an amorphous phase. In addition, the claimed particles consist essentially of the drug substance and surface modifier. By "consisting essentially of" it is meant that the claimed particles are essentially free of solvent contamination and other toxic materials, e.g., monomer or initiator, resulting from solvent precipitation and polymerization methods of particle preparation. As discussed further below, solvent contamination is characteristic of particles prepared by solvent precipitation techniques. Claim 1 has also been amended to recite that the drug substance is present in a specific amount, i.e., 99.9-10% by weight based on the total weight of the particle. Support for this amendment can be found on page 12, lines 7-9 of the instant specification.

Claim 1 has been further amended to specify that the "surface modifier" is non-crosslinked and present in a specific amount, i.e., 0.1-90% by weight based on the total weight of the particle. Applicants' specification teaches

that a wide variety of surface modifiers are useful within this range of amounts (see page 7, line 24 - page 9, line 2 and the examples). Indeed, applicants have shown that the invention can be practiced with a wide variety of surface modifiers. In addition to the numerous surface modifiers exemplified in applicants' specification and working examples, Table 1 in the Rule 132 Declaration of Eugene Cooper, Ph.D., illustrates eleven surface modifiers in varying amounts useful in the practice of this invention. This evidence demonstrates conclusively that the instant invention can be practiced with a wide variety of surface modifiers. Moreover, applicants' specification (page 12, lines 10-31) readily enables one skilled in the art to select surface modifiers for any particular poorly soluble drug substance.

Therefore, it is urged that claim 1 as amended including the terms "crystalline drug" and "surface modifier" is distinct and definite, particularly when read by one skilled in the art in light of the teaching in applicants' specification. In the event that the Examiner maintains this basis for the rejection, it is respectfully requested that a more detailed explanation be provided as to why such terms are deemed vague or indefinite.

It is noted that claims 4, 7 and 8 are dependent on claim 1 which now recites specific amounts of drug substance and surface modifier as suggested in the Office Action.

Claims 12-14 are directed to a dispersion containing the particles of claim 1. Claim 15 is directed to a pharmaceutical composition comprising the particles of claim 1. Claim 16 is directed to a method of treating a mammal using a pharmaceutical composition comprising the particles of claim 1. The inventive feature in each of these claims is the specific particles recited which have been more specifically defined in claim 1. Such particles are believed to be distinctly and adequately defined. As is taught in applicants' specification, appropriate media, carriers, dosages, effective amounts and treatment methods for particular drug substances are parameters which can be readily determined by one of ordinary skill in the art. Consequently, it is respectfully urged that claims 12-16, like claim 1, are distinct and definite.

Claims 17 and 18 have been amended to recite "wet grinding" instead of "applying mechanical means" and to specify that the grinding media are rigid and have an average size of less than 3 mm. Wet grinding in the presence of rigid grinding media indeed is necessary to reduce particle size to the critical level claimed. Applicants' specification teaches that the rigid grinding media for particle size reduction preferably have an average particle size of less than 3 mm, and more preferably, of less than 1 mm. In addition, the media preferably have a density greater that 3 g/cm^3. Claims 20-21 added by this amendment are

directed to specific preferred embodiments. Claims 20-21 are believed to be patentable over the art for the reasons discussed below.

Claim 18 has also been amended to indicate that the drug substance, after wet grinding, can be contacted with a surface modifier by mixing the surface modifier with the dispersion medium.

Claim 19 has been amended to clarify that the dispersion medium containing the drug substance and surface modifier is subjected to ultrasonic energy. It is noted that claim 19 is directed to a method of preparing the particles of the invention. Applicants have found that the claimed particles can be prepared by two related wet grinding techniques, i.e., the surface modifier can be present in the liquid dispersion medium during wet grinding or added to the dispersion medium thereafter. If the surface modifier is added to the dispersion medium after wet grinding, then the dispersion medium preferably is subjected to ultrasonic energy. Thus, the drug substance and surface modifier are in the dispersion medium during sonification.

The phrase "discrete phase of danazol" in claim 10 has been replace by "crystalline danazol". In addition, relative amounts of danazol and PVP have been specified.

Claim 11 has been amended to recite relative amounts of drug substance and surface modifier. Applicants do not completely understand what causes the resulting

differing particle sizes, e.g., those specified in claims 10 and 11. Indeed, as suggested in the Office Action, the compositions specified in claims 10 and 11 include different drug substances and thus have different purposes as is readily apparent to one skilled in the art.

Claim 6 has been rewritten in independent form and is believed to be allowable.

Claims 10 and 11 have been amended to overcome the rejection under §112 and are believed to be in allowable form.

In view of the above amendments and remarks, it is believed that the §112 rejection has been overcome and/or is no longer applicable. Accordingly, it is requested that such rejection be withdrawn.

The rejection under 35 U.S.C. §103 over Violante (U. S. Patent 4,826,689) in view of Oppenheim (U. S. Patent 4,107,280) and Motoyama (U. S. Patent 4,540,602) is respectfully traversed. It is respectfully submitted that applicants' claims are patentable over these references taken individually or in combination.

Violante (U. S. Patent 4,826,689) relates to a method of making uniformly sized particles from water-insoluble organic compounds. However, the particles and methods described by Violante are radically different than the applicants' particles and methods. Violante describes a

solvent precipitation technique wherein the organic compound is dissolved in an organic solvent. Violante teaches that

> "The particles are formed by a carefully controlled precipitation of the compound into an aqueous phase from an organic solvent in which the compound is soluble (column 4, lines 34-37)."

The Office Action indicates that Violante teaches a crystalline drug substance. However, Violante teaches a solvent precipitation technique for preparing amorphous, non-crystalline particles. In fact, Violante specifically teaches that the crystalline state is to be avoided. For example, Violante teaches

> "When properly practiced, this invention enables the trapping of a compound in the metastable particle state, precluding transformation to the crystalline state (column 4,lines 48-51)." (emphasis added)

Consequently, Violante teaches away from applicants' claimed particles and wet grinding method. This is affirmed in column 14, lines 5-24 wherein Violante states that:

> "Physical methods for modifying and controlling particle size, such as ball milling, grinding or sonication result in preparation[s] with a very broad range of particle diameters... These methods are commonly used to eliminate large particles (greater than 4-5 microns) ...but generally some particles of submicron size are also produced; these very small particles have been shown to be more toxic than 1-2 micron particles... A chemical precipitation procedure for producing particles of a given size was developed to avoid these problems."

Moreover, applicants' invention provides significant commercial advantages compared to Violante and other prior

art solvent precipitation techniques for preparing particles such as those described in EPO 275,796, and U. K. Patent Application GB 2,220,048. Such solvent precipitation techniques result in the formation of particles contaminated with solvents. Such solvents are often toxic and can be very difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels to be practical. This problem is noted in U. K. Patent Application 2,200,048 which suggests that it is not possible to completely remove solvent by conventional techniques. Thus, Violante in no way suggests applicants' claimed particles or method. To further evidence the nonobviousness of applicants' claimed particles, the attached Rule 132 Declaration of Eugene Cooper, Ph.D., points out that the above-noted differences between the particles of the claimed invention and particles prepared by prior art techniques such as solvent precipitation techniques are commercially significant.

Motoyama is primarily directed to processes (radically different than applicants' method) which involve emulsification steps and/or heating and gelling, i.e., crosslinking, reactions. In one embodiment, Motoyama teaches that a solid drug can be pulverized in an aqueous solution of a water-soluble high molecular substance, and that as a result of such wet grinding, the drug is formed into finely divided particles ranging from 0.5 μm or less to 5μ in diameter (column 9, line 43 - column 10, line 18). However,

there is no suggestion by Motoyama that particles in the claimed size range, i.e., less than about 400 nm can be obtained. Motoyama exemplifies such wet grinding in Example 15 and 18. It is noted that Example 18 involves wet grinding, in the absence of grinding media, by means of a Politron®. The procedure set forth in Example 15 includes wet grinding in the presence of grinding media, i.e., glass beads having diameters of 2 to 5 mm. However, as indicated in the Rule 132 Declaration of John F. Bishop submitted herewith, reproductions of the procedures described in Example 15 and Example 18 in Motoyama resulted in particles much larger than the size specified in applicants' claims. Moreover, the Rule 132 Declaration of John F. Bishop demonstrates that particles in the claimed size range were not produced even when extraordinarily longer grinding times and higher rotational disk speeds than suggested in Motoyama were employed in an effort to produce particles as small as possible. Thus, Motoyama does not disclose or suggest, and, particularly in view of the Rule 132 Declaration of John F. Bishop, can not be construed to disclose or suggest, applicants' claimed particles having an average effective particle size of less than about 400 nm or method for the preparation thereof.

Oppenheim teaches particles in the size range from about 10 to about 1000 nm which comprise a crosslinked matrix of macromolecules. A pharmaceutically active material can be

supported on or incorporated into the matrix. Such particles require the presence of a crosslinker or hardening agent and differ substantially from applicants' claimed particles. Oppenheim's crosslinkers, such as formaldehyde or glutaraldehyde, like polymerization initiations, can be toxic and must be removed from solution (col. 4, lines 28-42). Moreover, when the crosslinked particles of Oppenheim contain a pharmacodynamically active material, such material must be water soluble or introduced from a solvent solution (col. 3, lines 55-56; column 7, lines 38-43). Thus the particles of Oppenheim suffer from the same disadvantages (discussed above) which result from solvent precipitation techniques. Nevertheless, to further distinguish applicants' claims from the teaching of Oppenheim, claim 1 has been amended to recite that the particles include a non-crosslinked surface modifier. In any event, Oppenheim in no way suggests applicants' claimed particles or methods.

Thus it is urged that the references individually do not suggest applicants' claimed particles or method for the preparation thereof. Furthermore, applicants respectfully submit that the combined teachings of the references in no way suggest the claimed invention. As noted, both Violante and Oppenheim involve solvent precipitation techniques and particles prepared therefrom which are radically different than applicants' claimed method and particles. Accordingly, it is respectfully urged that

the teachings of Violante and Oppenheim are so far removed from the applicants' invention that even if it were proper to combine them with Motoyama, the combination would lack any suggestion of applicants' invention.

As for the propriety of combining them, the opinion of the Court of Appeals for the Federal Circuit in Uniroyal v. Rudkin-Wiley 5 USPQ 2d 1434 is relevant. As the court said, "Something in the prior art as a whole must suggest the desirability, and thus the obviousness, of making the combination." Nothing in the cited references or the art offers any suggestion that the teachings could be so modified and combined to yield anything resembling applicants' claimed particles or method. While Motoyama incidentally discloses a method more similar to the applicants' method than the methods of Oppenheim or Violante, there is no reason for one skilled in the art to expect that particles of the sizes described by Oppenheim or Violante could be prepared according to the teaching of Motoyama. Thus, even when combined, the teachings of the references in no way suggest the claimed invention.

Furthermore, there is no suggestion whatsoever in the cited references or in the art as a whole of the unexpected, advantageous properties associated with the claimed particles. The particles of the invention can be formulated into pharmaceutical compositions exhibiting unexpectedly increased bioavailability. It is noted that

applicants have shown 7, 16 and 37-fold improvements in bioavailability for pharmaceutical compositions containing particles (comprising respectively as the drug substance Steroid A, Danazol and WIN 63,394) of the invention compared to conventional formulations. As pointed out in the Rule 132 Declaration of Eugene Cooper, Ph.D., submitted herewith, such dramatic improvements in bioavailability are completely unexpected and commercially advantageous inasmuch as drug substances in the form of the particles of the invention can achieve the same therapeutic effect as substantially greater dosages of drug substances prepared by prior art techniques. Further, pharmaceutical compositions according to this invention have advantageously exhibited improved dose proportionality and decreased fed-fasted variability. Moreover, certain particles of the present invention have been found to be extraordinarily useful in x-ray contrast compositions. Still other particles of the present invention, e.g., particles comprising naproxen and indomethacin, have resulted in more rapid onset of action when orally administered compared to conventional naproxen and indomethacin formulations.

For all of the reasons set forth above it is respectfully urged that all of applicants' claims are patentable over the prior art. If it therefore requested that the rejection under § 103 be withdrawn.

In conclusion, applicants' claims distinctly point out and define the invention by virtue of amendments made herein. The invention is nonobvious over the prior art because the art does not suggest the invention or its unexpected, advantageous properties. In view of the foregoing amendment, remarks, and the attached Rule 132 Declarations, it is respectfully asserted that the Examiner's rejections cannot be sustained and should be withdrawn. It is respectfully urged that applicants' claims 1-8 and 10-21 are patentable and in condition for allowance. Notice of Allowance or early action to that end is earnestly solicited.

Respectfully submitted,

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Registration No. 24,352

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EXHIBIT G

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RECEIVED NOV 12 1992 GROUP 1800

#2 D. Gassaway 11-17-92

In re Application of
Elaine Liversidge et al

Serial No. : 07/908,125 : Group Art Unit: ~~2305~~

Filed: July 1, 1992 :

: Malvern, Pennsylvania 19355

Title: Surface Modifier :
Anticancer Nanoparticles :

CERTIFICATE UNDER 37 CFR 1.8 (a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents and Trademarks Washington, D.C. 20231, on 26 Oct. 1992
(Date of Deposit)

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

William Davis 30744
(Reg. No.)

William Davis 26 Oct 1992
(Date of Signature)

Sir:

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 CFR 1.97 - 1.99

For the convenience of the Patent Office, record is made below to published art for consideration by the Office in connection with the above-identified application. No representation is made or intended that a search has been made, or, if made, was complete, or that no more pertinent art than that listed is available. It is expected that the Patent Office will conduct independently a complete search for relevant prior art.

In compliance with MPEP Sec. 609 and 37 CFR 1.97 through 1.99, accompanying each citation is a discussion indicating and including reference to specifically identified portions (e.g., page and line) of the above-identified application, where appropriate. Unless otherwise indicated, a full text copy of the entire item of published art and, in the case of references not in English, a translation thereof is attached. Where specifically indicated, an equivalent

English language patent or publication is attached in lieu of a translation.

In accordance with the Patent Office Notice (998 OG 9) dated August 15, 1980, also enclosed is a completed form PTO-1449.

CITED ART

AA U.S. Patent 2,671,750 relates to an aqueous suspension of cortisone acetate and a method for the preparation thereof which comprises milling an aqueous vehicle and cortisone acetate. However, the aqueous vehicle contains a surface active agent, which functions to prevent individual particles from coalescing (column 4, lines 25-28), a suspending agent and a bacteriological preservative. Moreover, suspensions reported contain a substantial percentage of particles greater than 5 microns (5,000 nm) in size.

AB U.S. Patent 4,107,288 describes particles in the size range from 10 to 1,000 nm containing a biologically or pharmacodynamically active material. However, the particles comprise a cross-linked matrix of macromolecules having the active material supported on or incorporated into the matrix.

AC U.S. Patent No. 4,540,602 discloses that a solid drug can be pulverized in an aqueous solution of a water-soluble high

molecular substance, and that as a result of such wet grinding, the drug is formed into finely divided particles ranging from 0.5 µm or less to 5 µm in diameter. However, there is no suggestion that particles having an average particle size of less than about 400 nm can be obtained. Indeed, attempts on behalf of the applicants to reproduce the wet grinding procedures described in U.S. Patent No. 4,540,602 resulted in particles having an average particle size of much greater than 1 µm.

AD U.S. Patent No. 4,826,689 discloses a method of making uniformly sized non-crystalline amorphous particles from water-insoluble organic compounds wherein the organic compound is dissolved in an organic solvent. However, solvent precipitation techniques such as described in U.S. Patent No. 4,826,689 for preparing particles tend to provide solvent contaminated particles. Such solvents are often toxic and can be very difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels for diagnostic imaging. Additionally, amorphous materials and formulations tend to exhibit unacceptably poor stability and/or short shelf-lives.

AE U.S. Patent No. 4,851,421 describes an agricultural suspension containing a

biocidal fine powder and a specific adjuvant, but does not suggest applicants' surface modified anticancer nanoparticles.

AL EPO 411,629 (indicating a publication date of February 6, 1991, after the applicants' priority date) discloses a process for micronizing slightly soluble drugs by subjecting a mixture of the drug and a sugar or sugar alcohol to high speed stirring comminution or impact comminution. There is no suggestion of the applicants' claimed particles or method.

AM Japanese Patent Application 2,282,330 relates to a danazol composition containing danazol microcrystals prepared by solvent precipitation, a surfactant and optionally a carrier. There is no suggestion of applicants' claimed particles or method.

AN U.K. Patent Application 2,185,397 discloses a drug delivery system wherein particles of drug are directed away from the reticuloendothelial system by the use of a surface coating which prevents the take up of the composite particles.

AO U. K. Patent Application 2,200,048 discloses pharmaceutical colloidal hydrosols for injection. The hydrosols are formed by a solvent precipitation

technique (page 11, lines 8-14), and thus exhibit the problems, e.g., solvent contamination, associated with particles prepared by solvent precipitation techniques such as described in U.S. Patent 4,826,689. U.K. Patent Application 2,200,048 suggests that it is not possible to completely remove the solvent, noting that the suspension contains residual organic solvent after solvent removal in a rotary evaporator (page 11, lines 11-14). There is no suggestion of applicants' claimed particles or method.

AP

EPO 275,796 describes the production of colloidally dispersible systems comprising a substance in the form of spherical particles smaller than 500 nm. However, the method involves a precipitation effected by mixing a solution of the substance and a miscible non-solvent for the substance and results in the formation of non-crystalline nanoparticle. Furthermore, precipitation techniques for preparing particles tend to provide particles contaminated with solvents. Such solvents are often toxic and can be very difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels to be practical.

AR
AS

These publications provide a background on industrial milling and colloidal dispersions but do not suggest applicants' surface modified anticancer nanoparticles.

AT

See applications page 1.

Respectfully submitted,

William J. Davis

Date: 26 October 1992

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#6/a
10/5/93

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Liversidge et al	:	Group Art Unit: 1502
	:	
Title: SURFACE MODIFIED ANTICANCER NANOPARTICLES	:	Examiner: W. Benston, Jr.
	:	
Serial No.: 07/908,125	:	Malvern, Pa 19355
	:	
Filed: July 1, 1992	:	

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GROUP 1500

Honorable Commissioner of Patents and Trademarks
Washington, DC 20231

AMENDMENT

Sir:

In response to the Office Action mailed March 11, 1993, the period for response having been extended by 3 months to expire on September 11, 1993, by the petition and fee authorization enclosed herewith, please amend this application, without prejudice, as follows:

In the Claims

Please rewrite claim 1 as follows:

1. (Amended) Particles consisting essentially of 99.9-10% by weight of a crystalline anticancer agent having a solubility in water of less than 10 mg/ml, said anticancer agent having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an average effective particle size of less than about 1000 nm.

P 30133 10/06/93 07908125 19-4325 030 103 132.00CH

Please add claims 28-33 as follows:

12. The particles of claim 1 wherein said surface modifier is a surfactant.

13. The particles of claim 1 wherein said surface modifier is a nonionic surfactant.

14. The particles of claim 1 wherein said surface modifier is an anionic surfactant.

15. The particles of claim 1 wherein said surface modifier is selected from the group consisting of gelatin, casein, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, poloxomers, tyloxapol, poloxamines, dextran, a dioctyl ester of sodium sulfosuccinic acid, sodium lauryl sulfate, an alkyl aryl polyether sulfonate, a mixture of sucrose stearate and sucrose distearate, hexyldecyl trimethyl ammonium chloride, bovine serum albumin and $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$.

16. The particles of claim 1 wherein said surface modifier is present in an amount of 10 to 60% by weight based on the total weight of the dry particle.

23. The particles of claim 1 wherein said surface modifier is present in an amount of 10 to 30% by weight based on the total weight of the dry particle.

REMARKS

Claims 1-27 are pending in this application. Claims 7-15 and 17-23 have been withdrawn from consideration. Claims 28-33 have been added by this amendment. The present application is a continuation-in-part of U.S. Patent Application Ser. No. 647,105 filed January 25, 1991, now U.S. Patent No. 5,145,684. Favorable reconsideration of this application is respectfully requested in view of this amendment and the accompanying remarks.

Applicants' claims are directed to particles consisting essentially of a crystalline anticancer agent having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 1000 nm. Applicants' claims are further directed to anticancer compositions comprising such particles and to methods of treating mammals comprising administering the particles, whereby the efficacy of the anticancer agent is enhanced and/or the toxicity of the agent is reduced. The particles are prepared by wet grinding coarse crystals of the anticancer agent in the presence of fine grinding media and in conjunction with a surface modifier.

The rejection of claims 1-6, 16 and 24-27 under 35 U.S.C. § 103 as being unpatentable over King (U.S. Patent No. 5,124,338) in view of Devissaguet et al (U.S. Patent No. 5,049,322) is respectfully traversed. It is respectfully submitted that applicants' claims are patentable over these references taken individually or in combination.

King describes potentiating agents, i.e., specific acrivatine esters, which enhance the efficacy of antineoplastic agents. However, the pharmaceutical formulations of King are conventional, i.e., they are prepared by methods known in the

art (column 5, lines 55-58). Consequently, as noted in the Office Action, King does not suggest a particulate anticancer agent having an average effective particle size of less than 1000 nm.

Devissaguet et al describe a process for preparing nanocapsules. However, the nanocapsules described by Devissaguet et al are radically different than applicants' particles. The Devissaguet et al nanocapsules feature a wall of film-forming polymer (substance A) and a core (substance B), which can be a medicinal. On the other hand, applicants' claimed particles consist essentially of a crystalline anticancer agent and a non-crosslinked surface modifier adsorbed on the surface thereof. Moreover, the only medicinal containing nanocapsules described by Devissaguet et al are prepared by a precipitation technique, i.e., substance B is first dissolved in a solvent. For example, as illustrated in Example 2 therein, indomethecin is dissolved in acetone. Such solvent precipitation techniques provide non-crystalline, e.g., amorphous, cores which are contaminated with solvent. Such solvents are often toxic and can be very difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels to be practical. On the other hand, applicants' crystalline particles, prepared by wet grinding, a technique entirely different than the technique of Devissaguet et al, are essentially free of solvent contamination.

Thus, it is urged that the references individually do not suggest applicants' claimed particles. Furthermore, applicants' respectfully submit that the combined teachings of the references in no way suggest the claimed invention. Nothing in the cited references or the prior art as a whole offers any suggestion that the teachings could be so modified and combined to yield anything resembling applicants' claimed particles of a crystalline anticancer agent. Devissaguet et al disclose nanocapsules, radically different than applicants' crystalline particles, of a size embraced by the range specified in applicants' claims. However, there is no reason for one skilled

in the art to expect that the claimed crystalline particles could be prepared according to the teaching of Devissaguet et al. Nor does King, teaching conventional pharmaceutical formulation preparation techniques, remedy the deficiency in the teaching of Devissaguet et al. Even if the teaching of Devissaguet et al were applied to the anticancer agents listed by King, the resulting nanocapsules, as discussed above, would be radically different than the claimed crystalline particles.

Further, applicants' crystalline nanoparticles are obtained by a specific wet grinding technique which is not disclosed or suggested in the prior art. As stated in EPO 411,629 (cited in applicants' disclosure statement) "preparation of submicron particles of less than 1 µm in diameter is almost impossible". The reason for this is the inherent tendency of such particles to agglomerate, aggregate, solidify and/or adhere to the particle size decreases. Additionally, as discussed in the instant specification, applicants' attempts to reproduce the closest prior art wet grinding technique described by Motoyama et al in U.S. Patent No. 4,540,602 resulted in particles having an average particle size of much greater than 1 micron. Thus, the prior art as a whole does not disclose or suggest, and cannot be construed to disclose or suggest, applicants' claimed crystalline particles having an average effective particle size of less than 1000 nm.

Furthermore, there is no suggestion in the cited references or in the art as a whole of the unexpected advantageous properties associated with the claimed crystalline anticancer particles. The particles of this invention have been formulated into anticancer compositions which demonstrate enhanced efficacy and/or reduced toxicity and which can be administered by IV bolus injection. These advantages are illustrated in the working examples set forth on pages 13-22 of the instant specification.

To further evidence the nonobviousness of the claimed particles, applicants' proffer herewith the attached Rule 132 Declaration of Gary G. Liversidge, Ph.D., pointing out that the

above-noted differences between the particles of the claimed invention and particles prepared by prior art techniques, such as solvent precipitation techniques, are commercially significant.

For all the reasons set forth above, it is respectfully urged that all of the applicants' claims are patentable over the prior art. It is therefore requested that the rejection under § 103 be withdrawn.

Claim 1 has been amended consonant with the amendment to the claims in parent U.S. Patent No. 5,145,684 for reasons discussed fully in the parent relating to the § 112 rejection and prior art cited therein. More specifically, claim 1 has been amended to recite minimal and maximal amounts of the crystalline anticancer agent and surface modifier present, that the surface modifier is non-crosslinked, and that the anticancer agent has a specific, i.e., low, solubility in water. Ample support for such amendments can be found, e.g., on page 9, lines 1-5; page 7, lines 19-21; and page 3, lines 23-29 of the instant specification.

New claims 28-33 added by this amendment are directed to various embodiments of the surface modifier component of the particles of this invention. Ample support for such claims can be found, e.g., on page 5, line 32 - page 7, line 5 of the instant specification. The new claims are dependent from claim 1 and thus are patentable over the prior art for the reasons discussed above.

In conclusion, applicants' claimed invention is nonobvious over the prior art because the art does not suggest the invention or its unexpected, advantageous properties. In view of the foregoing amendment, remarks and the attached Rule 132 Declaration, it is respectfully asserted that the Examiner's rejection cannot be sustained and should be withdrawn. It is respectfully urged that claims 1-6, 16 and 24-33 are patentable and in condition for allowance. Notice of Allowance or early action to that end is earnestly solicited.

In the event that the Examiner believes that a telephone and/or personal interview would be helpful in resolving any remaining issues, it is requested that the Examiner telephone applicants' undersigned attorney.

Respectfully submitted,

William J Davis

William J. Davis
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CERTIFICATE UNDER 37 CFR 1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents and Trademarks Washington, D.C 20231, on September 10, 1993
(Date of Deposit)

William J Davis 30,744
(Reg. No.)

September 9, 1993
(Date Signature)

#1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

MAIL ROOM SEP 13 1993 PAT. & TRADEMARK OFF.

In re Application of	:	
Liversidge et al	:	Group Art Unit: 1502
	:	
Title: Surface Modified	:	Examiner: W. Benston, Jr.
Anticancer Nanoparticles	:	
	:	
Serial No.: 07/908,125	:	Malvern, PA 19355
	:	
Filed: July 1, 1992	:	

.. ..

Honorable Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

RULE 132 DECLARATION

I, Gary G. Liversidge, hereby say and declare that:

1) I am currently employed by Sterling Winthrop Inc. as Director of Oral Products. My primary responsibilities include managing research and development work associated with pharmaceutical sciences including drug delivery. I received a B. Pharm. degree in Pharmacy from the University of Bradford in 1978, and a Ph.D. degree in Pharmaceutical Chemistry from the University of Nottingham in 1981.

2) I am a coinventor of the subject matter disclosed and claimed in U.S. Patent Application Serial No. 908,125.

3) I have read and understood and I am quite familiar with the teaching and subject matter of U.S. Patent No. 5,049,322 of Devissaguet et al.

4) U.S. Patent No. 5,049,322 discloses a solvent precipitation technique for preparing nanocapsules. Solvent precipitation techniques ordinarily result in the formation of non-crystalline or amorphous particles. Particles prepared by solvent precipitation techniques provide particles which are contaminated with solvent. Such solvents are often pharmaceutically unacceptable and can be difficult, if not impossible, to adequately remove to pharmaceutically acceptable levels to be practical. This problem is noted in U.K. Patent Application 2,200,048 of record which suggest that it is not possible to completely remove solvent by conventional techniques.

5) The crystalline particles described in U.S. Patent Application Serial No. 908,125 are essentially free of solvent contamination. Such essentially solvent free particles prepared according to the method described in U.S. Patent Application Serial No. 908,125 and parent U.S. Patent No. 5,145,684 provide a significant commercial advantage compared to the solvent contaminated particles prepared according to the teaching of Devissaguet et al and other prior art solvent precipitation techniques such as those described in U.S. Patent No. 4,826,689 of record.

6) Example 7 in U.S. Patent No. 5,049,322 describes nanocapsules of a polymer containing an inorganic mineral solid, i.e., particulate silicon carbide. However, there is no exemplification of a particulate organic medicine. Moreover, I believe that the prior art does not teach one skilled in the art how to prepare crystalline particles of organic medicines, e.g., anticancer agents, in the size range specified in U.S. Patent No. 5,049,322.

7) Furthermore, laboratory work has demonstrated that particles prepared according to the teaching of U.S. Patent Application Serial No. 908,125 have exhibited unexpected

advantageous properties, e.g., with respect to toxicity and/or efficacy.

8) All statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and

9) These statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

9/8/93
Date

Gary G. Liversidge, Ph.D.

CERTIFICATE UNDER 37 CFR 1.8 (a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents and Trademarks Washington, D.C 20231, on September 10, 1993
(Date of Deposit)

William J Davis 30,744
(Reg. No.)

September 9, 1993
(Date Signature)

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
07/908,125	07/01/92	LIVERSIDGE G	PRF-328/92

EXAMINER
BENSTON, JR, W

15M1/1222

PATENT DEPARTMENT
STERLING WINTHROP, INC.
9 GREAT VALLEY PARKWAY
MALVERN, PENNSYLVANIA 19355

ART UNIT	PAPER NUMBER
1502	9

DATE MAILED: 12/22/93

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☒ Responsive to communication filed on 9/13/93 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire -3- month(s), -0- days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____

Part II SUMMARY OF ACTION

1. ☒ Claims 1-33 are pending in the application.
   Of the above, claims 7-15 AND 17-23 are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-6, 16 And 24-33 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed on _____, has been ☐ approved. ☐ disapproved (see explanation).
12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received
    ☐ been filed in parent application, serial no. _____; filed on _____
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

PTOL-326 (Rev. 9-89)

15.

Receipt of amendment "A" dated 9/13/93 is acknowledged.

16.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

17.

Claims 1-6, 16 and 24-33 would be allowable if rewritten or amended to overcome the rejection under 35 U.S.C. § 112.

18.

35 U.S.C. § 101 reads as follows:

> "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

19.

Claims 1-6 and 24-33 are rejected under 35 U.S.C. § 101 because the claimed inventive concept lacks a patentable utility in view of the use of the word "anti-cancer".

20.

Claims 1-6, 16 and 24-33 are rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is enabling only for claims limited to the following:

A) Concentration of the surface modifier can vary from about 0.1 to about 90% and preferably is 1-75%, more preferably 20-60% by weight. (Page 9, lines 1-6).

B) The preferred surface modifiers include polyvinylpyrrolidone, pluronic F108, polyvinyl alcohol and gum acacia. See M.P.E.P. §§ 706.03(n) and 706.03(z).

Claims 1-6, 16 and 24-33 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The phrase "anti-cancer" agent is vague and indefinite as said phrase implies/suggests that said "phrase is a cancer cure". The examiner suggests the words "medicament" or "drug" be considered as an alternative for use of "anti-cancer" in said composition claims. A correction is requested.

No claim is allowed.

21.

Any inquiry concerning this communication should be directed to William E. Benston, Jr. at telephone number (703) 308-2351.

W.E. Benston:jaw
December 19, 1993

THURMAN K. PAGE
SUPERVISORY PATENT EXAMINER

Gp 1502 #12/B 8/23

EK DN 62,937 PATENT



CERTIFICATE UNDER 37 CFR 1.8(a)

I hereby certify that this correspondence (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on: June 22, 1994
(Date of Deposit)

William J. Davis
Attorney/Agent for Applicant
William J. Davis 6/22/94
(Date)

RECEIVED JUL 18 1994 GROUP 150

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
G. Liversidge et al	:	Examiner: W. Benston, Jr.
	:	
Title: Surface Modified	:	Group Art Unit: 1502
Anticancer Nanoparticles	:	
	:	
Serial No.: 07/908,125	:	Malvern, Pa 19355
	:	
Filed: July 1, 1992	:	

Honorable Commissioner of Patents and Trademarks
Washington, DC 20231

AMENDMENT

Sir:

In response to the Office Action mailed December 22, 1993, the period for response having been extended by three months to expire on June 22, 1994 by virtue of the petition and fee authorization enclosed herewith, please amend this application, without prejudice, as follows:

IN THE CLAIMS

Please rewrite claim 1 as follows:

1. (Twice Amended) Particles consisting essentially of 99.9% by weight of a crystalline [anticancer agent] medicament useful in treating cancer susceptible to treatment with said medicament, said medicament having a solubility in water of less than 10 mg/ml, [said anticancer agent] and having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1-90% by weight and sufficient to maintain an average effective particle size of less than [about] 1000 nm, wherein said medicament is selected from the group consisting of alkylating agents selected from the group consisting of alkylating agents having a bis-(2-chloroethyl)-amine group, alkylating agents having a substituted aziridine group, alkyl sulfonates, and N-alkyl-N-nitrosoureas; antimetabolites; natural products selected from the group consisting of vinca alkaloids, epipophylotoxins, adriamycine, daunomycine, doctinomycine, daunorubicin, doxorubicin, mithramycin, bleomycin, mitomycin, enzymes, biological response modifiers, camptothecin, taxol and retinoids; hormones and antagonists; radiosensitizers; platinum coordination complexes; anthracenediones; and adrenocortical suppressants.

B1

In claim 4, line 2, please delete "0.1 to 90%" and insert therefor -- 1 to 75% --.

REMARKS

Claims 1-33 are pending in this application. Claims 7-15 and 17-23 have been withdrawn from consideration. Accordingly, claims 1-6, 16 and 24-33 are under consideration.

The indication in the Office Action that claims 1-6, 16 and 24-33 would be allowable if rewritten or amended to overcome the rejection under 35 U.S.C. §112 is acknowledged with appreciation. It is believed that Applicants' claims, as amended, overcome such rejection.

Claims 1-6 and 24-33 stand rejected under 35 U.S.C. §101 on the basis that the claimed invention lacks patentable utility in view of the use of the word "anticancer". Claims

1-6, 16 and 24-33 also stand rejected under 35 U.S.C. §112, second paragraph, on the basis that the phrase "anticancer" agent is vague and indefinite and implies/suggests a cancer cure. The Examiner suggests that the words "medicament" or "drug" be considered as an alternative for "anticancer". Responsive to the Examiner's suggestion, the claims have been amended to recite that the anticancer agent is a specific medicament useful in treating a cancer susceptible to treatment with such medicament. Support for this amendment can be found on pages 4 and 5 of the instant specification. It is noted that this invention can be practiced with poorly soluble crystalline anticancer agents known in the art to be useful in treating susceptible cancers. It is believed that the amended claims are distinct and definite. Further, the data in Applicants' working examples demonstrates the remarkable utility of this invention. Accordingly, withdrawal of the rejections under §101 and §112, second paragraph, is respectfully requested.

Claims 1-6, 16 and 24-33 stand rejected under 35 U.S.C. §112, first paragraph. The Office Action indicates that the disclosure is enabling only for claims limited to the following:

A) Concentration of the surface modifier can vary from about 0.1 to 90% and preferably is 1-75%, more preferably 20-60% by weight.

B) The preferred surface modifiers include polyvinylpyrrolidone, Pluronic F108, polyvinyl alcohol and gum acacia.

With respect to A, Applicants' claims specifically recite surface modifier concentration, i.e., 0.1 to 90% by weight. Claim 4 is directed to a preferred embodiment, i.e., wherein the surface modifier is present in an amount of 1-75%. With respect to B, Applicants' specification teaches that a wide variety of surface modifiers are useful. Indeed, Applicants have shown that the invention can be practiced with a wide variety of surface modifiers. In addition to the numerous surface modifiers exemplified in Applicants' specification and working examples, additional surface modifiers tested in varying amounts have been demonstrated to be useful. Moreover, Applicants' specification (page 10, line 24 - page

12, line 13) readily enables one skilled in the art to select surface modifiers for any poorly soluble medicament. The evidence demonstrates conclusively that the instant invention can be practiced with a wide variety of surface modifiers. Thus, one skilled in the art is readily able to practice the claimed invention, particularly in view of the teaching in Applicants' specification. Accordingly, withdrawal of the rejection under §112, first paragraph, is respectfully requested.

In the event that the Examiner believes that a telephone and/or personal interview would be helpful in resolving any remaining issues, the Examiner is respectfully invited to telephone Applicants' undersigned attorney.

In conclusion, Applicants' claims distinctly point out and define the invention. The invention demonstrates patentable utility. In view of the foregoing amendment and remarks, it is respectfully urged that claims 1-6, 16 and 24-33 are patentable and in condition for allowance. Notice of Allowance or early action to that end is earnestly solicited.

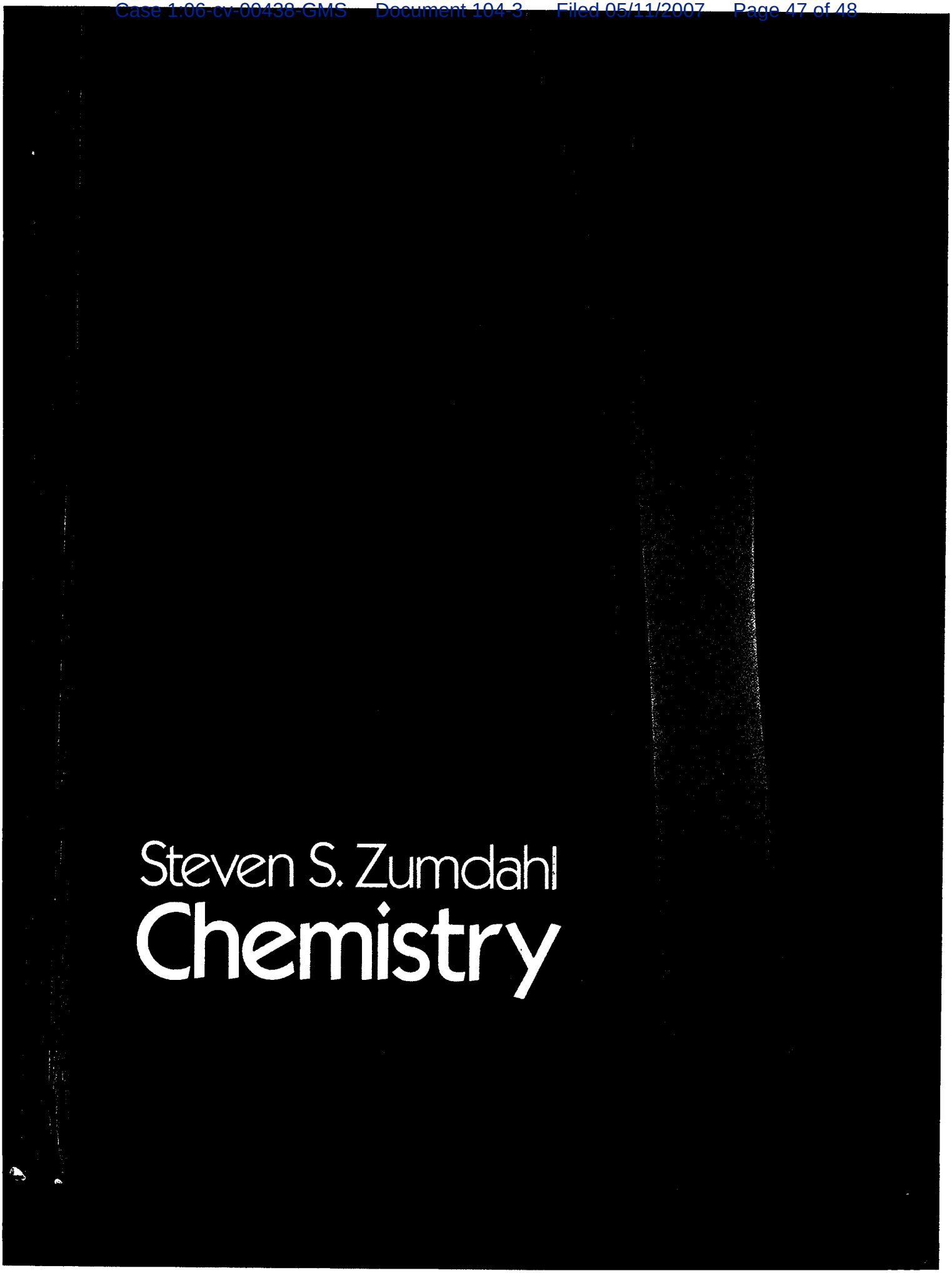
Respectfully submitted,

William J. Davis
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Date: June 22, 1994

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EXHIBIT H


Steven S. Zumdahl
Chemistry

GLOSSARY

Accuracy the agreement of a particular value with the true value. (1.3)

Acid a substance that produces hydrogen ions in solution; a proton donor. (4.2)

Acid-base indicator a substance that marks the end point of an acid-base titration by changing color. (15.4)

Acid rain a result of air pollution by sulfur dioxide. (5.9)

Acid dissociation constant (K_a) the equilibrium constant for a reaction in which a proton is removed from an acid by H_2O to form the conjugate base and H_3O^+. (14.1)

Acidic oxide a covalent oxide that dissolves in water to give an acidic solution. (14.10)

Actinide series a group of fourteen elements following actinium in the periodic table, in which the 5*f* orbitals are being filled. (7.11; 18.1)

Activated complex (transition state) the arrangement of atoms found at the top of the potential energy barrier as a reaction proceeds from reactants to products. (12.5)

Activation energy the threshold energy that must be overcome to produce a chemical reaction. (12.5)

Addition polymerization a type of polymerization in which the monomers simply add together to form the polymer, with no other products. (22.5)

Addition reaction a reaction in which atoms add to a carbon-carbon multiple bond. (22.2)

Adsorption the collection of one substance on the surface of another. (12.6)

Air pollution contamination of the atmosphere, mainly by the gaseous products of transportation and production of electricity. (5.9)

Alcohol an organic compound in which the hydroxyl group is a substituent on a hydrocarbon. (22.4)

Aldehyde an organic compound containing the carbonyl group bonded to at least one hydrogen atom. (22.4)

Alkali metal a Group 1A metal. (2.7; 18.2)

Alkaline earth metal a Group 2A metal. (2.7; 18.4)

Alkane a saturated hydrocarbon with the general formula C_nH_{2n+2}. (22.1)

Alkene an unsaturated hydrocarbon containing a carbon-carbon double bond. The general formula is C_nH_{2n}. (22.2)

Alkyne an unsaturated hydrocarbon containing a triple carbon-carbon bond. The general formula is C_nH_{2n-2}. (22.2)

Alloy a substance that contains a mixture of elements and has metallic properties. (10.4)

Alloy steel a form of steel containing carbon plus other metals such as chromium, cobalt, manganese, and molybdenum. (24.4)

Alpha (α) particle a helium nucleus. (21.1)

Alpha particle production a common mode of decay for radioactive nuclides in which the mass number changes. (21.1)

Amine an organic base derived from ammonia in which one or more of the hydrogen atoms are replaced by organic groups. (14.6; 22.4)

α-Amino acid an organic acid in which an amino group and an R group are attached to the carbon atom next to the carboxyl group. (23.1)

Amorphous solid a solid with considerable disorder in its structure. (10.3)

Ampere the unit of electrical current equal to one coulomb of charge per second. (17.7)

Amphoteric substance a substance that can behave either as an acid or as a base. (14.2)

Anion a negative ion. (2.6)

Anode the electrode in a galvanic cell at which oxidation occurs. (17.1)

Antibonding molecular orbital an orbital higher in energy than the atomic orbitals of which it is composed. (9.2)

Aromatic hydrocarbon one of a special class of cyclic unsaturated hydrocarbons, the simplest of which is benzene. (22.3)

Arrhenius concept a concept postulating that acids produce hydrogen ions in aqueous solution, while bases produce hydroxide ions. (14.1)

Arrhenius equation the equation representing the rate constant as $k = Ae^{-E_a/RT}$ where A represents the product of the collision frequency and the steric factor, and $e^{-E_a/RT}$ is the fraction of collisions with sufficient energy to produce a reaction. (12.5)

Aqueous solution a solution in which water is the dissolving medium or solvent. (4.0)

Atactic chain a polymer chain in which the substituent groups such as CH_3 are randomly distributed along the chain. (24.2)

Atmosphere the mixture of gases that surrounds the earth's surface. (5.9)

Atomic number the number of protons in the nucleus of an atom. (2.5; 21)

EXHIBIT I

International Journal of Pharmaceutics, 24 (1985) 1–17
Elsevier

IJP 00802

Research Papers

Evaluation of two concepts of crystallinity using calcium gluceptate as a model compound

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(Received June 22nd, 1984)
(Modified version received October 26th, 1984)
(Accepted November 9th, 1984)

Key words: calcium gluceptate – models of crystallinity – quantitation of crystallinity; X-ray, calorimetry, density – effect of grinding on crystallinity – effect of crystallinity on solubility – effect of dehydration on solubility

Summary

According to the USP, solids are either crystalline, non-crystalline (amorphous) or a mixture of the two. The degree of crystallinity depends on the fraction of crystalline material in the mixture (two-state model). An alternative concept is that the degree of crystallinity has a value between 0% (amorphous) and 100% (perfect crystal) depending on the state of disorder in the lattice (one-state model). On grinding dehydrated calcium gluceptate (II) for increasing times, there were marked increases in apparent water solubility, decreases in the intensity of X-ray diffraction peaks, and heats of solution changed from endothermic to exothermic. The results are attributed to decreases in crystallinity, since surface area measurements showed that they could not be due to particle size reduction. Density values obtained for II using a liquid suspension method changed progressively with grinding. Lack of separation into two fractions on dispersion in the suspending liquid suggests that the decrease in crystallinity was not due to a decrease in the proportion of crystalline material in a crystalline-amorphous mixture. It is concluded that grinding decreases the crystallinity of II by increasing lattice disorder according to the one-state model, and thereby increases the apparent water solubility.

Correspondence: A.G. Mitchell, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

0378-5173/85/$03.30 © 1985 Elsevier Science Publishers B.V. (Biomedical Division)

Introduction

It is becoming increasingly recognized that the degree of crystallinity has an important bearing on the properties of pharmaceutical solids. Many properties such as chemical stability, water uptake and loss, solubility, dissolution rate, mixing, flow and compaction depend on the degree of crystallinity (Huttenrauch, 1978; York, 1983).

There appear to be two concepts regarding degree of crystallinity, and these are illustrated schematically in Fig. 1. According to the USP, solids are either crystalline, non-crystalline (amorphous) or a mixture of the two states (USP XX, 1980a); the degree of crystallinity depends on the fraction of crystalline material in the mixture. This can be called the two-state model. An alternative concept is that the degree of crystallinity has a value located on a continuous scale which varies between 100% (perfect crystal) and 0% (non-crystalline or amorphous) depending on the state of order/disorder in the lattice (Huttenrauch, 1978). Since there is no sharp distinction between the crystalline and amorphous states, this model is referred to as a one-state model. It is recognized that these two models represent, in a highly simplified way, the complex transition from the crystalline to the amorphous state and that other models or combinations of models are possible.

Powder X-ray diffraction is the method most widely used to determine the degree of crystallinity of pharmaceuticals (Black and Lovering, 1977; Nakai et al., 1977, 1982). A major limitation is the possible effect of particle size and sample orientation on X-ray intensity. Moreover, the separation of amorphous scattering from the total diffraction pattern is at best arbitrary (Alexander, 1969). Provided the energy difference between the crystalline and amorphous states is large, heat of solution measurements are more precise and less subject to artefacts than powder X-ray diffraction methods (Pikal et al., 1978). However, neither of these methods can

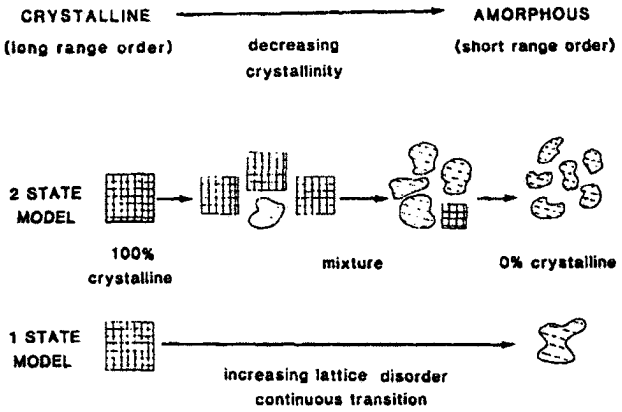


Fig. 1. Schematic representation of the two models of crystallinity. Squares represent the lattices of perfect crystals, while irregular shapes represent the amorphous state; no assumptions are implied regarding the structure of the latter.

distinguish between the two concepts of crystallinity, and in calculating the degree of crystallinity, the two-state model is implicitly assumed. Both X-ray line broadening and a decreased enthalpy of solution suggest a decrease in crystallinity but give no indication of the extent of lattice disorder of the individual particles in the sample under investigation.

The density of dislocations (line defects) provides some insight into the state of order of single crystals. Because of the localized energy associated with dislocations, two-dimensional nucleation occurs more rapidly at the site where a dislocation emerges on a crystal surface than elsewhere. Treating a cleaved surface with an etching solution reveals the dislocation sites as etch pits which can be seen and counted under a microscope (Burt and Mitchell, 1981; Friesen et al., 1981). This method has several limitations: (i) it is restricted to large well-formed crystals; (ii) only up to about 10^8 dislocations/cm^2 can be visually counted; and (iii) the method is restricted to quantitating dislocations; other types of crystal imperfections such as point defects are not included.

Density measurements can also provide an indication of the state of order of a solid. Although there are exceptions, crystalline materials in general have a higher density than their amorphous counterparts because the atoms in the crystal lattice are located at the minimum possible distance from each other. An increase in lattice disorder (decreasing crystallinity) will usually result in an increase in volume and therefore a decrease in density. Among the several techniques used to determine the density of solids, the suspension density method was selected because it is not only possible to differentiate between samples having very small differences in density (Johnston and Hutchison, 1940) but it may also provide a method of distinguishing between the two models of crystallinity. A liquid is chosen which has a density close to that of the solid and which neither reacts with or nor dissolves the solid. The solid is dispersed in the liquid and the temperature altered until the solid is suspended, at which temperature, the density of the solid is equal to that of the liquid. Since the temperature coefficient of expansion of a solid is generally much less than that of a liquid, the effect of temperature on the density of the solid is considered negligible (Estermann et al., 1949). If the simple two-state model is valid, then on dispersion in the suspending liquid, a partially crystalline sample would separate into two fractions as a result of the difference in density between the crystalline and amorphous states. On the other hand, if the one-state model is applicable, then progressive changes in crystallinity should be accompanied by gradual, progressive changes in density.

The suspension density method was used by Vaughan et al. (1958) to measure the decrease in density on compressing potassium chloride crystals, and by Huttenrauch (1978) to determine changes in the degree of crystallinity of microcrystalline cellulose and lactose on grinding, drying and compaction.

The objective of this work was to evaluate the two concepts of degree of crystallinity using calcium gluceptate as a model compound. Calcium gluceptate is official in the USP (1980b) as the calcium salt of D-glycero-D-guco-heptonic acid, which is the α-epimer of glucoheptonic acid. Calcium gluceptate was formerly available as an amorphous anhydrate which was very soluble in water, but the

solutions were unstable, and the crystalline hydrate precipitated on storage (Suryanarayanan and Mitchell, 1981, 1984). Synthesis of amorphous calcium gluceptate has been impossible in recent years [1], and we have found that commercial material from a number of sources is a crystalline hydrate containing 3.5 molecules of water of crystallization. The crystalline hydrate was used in this work to investigate the effects of dehydration and grinding on increased apparent water solubility and decreased crystallinity.

Materials and Methods

Materials

Acetonitrile (Caledon), carbon tetrachloride (BDH), ethylene dibromide (BDH), hydrochloric acid (Allied Chemicals), lithium fluoride (Fisher), methanol (Fisher), methylene chloride (Caledon) and tris(hydroxymethyl)aminomethane (Parr) were used as received. Calcium gluceptate (Pfanstiehl, lot 12953-D; amorphous anhydrate) has been described previously (Suryanarayanan and Mitchell, 1984). Calcium gluceptate, (I), (Pfanstiehl, lot 13313-E; crystalline hydrate) was used as received or was dried at 60°C under vacuum (pressure < 130 Pa) for 16 h (USP 1980b), to give the anhydrate (II).

Gas-liquid chromatography (GLC)

The USP monograph for calcium gluceptate specifies calcium α-glucoheptonate, but the analytical procedures described in the USP for the identification and assay of calcium gluceptate do not distinguish between the calcium salts of α- and β-glucoheptonic acids. Using a GLC technique, Suryanarayanan and Mitchell (1984) have shown that some commercial samples of calcium gluceptate contain mixtures of the α and β epimers. The material used in this work was calcium α-glucoheptonate, and thus complied with USP specifications. GLC analysis of II, and II ground for 4 h, showed no evidence of degradation on drying or grinding.

Thermogravimetric analysis (TGA)

The weight loss on heating I up to 140°C at $10°C \cdot min^{-1}$ in a thermogravimetric analyzer (Du Pont 900/950) was 11.3% w/w.

Grinding

Both I and II were ground in a mechanical agate mortar and pestle (Pulverisette 2, Fritsch) for varying times.

Apparent solubility

Unground and ground samples of I or II were added to about 0.5 ml of distilled water in a culture tube and dissolved by vigorous shaking at room temperature

[1] Dr. A.G. Holstein, Pfanstiehl Laboratories, Waukegan, IL, U.S.A.; personal communication, 1980.

(~ 22°C) using a vortex mixer. At the first sign of persistent turbidity, the addition of solid was stopped and the solution weighed. The water was evaporated off at 60°C under vacuum until the residual solid reached a constant weight.

Surface area

About 100 mg of each sample was accurately weighed into the sample cell of a surface area analyzer (Quantasorb Sorption System, Quantachrome). The specific surface area was determined by the multipoint BET method (Lowell, 1973) using 0.072, 0.104 and 0.184 mol% krypton (adsorbate) in helium (carrier).

Polarized light microscopy

The samples were mounted in mineral oil and examined by means of a polarizing microscope (Standard 14, Zeiss). The birefringence and extinction positions of II ground for varying times were compared with unground II and the commercial amorphous anhydrate.

Determination of degree of crystallinity

Powder X-ray diffraction

The samples were exposed to Ni filtered CuKα radiation (35 kV × 15 mA) at a scanning rate of 1° $2\theta \cdot \text{min}^{-1}$ in a wide angle X-ray diffractometer (Philips) with a xenon proportional counter. Degree of crystallinity determinations were carried out only on ground samples of II. Around a 2θ value of 20°, II intensely diffracts X-rays, and the peak at 20.1°, which exhibited the largest deviation from baseline, was chosen for crystallinity calculations (Fig. 2a). Crystalline and amorphous standards are required for quantitative estimates of percent crystallinity. Unground II was chosen as the crystalline standard (100% crystallinity). After grinding for 4 h,

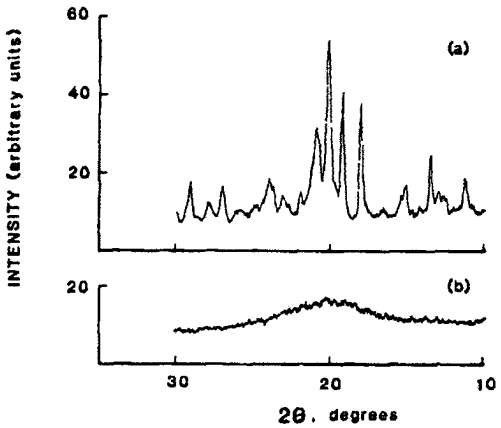


Fig. 2. Powder X-ray diffraction patterns of (a) II (100% crystalline standard), and (b) II ground for 4 h (0% crystalline standard).

II became amorphous to X-rays (Fig. 2b) and was used as the amorphous standard (0% crystallinity). Various proportions of the amorphous and crystalline standards were mixed to yield samples of known crystallinity from 0% to 100%. To an accurately weighed sample of each mixture, 11% w/w lithium fluoride was added as an internal standard, mixed well and the mixture redried at 60°C under vacuum to constant weight (Imaizumi et al., 1980). Lithium fluoride was chosen as the internal standard because the 2θ values at which it diffracts X-rays do not coincide with those of II. The ratio of the X-ray diffraction intensity of II at 20.1° 2θ to that of lithium fluoride at 45.0° 2θ was calculated for samples of varying crystallinity from 0 to 100%. This ratio was plotted as a function of percent crystallinity and used as a standard curve (Fig. 3). Fig. 4 is a representative X-ray diffraction pattern of a 20% crystalline sample prepared by mixing appropriate weights of the crystalline and amorphous reference standards.

To investigate the effect of grinding time on the degree of crystallinity, II was ground for varying periods of time. To an accurately weighed ground sample, 11% w/w lithium fluoride was added, the mixture was redried and the ratio of X-ray diffraction intensities determined as above. The degree of crystallinity at each grinding time was determined from the standard curve.

In an attempt to eliminate the effect of particle size on the intensity of diffracted peaks, the samples were initially sieved and a -100 $+250$ sieve fraction used for X-ray analysis. However, examination using a scanning electron microscope showed that after grinding, the powder consisted of aggregates of particles, and sieving was

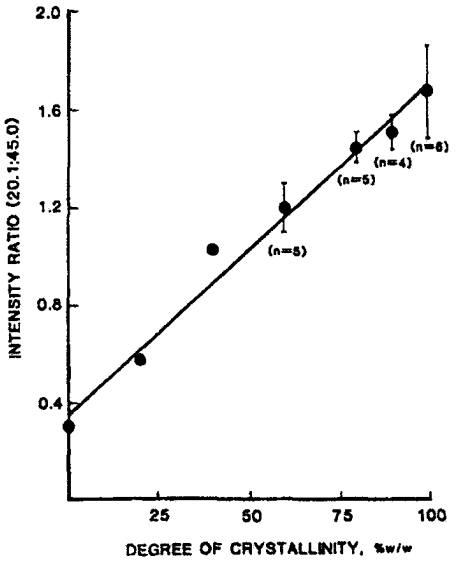


Fig. 3. Standard curve relating the degree of crystallinity of II and intensity ratio of the X-ray diffraction peak of II at 20.1° 2θ to that of lithium fluoride at 45.0° 2θ. Mean $\pm$ standard deviation are shown for selected values; other values are averages of two determinations.

discontinued. The intensity of the diffraction peaks was unaffected by the method of packing the powders into the sample holder, showing that the particles did not exhibit a preferred orientation.

Calculating the degree of crystallinity from an X-ray diffraction pattern usually involves measuring the total area under the curve and subtracting the contribution due to amorphous scattering. This method is arbitrary because of the inherent difficulty in separating the amorphous scattering from the crystalline diffraction. For example, Black and Lovering (1977) found that a digoxin sample judged to be crystalline by polarized-light microscopy was only 50% crystalline according to the above method of calculating crystallinity. They attributed the low value to overlap of crystalline peaks and proceeded to assume that the sample was 100% crystalline. The method of Imaizumi et al. (1980) successfully overcomes the problem by adding an internal standard and calculating intensity ratios rather than the areas under the curve. The effects of factors like background scattering need not be considered because the experimental crystallinity was determined from the standard curve (Fig. 3).

Solution calorimetry

Heats of solution were determined at room temperature (~ 22°C) using a solution calorimeter (Model 1451, Parr) with distilled water as the solvent. The energy equivalent of the calorimeter and its contents was determined by dissolving an accurately weighed amount of tris(hydroxymethyl)aminomethane in 100 g of 0.1 M hydrochloric acid and measuring the temperature change. The calorimeter was standardized as described previously (Suryanarayanan and Mitchell, 1984).

The crystalline and amorphous standards were mixed in various proportions to give samples of known percent crystallinity as before, and their heats of solution determined. A standard curve was plotted of the heat of solution as a function of percent crystallinity (Fig. 5). Samples of II ground for different times were redried at 60°C under vacuum to constant weight before their heat of solution values were measured and the percent crystallinities were determined from the standard curve. The final solution concentrations were normally less than 0.01 M.

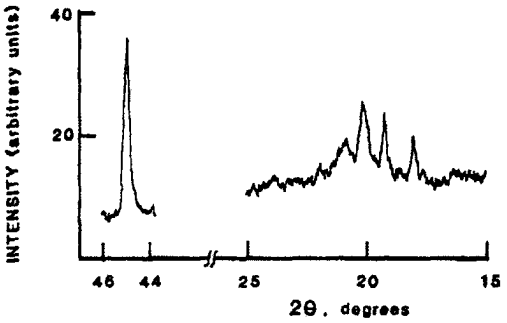


Fig. 4. Powder X-ray diffraction pattern of 20% crystalline II containing 11% w/w lithium fluoride as the internal standard.

Suspension density method

Carbon tetrachloride and ethylene dibromide were chosen as the suspending liquids because neither of them reacted with nor dissolved II. The density of carbon tetrachloride was lower than that of II, while that of ethylene dibromide was higher. The two liquids were mixed in varying proportions until a mixture was obtained with approximately the same density as II.

Density determinations were carried out on unground and ground samples of II. After grinding, the samples were dried at 60°C under vacuum to constant weight. Each sample was transferred to a Quantasorb sample cell, and a stream of nitrogen passed over it at room temperature for 4 h to dry the powder and remove surface impurities. The cell is automatically sealed on disconnection from the Quantasorb Sorption System so that the contents do not come in contact with the atmosphere. The cell was transferred to a 'dry chamber' (maintained at $< 1\%$ RH with phosphorus pentoxide). Here the solid was dispersed in the carbon tetrachloride–ethylene dibromide mixture contained in a borosilicate glass tube (Kimax, Owens-Illinois). The tube was closed tightly with a polytetrafluoroethylene-lined screwcap and transferred to a jacketed cell containing water (Fig. 6). Water was pumped from a thermostatically controlled water bath (Magniwhirl, Blue M Electric) through the double-wall of the cell and then through the outer jacket surrounding the oscillating tube of a digital density meter (Model DMA 45, Paar). The temperature of the water bath was altered until the dispersed sample was suspended. A sample of the pure liquid mixture was immediately injected into the oscillating tube of the density meter. After equilibration (density readings constant to ± 0.0001), the density of the suspension liquid gave the density of the suspended solid. Before using the suspension cell to determine the density of samples of II, the density meter was calibrated

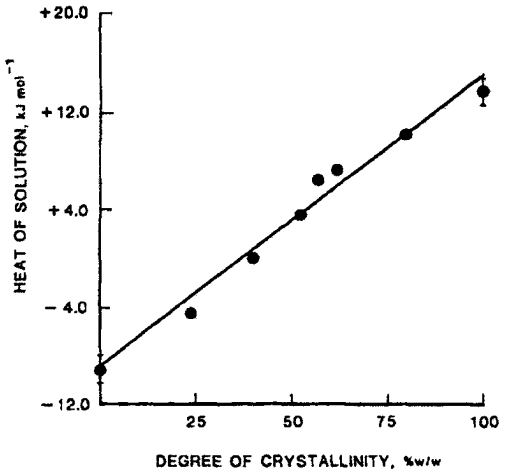


Fig. 5. Standard curve relating the degree of crystallinity and the heat of solution of II in water at room temperature (~ 22°C). The mean ± standard deviation of 100% and 0% crystalline standards (n = 4) are shown.

at 20°C with air and distilled water and its accuracy was checked by measuring the density of a number of liquids and comparing the experimental with the literature values. Experimentally determined density values (in $g \cdot cm^{-3}$) were: acetonitrile 0.7823 [0.7822 (Lange's Handbook of Chemistry, 1979)], methanol 0.7920 [0.7915 (Merck Index, 1983a)] and methylene chloride 1.3255 [1.3255 (Merck Index, 1983b)], which agree closely with the literature values given in parentheses.

The densities of the reference liquids were first determined with the flow of water in the direction shown in Fig. 6, then the direction of flow was reversed. The densities were independent of the direction of flow, showing that there was no significant difference in the temperature between the suspension cell and the density meter. The temperature of the water bath was accurately controlled to ±0.005°C by means of a proportional temperature controller (Model 76, YSI). Precise temperature control was necessary because a decrease in crystallinity from 100% to 0% caused a change in suspension temperature of only about 7°C. The density values of II ground for varying time periods were determined and the degree of crystallinity calculated from:

$$\% \text{ crystallinity} = \frac{\rho - \rho_a}{\rho_c - \rho_a} \times 100 \qquad (1)$$

where ρ = density of sample under investigation, ρ_a = density of amorphous standard (II ground for 4 h), and ρ_c = density of crystalline standard (unground II).

Results and Discussion

According to the U.S.P. (1980b), calcium gluceptate is an anhydrate or an hydrate containing 2 molecules or 3.5 molecules of water of crystallization. The weight loss of I determined by TGA (11.3% w/w) is close to the theoretical weight loss of 11.4% w/w expected for the 3.5 hydrate. I had a low aqueous solubility of 0.07 molal, but

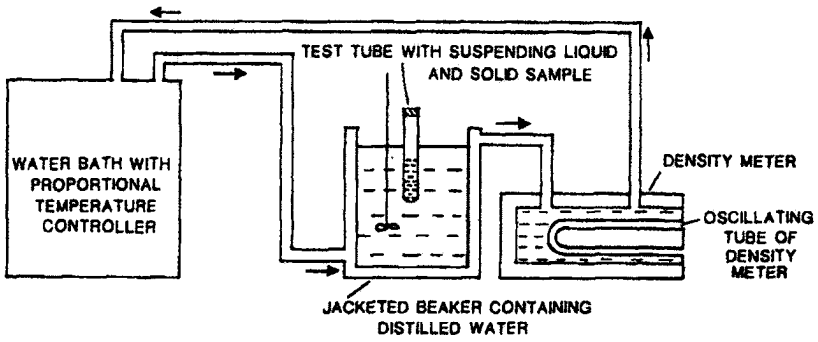


Fig. 6. Schematic diagram of apparatus for suspension density determinations. Arrows indicate the direction of water flow.

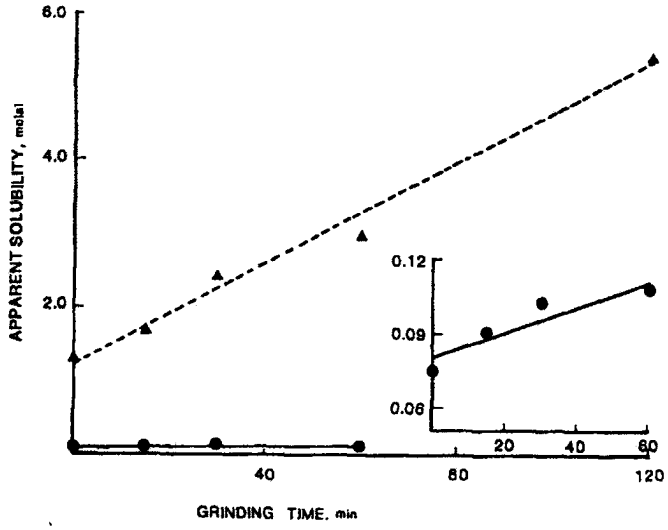


Fig. 7. Effect of grinding on the apparent solubility of I (●——●) and II (▲--▲) in water at room temperature (~ 22°C). Inset: solubility values of I plotted on an expanded scale.

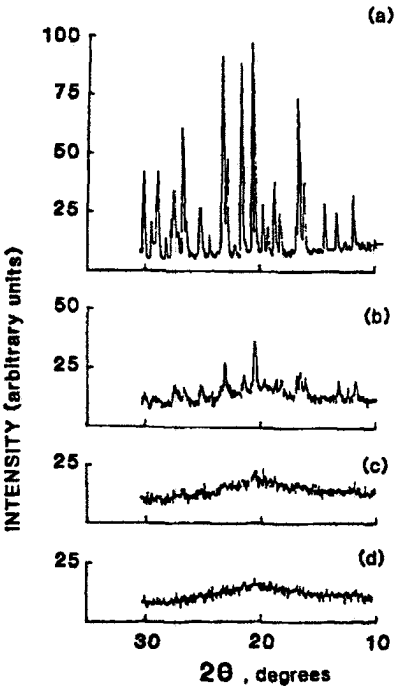


Fig. 8. Powder X-ray diffraction patterns of I (a) unground; (b), (c) and (d) ground for 15, 30 and 60 min, respectively.

TABLE 1

EFFECT OF GRINDING ON SOME PROPERTIES OF II

Grinding time (min)	Apparent solubility (molal)	Surface area ($m^2 \cdot g^{-1}$)	Density ($m^2 \cdot g^{-3}$)	Particle diameter (assuming spherical particles) (Å)	Heat of solution ($kJ \cdot mol^{-1}$)	% crystallinity by:		
						X-ray	heat of solution	density
0	1.29	5.45	1.6621	6620	+13.77	100 *	100 *	100 *
15	1.67	8.16	1.6720	4400	+5.91	72.4	61.8	68.3
30	2.41	8.05	1.6782	4380	−1.46	32.2	31.3	48.4
60	2.96	10.51	1.6822	3400	−6.09	24.8	12.2	35.6
240	ND	14.08	1.6933	2500	−9.13	0.0 *	0.0 *	0.0 *

* assumed.

ND = not determined.

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grinding for 1 h increased the apparent solubility to 0.11 molal (Fig. 7). There was a progressive decrease in the intensity of the X-ray diffraction peaks with increase in grinding time, suggesting a decrease in the crystallinity of the sample (Fig. 8). When I was dehydrated to II, the apparent solubility increased dramatically from 0.07 to 1.3 molal. Grinding II resulted in further increases in apparent solubility. Fig. 7 shows that the effect of grinding on the apparent solubility of II was much more marked than on I. The apparent solubility of II ground for longer than 2 h could not be determined because the solutions were extremely unstable and precipitated very rapidly. The precipitate had a powder X-ray diffraction pattern identical with that of I, and is therefore the crystalline 3.5 hydrate. The precipitate from solutions of ground II is also the same as that obtained previously from solutions of the commercial amorphous anhydrate (Suryanarayanan and Mitchell, 1984).

The crystallinity of II decreased with duration of grinding, but Table 1 shows poor agreement between the percent crystallinity values determined using X-ray diffraction, heat of solution and density methods. The degree of crystallinity determinations were confined to unground and ground II because of the significant effect of grinding on apparent water solubility. The relationship between degree of crystallinity and apparent solubility is shown in Fig. 9.

When examined by means of a polarizing microscope, the commercial amorphous anhydrate was non-birefringent compared with II, which consisted of highly birefringent needle crystals. The size of the needle crystals decreased with grinding time, but after about 30 min grinding, the particles started to form aggregates which were readily redispersed in the mineral oil mounting liquid. The deaggregated material retained its birefringent character for up to 2 h of grinding, but after 4 h grinding, many of the particles were non-birefringent (i.e. amorphous). Although the 4-h

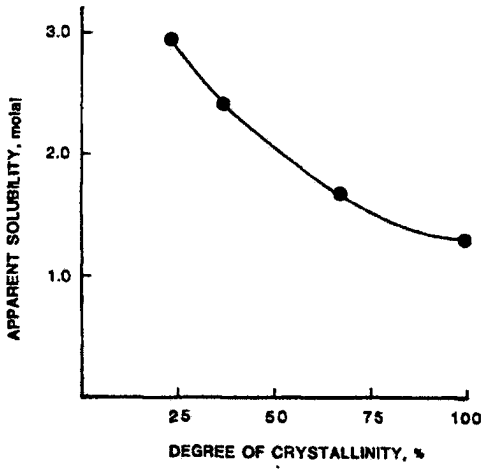


Fig. 9. Relationship between the degree of crystallinity and apparent solubility of II in water at room temperature (~ 22°C). The individual crystallinity values are averages of the values determined by X-ray, calorimetry and density for each grinding time (Table 1).

ground II was amorphous to X-rays, Fig. 2b, it is apparent that polarized light microscopy was a more sensitive indicator of crystallinity than X-ray diffraction, since some particles still showed birefringence and extinction positions. However, examination by means of the polarizing microscope gave no indication of the progressive decrease in crystallinity with grinding time.

In the pharmaceutical literature, the degree of crystallinity has usually been calculated on the assumption that the two-state model is applicable (Black and lovering, 1977; Pikal et al., 1978; Nakai et al., 1982). The X-ray crystallinity is often calculated according to the following relationship (Klug and Alexander, 1974):

$$\text{Percent crystallinity} = \frac{Ic}{Ic + Ia} \times 100 \tag{2}$$

where Ic and Ia are respectively the crystalline and amorphous intensities of diffracted X-rays. Thus the total energy of diffracted radiation from the crystalline and amorphous components may be considered as proportional to the quantity of crystalline and amorphous phases present, respectively (Clark and Terford, 1955).

Using the calorimetric method, percent crystallinity is given by:

$$\frac{\Delta H_s - \Delta H_a}{\Delta H_c - \Delta H_a} \times 100 \tag{3}$$

where ΔH_s, ΔH_c and ΔH_a are the heats of solution to infinite dilution in any fixed solvent, of the sample, the 100% crystalline standard and the 0% crystalline (amorphous) standard, respectively. In this work, percent crystallinity by both X-ray diffraction and calorimetry was determined from standard curves obtained by mixing various proportions of the crystalline and amorphous standards (Figs. 3 and 5). Eqns. 2 and 3 are based on a two-state model for polymers, according to which small but perfect crystalline regions (crystallites) are embedded within a continuous amorphous matrix (Miller, 1966a). However, even in polymers, such a model is recognized as a gross oversimplification (Miller, 1966b), and its relevance to most pharmaceuticals is questionable because of the differences between polymers and other crystalline materials.

Density measurements of ground II were undertaken both as an alternative method of measuring the degree of crystallinity and as a method of testing the models of crystallinity. Contrary to expectations, the results in Table 1 show that the density gradually increased with grinding time, suggesting that the crystals initially have an open lattice which gradually collapses under mechanical stress. According to the two-state model illustrated in Fig. 1, the decrease in crystallinity with grinding is due to a progressive conversion of crystalline material to the amorphous state. However, dispersion of ground II in the carbon tetrachloride–ethylene dibromide mixture did not result in separation into two fractions corresponding to the crystalline and amorphous states. Instead, there was a gradual and progressive change in the density of II with increasing grinding time, which suggests the one-state model. However, as discussed above, other models of crystallinity are

possible. For example, the surface of a crystal may become amorphous on grinding (Khodakov and Rebinder, 1961) and the thickness of the amorphous layer may increase with grinding time until the whole particle is amorphous. When dispersed in the suspending liquid, the solid would not separate into two fractions even though each particle contains both amorphous and crystalline phases. Hence, by itself, the suspension density method does not provide unequivocal evidence for a particular model.

Microscopical examination using polarized light showed that all particles were birefringent even after 2 h of grinding. If the surface amorphization model were correct, birefringency would be expected to disappear very quickly on grinding. It would be expected that the increasingly thick amorphous layer would cause the disappearance of birefringence after a few minutes of grinding. Hence, the formation of an amorphous layer surrounding a crystalline core does not describe the decreasing crystallinity of II as grinding continues. It is probable that grinding causes more disorder in the surface layers of a particle than in the bulk, and that the disorder progressively decreases towards the core of each particle. If the two-state model were correct, the majority of the particles should be non-birefringent after 1 h of grinding because the crystallinity value is less than 50% (Table 1).

Although the density of a solid is independent of particle size, it was important to verify that the X-ray line broadening and changes in the heat of solution values were not simply due to a decrease in particle size on grinding. From the surface area determined by krypton adsorption and the density, a hypothetical particle size was determined for each sample, assuming that all the particles were spherical in shape and of uniform size (Table 1). The effect of particle size on X-ray line breadth usually becomes apparent only when the crystal size is below 1000 Å (Cullity, 1978). Since, after 4 h grinding, the hypothetical particle diameter of II was about 2500 Å, it can be concluded that the X-ray line broadening is mainly a consequence of distortion of the crystal lattice rather than particle size reduction.

Brunauer et al. (1956a) observed that a 26-fold increase in surface area of calcium oxide (from 0.3 to 7.8 $m^2\ g^{-1}$) produced a decrease in total enthalpy of 0.56 $kJ \cdot mol^{-1}$ (from $-198.19\ kJ \cdot mol^{-1}$ to $-198.75\ kJ \cdot mol^{-1}$). Further work by Brunauer et al. (1956b, 1959) confirmed that very large increases in surface area produced only small changes in enthalpy. The results in Table 1 show that crystalline II with an endothermic heat of solution was rendered X-ray amorphous on grinding with an accompanying exothermic heat of solution, i.e. a very large enthalpy change for a small increase in surface area. Dialer and Kuessner (1973) observed a similar effect on milling crystalline sucrose, which resulted in its transformation into a glass-like material. The accompanying change in enthalpy could not be accounted for by the increase in surface area alone. According to Calvet and Prat (1963), crystal dissolution is preceded by the exothermic adsorption of solvent molecules on the solute surface, followed by an endothermic breakup of the crystal lattice. Amorphous solids are characterized by the absence of long range order in their crystal lattice. When they dissolve in a solvent, less energy is required to break up the lattice and the overall heat of solution generally becomes exothermic. Thus the change from the endothermic to exothermic heat of solution on grinding II

is attributed to a progressive change from an ordered lattice to a highly disordered lattice containing excess free energy.

The increase in apparent solubility of II on grinding is also attributed to the increase in lattice disorder. Because of the method by which the apparent solubility was determined, the experimental value will depend on the dissolution rate of II as well as equilibrium solubility of I. Burt and Mitchell (1981) showed that differences in dislocation density in potassium perchlorate crystals, induced by changes in crystal growth rate, caused a significant increase in the dissolution rate constant. Friesen et al. (1981) increased the number of dislocations from an initial value of $3.5 \times 10^3\ cm^{-2}$ up to approximately $2.5 \times 10^5\ cm^{-2}$ by mechanically stressing single crystals of potassium perchlorate, and produced a 40% increase in the dissolution rate constant. The much greater stress of grinding can be expected to introduce much larger numbers of dislocations and other defects into a crystal, and it is suggested that the resulting decrease in crystallinity is responsible for the increase in apparent solubility of II.

In addition to the creation of lattice disorder, grinding may increase apparent solubility both as a result of particle size reduction and by exposing more reactive crystal faces to the dissolution medium. The effect of particle size reduction on solubility can be calculated from the Ostwald-Freundlich equation (Florence and Attwood, 1982), but the 2.3-fold increase in apparent solubility of II after grinding for 60 min is far greater than could be accounted for by the 2.0-fold reduction in hypothetical particle size [2]. Since II occurred as a needle habit, the surfaces created on fracture may be more reactive than surfaces parallel to the needle axis. The effects of crystal anisotropy and habit modification in nickel sulfate hexahydrate crystals have been studied by Burt and Mitchell (1979, 1980), and although significant effects on dissolution rate constants were observed, the contribution of dissolution anisotropy towards the overall increase in apparent solubility found in this work is likely to be minimal compared with the effect due to increased disorder.

A major difficulty with calculating percent crystallinity is the selection of appropriate crystalline and amorphous standards. The perfect crystal does not exist, and the material used as the 100% crystalline standard (unground II) will contain numerous defects (reduced crystallinity) as a result of its preparation from I by dehydration. Similarly, II ground for 4 h, selected as the 0% crystalline standard because it was X-ray amorphous, does not represent a true amorphous state since some particles still showed birefringence when examined by means of polarized light microscopy [3]. Degree of crystallinity values obtained using one set of standards and a particular experimental method are not likely to agree with values obtained using

[2] Assuming arbitrary surface energy values ranging from $1 \times 10^{-6}\ J \cdot cm^{-2}$ to $5 \times 10^{-5}\ J \cdot cm^{-2}$ for II, the calculated solubility ratio at 25°C of the 1 h ground (hypothetical particle diameter 3400 Å) to unground sample (hypothetical particle diameter 6620 Å) would range from 1.01 : 1.00 to 1.41 : 1.00.

[3] Amorphous anhydrous calcium gluceptate (Pfanstiehl Lot 12953-D) could have been used as the 0% crystalline standard, but we had only a very small quantity of this material and it is no longer commercially available.

either other standards or another method, and Otsuka and Kaneniwa (1983) have shown that even using two X-ray diffraction methods resulted in different values for the crystallinity of cephalexin. Hence, too much importance should not be attached to the numerical values of percent crystallinities. Nevertheless, they provide a useful indication of the state of order of a solid and can be correlated with other properties of the solid state which are profoundly influenced by changes in the state of order.

Conclusions

(1) Dehydrating calcium gluceptate hydrate produced a dramatic increase in apparent water solubility.

(2) Grinding dehydrated calcium gluceptate caused further increases in apparent water solubility.

(3) Grinding reduced particle size and decreased the degree of crystallinity, but only the latter was important with respect to the increases in apparent solubility.

(4) The degree of crystallinity was assessed by X-ray, heat of solution and density measurements, but the values were not in good agreement.

(5) It is suggested that the decrease in crystallinity with grinding is due to an increase in lattice disorder throughout the entire sample (one-state model) rather than to a progressive increase in the proportion of amorphous material in a crystalline-amorphous mixture (two-state model).

Acknowledgements

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EXHIBIT J

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centres. Therefore it seems that D^+ is not an absolute requirement for photosynthetic activity but does play some role in optimizing metabolic activity.

The very important conclusion which can be drawn from this work, given that D^+ is a tyrosine radical at position 160 on the D2 polypeptide of *Synechocystis*, is that Z is almost certainly the symmetrically related tyrosine on the D1 polypeptide. By analogy with the structure of the L and M subunits, it seems that the tyrosines are probably located in the third membrane helices of the two reaction-centre polypeptides and are at distances from the membrane surface equivalent to that expected for P680, assuming histidines 198 on D1 and D2 form ligands with the chlorophylls which constitute this primary electron donor.

It is particularly surprising that the similarity of the environments of two tyrosines of D1 and D2 can give rise to dramatically different redox kinetics of Z and D. This, therefore, is another striking example of major functional differences between structurally symmetrical components within photosynthetic reaction centres that has been so clearly shown for bacteriochlorophyll and bacteriopheophytin of the purple bacterial system[7,8]. □

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Materials science

Strategies to defeat brittleness

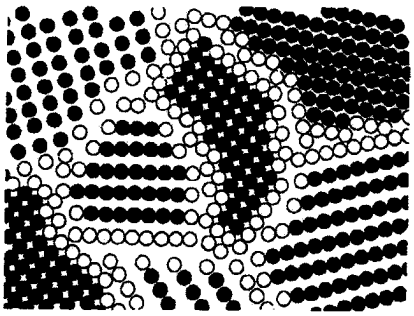
Robert W. Cahn

THE highest attainable strength is found in materials composed of light atoms linked into a network by strong ionic or covalent bonds — in other words, ceramics. These also have particularly high melting-points and so would be apt for engineering use at both ambient and high temperatures were it not for their universal brittleness, which is a synonym for the inability to deform plastically and thus to absorb mechanical or thermal shocks. No ultrabrittle material could ever be trusted, for instance, in an aero engine. Two recent papers[1,2] present radically new ways of overcoming brittleness in hard materials. Gleiter *et al.* show[1] that reducing the grain size of ceramics can allow them to flow plastically. And on page 139 of this issue[2], Brookes *et al.* show that even diamond, if pressurized in the right way, can deform without fracturing.

During the past 15 years particularly, attempts have been made to overcome the handicap of brittleness by improvements in processing and composition[3]. Macro-defect-free cement[4], chemically modified silicon nitrides[5] and transformation-toughened zirconia[6,7] represent successful improvements based on these two strategies. The last of these was originally proposed[8] with the provocative title "Ceramic steel?", because doped zirconia, like high-tensile steel, depends on a partial phase transformation to temper strength with shock resistance: an advancing crack triggers a local transformation which hinders further propagation of the crack.

None of these improvements has gone far enough. Jack[5] described with feeling the technological, economic and organizational problems that dimmed the promise of silicon nitride, which "has now been the ceramic of the decade for three decades". The ceramic engine, outside Japan at least, still seems a long way from practical realization. There is scope for radically new approaches.

One such approach, recently reported by Gleiter and co-workers in *Nature*[1], depends on the creation of what is virtually a new state of matter, intermediate between a polycrystal and a glass — the 'nanocrystal'. The structure of a nanocrystalline metal, made by evaporation or sputtering onto a cold substrate followed by compaction of the fine powder so produced, is shown schematically in the figure. The crystal grains are so small that almost half the atoms in the metal occupy intergranular sites; examination by X-ray diffraction of nanocrystalline gold shows[9] that the intergranular material is gas-like rather than glass-like — there is no short-range order. Mössbauer spectroscopy of nanocrystalline iron over a range of temperatures shows[10] that the intergranular matter has a lower Debye temperature (or elastic constant), and thus has a lower cohesion, than do the grains.



Structure of a nanocrystalline metal. Solid circles, atoms arranged in crystalline structures; open circles, loosely packed intergranular atoms that give the material its high self-diffusivity and plasticity. Under pressure, atoms on the compressed side of a grain rapidly self-diffuse through the intergranular circuit to the stretched side. (Courtesy of H. Gleiter.)

The solubility of hydrogen as a function of its concentration is a measure of the prevalence of microcavities, which are hydrogen traps; such solubility measurements on nanocrystalline palladium show[11] that the intergranular 'phase' is indeed full of cavity-traps. This in turn implies, as has been observed[11], that solute diffusion of small atoms along the intergranular short-circuits is extremely concentration-dependent and can be higher or lower than in the crystal. Self-diffusion, however, is not affected by traps and is spectacularly enhanced. Self-diffusion at 20–120 °C in nanocrystalline copper is enhanced by no less than 19 orders of magnitude compared with an ordinary polycrystal[12].

Gleiter and co-workers observe[1] easy plasticity, at 80–180 °C, of nanocrystalline TiO_2 (made by oxidation of nanocrystalline titanium) and CaF_2 with grain diameters of about 10 nm — smaller by a factor of about 1,000 than normal grains. This can be interpreted in terms of diffusion creep. In this process, the cations and anions diffuse from the compressed to the stretched side of each grain, round an intergranular circuit, thereby changing the shape of the grain. The authors estimate that the small grain size enhances the strain rate by a factor of 10^9, and the large self-diffusivity by a further factor of 1,000. In effect, these materials behave superplastically[13], although the superplastic strain rates that are shown to be feasible are very much higher than those normally encountered in the industrial superplastic forming of aluminium alloys.

Of course, a superplastic ceramic will be weak in a critical range of temperatures: but this can in principle be counteracted by heating the ceramic to make the grains grow slightly: the larger the grains, the higher the critical temperature range. As the authors point out, the core and surface of a ceramic block can be treated (perhaps by microwave heating) to have different grain sizes and plastic properties. The possibilities are intriguing.

Gleiter and colleagues found it necessary to deform their ceramics by compressing them in contact with a platen

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made of a soft metal, aluminium, backed by a harder metal; presumably, contact with a hard platen causes intragranular cracking before the diffusion creep can begin. This soft-plate technique is similar to that used in the very different study of Brookes *et al.* reported in this issue[2]. These authors achieve plastic deformation in a monocrystal of diamond, the hardest and most brittle of solids. Following earlier work with MgO and TiC, they reason that a conical indenter pressed on a smooth diamond surface under constant load will — so long as it is slightly softer than the diamond — slowly be blunted until the distributed contact stress has fallen sufficiently for deformation to cease. If the hardness of the indenter is chosen correctly, then the stress in the contact zone is too low to crack the diamond but high enough to deform it plastically. For this to be feasible, the temperature must be very high. An indenter of cubic boron nitride (second only to diamond in hardness) at 1,000 °C neatly does the trick. The diamond deforms by clearly visible multiple dislocation glide. This temperature is 500 °C lower than the hitherto-accepted transition from brittleness to slight ductility, as determined by three-point bending[14].

It would be interesting to know how the grain size of polycrystalline diamond affects its plastic deformability; indeed, it is conceivable that a sufficiently fine grain size could permit a measure of diffusion creep, not linked to the motion of dislocations. In view of the extensive recent work on the formation of microcrystalline-diamond surface coatings by vapour deposition (see ref. 15), such an investigation could be of great practical value. □

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Myth, science and art of Greek peonies

At an exhibition at the Natural History Museum, Cromwell Road, London SW7, UK until 17 April the peony is used to trace the development of scientific documentation and botanical illustration. Peonies grow wild on the mountains and hills of Greece (the example shown here is the species *Paeonia parnassica* from Mount Parnassos), and were used 2,000 years ago for medical purposes, being associated with the mythical physician Paeon. The exhibition contains twelve lithographs made from paintings by Niki Goulandris. The lithographer, Takis Katsoulis, used 12–16 zinc plates for each illustration, one for each colour variation. The lithographs were made by printing each plate on a stone or metal press and then superimposing by hand. □

Courtesy of Niki Goulandris

Developmental biology

Growth factors in amphibian cell differentiation

Hugh Woodland and Liz Jones

THE molecules that control development are being unveiled at an accelerating rate. What is more striking than anything else is that so many fall into a select band of molecular families. These families, identified by their first members, are growth factors and their receptors, oncogenes and DNA-binding proteins with either zinc fingers or homoeo domains. Very recent examples are *wingless*, a segmentation gene of the fruitfly *Drosophila*, related to *int-1*, a mouse oncogene activated by mammary tumour virus[1]; and *dorsal*, which is involved in formation of the fly's dorso-ventral axis, and has extensive homology with v-*rel*, an avian oncogene[2]. Growth factors have an even longer pedigree in this regard. For example, epidermal growth factor (EGF) relatives have a role in cell-fate switching in the nematode reproductive system (*lin-12*)[3] and between epidermis and nervous system in *Drosophila* (*notch*)[4].

Two papers, one by Kimelman and Kirschner[5] and the other by Weeks and Melton[6], published in a recent issue of *Cell*, suggest that growth factors or their relatives have a role in mesoderm formation and the establishment of the dorso-ventral axis in Amphibia.

Dorso-ventral polarity

When the amphibian egg is laid it has an obvious pigmented animal pole and a yolky vegetal pole (Fig. 1*a*). The animal cells develop into ectoderm and the vegetal cells into endoderm. These cells also interact in the blastula, the vegetal cells inducing neighbouring animal cells to become mesoderm. The animal and vegetal cells are the first types to appear and presumably their different properties are established by substances ('determinants') localized in the egg. Without polarization phenomena, triggered by the entry of the sperm, the embryo would develop into a tube of radially symmetrical ventral tissues.

The effect of the sperm is to cause a polarized contraction of the surface regions of the egg[7]. In turn, this somehow makes the vegetal cells on the dorsal side generate a dorsal mesoderm induction signal (B, Fig. 1*b*) rather than, or together with, the basal ventral signal (A, Fig 1*b*). The dorsal mesoderm subsequently o estrates many later developmental

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Assessment of disorder in crystalline solids

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Summary

In the processing of pharmaceutical solids, disruption or activation of the crystalline structure often leads to varying degrees of disorder through the formation of defects and amorphous regions. Since percent disorder of processed samples must be assessed quantitatively using premixed standard samples of highly crystalline and amorphous solids, in the past, a number of questions about the validity of such an approach have been raised. Using sucrose as a model solid, a comprehensive assessment of such disorder has been carried out on predetermined mixtures of crystalline and amorphous samples, as well as on crystalline samples mechanically milled for various periods of time. Particular emphasis was placed on determining low levels of disorder in highly crystalline samples, since most techniques can detect no less than about 10% disorder. With predetermined mixtures, measurements of X-ray powder diffraction, density and heats of crystallization revealed good linearity with the percent disorder and acceptable detectability down to about 10%, as expected. However, using water vapor sorption measurements under very carefully controlled conditions proved effective in being able to detect disorder as low as 1%. A comparison of these four methods for estimating the percent disorder of milled samples of sucrose gave very consistent results, once the underlying factors that make these techniques sensitive to the concentration of amorphous structure present were recognized and taken into account.

Introduction

In the processing of pharmaceutical solids (York, 1983), as in milling (Dialer and Küessner, 1973; Nakai et al., 1977; Hüttenrauch, 1978), heat drying (Hüttenrauch and Keiner, 1979), spray drying (Mumenthaler and Leuenberger, 1991) and lyophilization (Pikal et al., 1978), disruption or activation of the crystal structure often occurs, leading to various degrees of disorder in the form of crystal defects and/or amorphous regions. Such changes also have been reported to occur during the tablet compaction process (Down and McMullen, 1985; Hüttenrauch, 1988; Ahlneck and Alderborn, 1989), and they are very likely to occur during other pharmaceutical processes involving water and drying such as during wet granulation and polymer film coating. Low levels of

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disorder or disruption in a crystalline solid also can be brought about by the presence of small amounts of impurities or additives (Pikal and Grant, 1987).

The formation of disorder in a solid produces regions that are in a higher energy state than that of the crystal. This can result in more advantageous pharmaceutical properties, such as enhanced dissolution rate (Chiou and Kyle, 1979; Burt and Mitchell, 1981), as well as undesirable properties such as increased chemical instability (Pikal et al., 1978; Oberholzer and Brenner, 1979; Waltersson and Lundgren, 1985) and a potential for solid-state transformation to lower energy crystalline forms upon storage (Makower and Dye, 1956; Pikal et al., 1978; Kontny et al., 1987). Ahlneck and Zografi (1990) have demonstrated how water vapor taken up at relatively low overall levels, can concentrate in regions of disorder, thus producing significant effects on molecular mobility, which in turn, can result in enhanced physical and chemical instabilities.

In view of the significant effects that the state of disorder in crystalline solids can have on the properties of pharmaceutical solids, it would be important to be able to assess the extent of disorder in a solid quantitatively, down to very low levels. By far, the most widely used technique is X-ray powder diffraction (Klug and Alexander, 1974), where the characteristic peak intensities or integrated peak intensities are measured as the crystallline sample is mixed with various proportions of an amorphous sample (Hermans and Weidinger, 1948; Black and Lovering, 1977) to provide a calibration plot. Other techniques used in this way to assess the percent disorder in crystalline samples include: density (Suryanarayanan and Mitchell, 1985; Duncan-Hewitt and Grant, 1986), heat of solution (Pikal et al., 1978; Hendriksen, 1990), infrared spectroscopy (Black and Lovering, 1977), dissolution rate (Hendriksen, 1990) and water vapor absorption (Pikal et al., 1978). Once a calibration curve with the pure crystalline and amorphous samples and their known mixtures is established, similar measurements are then made on the sample of interest to assign the corresponding percent disorder.

In evaluating the various approaches used to assess disorder in crystalline solids, a number of questions or uncertainties arise. First, there is the question of what constitutes 100% crystallinity and 100% amorphous nature in a given sample. Second, most techniques reported so far rarely were able to discern percent disorder of a physically mixed system of less than about 10%. Third, in the few cases where more than one technique has been used with the same processed sample, very different estimates of percent disorder often have been determined (Pikal et al., 1978; Duncan-Hewitt and Grant, 1986). It has been suggested that part of this latter uncertainty may arise because of differences in what properties these techniques actually measure. X-ray powder diffraction and density, for example, reflect the 'average' degree of order directly detected by the measurement (throughout the bulk), while heats of solution or water vapor absorption are measurements of the higher state of energy associated with the disordered state. Thus, different techniques may be more sensitive to different characteristics of the solid. It also is possible that the exact nature of an amorphous region may be different depending on the method used to cause activation, and, therefore, various techniques could respond differently to such samples. It has been pointed out that the use of a physical mixture of amorphous and crystalline particles represents a 'two-state' model, where each particle is almost entirely crystalline or amorphous, whereas in processed samples, it is very likely that all or most of the solid particles are only partially crystalline or amorphous, in what is termed a 'one-state' model (Hüttenrauch et al., 1985).

In this study, we wish to examine these issues more closely, using a model substance, sucrose, capable of being prepared in highly anhydrous crystalline and amorphous forms, and processed by milling to various extents in a controlled manner. Four different techniques have been compared: X-ray powder diffraction, density from helium pycnometry, heats of crystallization from the amorphous state by DSC measurements and water vapor absorption. Particular attention has been given to establishing the extent of agreement or disagreement for the same samples, milled to different extents, using the four tech-

niques, and to probing the lower limits of detection and precision for each technique.

Experimental

Materials

Sucrose, analytical grade, 99 + % (Aldrich Chemical Co., Milwaukee, WI) and lithium fluoride, reagent grade (Aldrich Chemical Co.) were used as received. The salts chosen for preparation of saturated salt solutions used to control relative humidity were described previously (Oksanen and Zografi, 1990). Water used in these solutions and in any freeze-drying procedure was doubly distilled after treatment with a Millipore, Milli Q filtering system. Krypton, used in the surface area measurements, was obtained from Matheson Co. (Division of Searle Products U.S.A., Inc.) at greater than 99.999% purity.

Procedures

Sample preparation Amorphous samples of sucrose were obtained by first freezing a 10% sucrose solution in water at −45°C (T'_g of sucrose is −32°C). The solid formed was then evacuated for 48 h at this temperature in a freeze dryer (Dura-Stop DC, FTS Systems, Stone Ridge, NY), after which the lyophilization was continued by gradually increasing the temperature of this system. The excess water was removed to below 0.1% by further drying the cake at 60°C for at least 48 h. Samples taken to be 100% crystalline were milled in a high energy impact mill (Shatter Box, Spex Industries, Edison, NJ) for 5 s to reduce the particle size for X-ray powder diffraction measurements. The milled samples were then sieved and transferred to desiccators containing magnesium nitrate, at a relative humidity of 56% at 30°C (Nyqvist, 1983), where they were stored for 1 week. This relative humidity and storage time have been shown to result in complete recrystallization of any amorphous sucrose present (Makower and Dye, 1956). The crystalline samples were further dried at 90°C in a vacuum oven (Precision®, Precision Scientific, Chicago, IL) for 3 h and stored in desiccators containing phosphorus pentoxide (relative humidity ≈ 0%). Amorphous samples of sucrose were found to exhibit a glass transition temperature of 74°C by DSC measurements, in good agreement with a previously reported value (Korey, 1991).

Milled samples were prepared by placing approx. 10 g of dried sucrose in a high energy impact mill, described above, for various time periods up to 900 s. The milled samples were sieved and the portion passing through a 230 mesh screen ($d < 63\ \mu m$) was collected. Samples were stored over phosphorus pentoxide and later manipulations were carried out either in a glove box at a relative humidity close to 0% or in environments where the ambient relative humidities were less than 20% to prevent recrystallization of any amorphous material produced by milling.

X-ray powder diffraction X-ray powder diffraction procedures were carried out using an X-ray diffractometer (45 kV × 40 mA, Scintag, Scintag, Santa Clara, CA) in an atmosphere of below 20% relative humidity and at room temperature. Approx. 2 g of each sucrose sample was loaded into a sample holder with 2 mm thickness and scanned at a rate of 5° 2θ per min. No particle size effects on peak intensities and integrated peak intensities were observed with samples that had been sieved below the equivalent of 63 μm. For those systems where an internal standard was included, LiF, previously sieved to equivalent particle diameters of less than 63 μm, was used at a level of 11% w/w; its characteristic peak at 45° 2θ was shown not to interfere with the peaks of interest for sucrose.

Density measurement A helium pycnometer (Model MVP-1, Quantachrome Corp., Syosset, NY) was used to measure the true density of all samples. Each value reported is the mean of at least eight independent measurements. Measurements were carried out in an atmosphere of less than 10% relative humidity.

Differential scanning calorimetry Measurements of heat of crystallization were carried out on a differential scanning calorimeter (TCII/DSC 30, Mettler, Hightstown, NJ), using hermetically sealed aluminum pans, at a scanning rate of 10° 2θ per min. Manipulation of materials before

sealing the sample pans was carried out at less than 10% relative humidity.

Specific surface area measurement Specific surface area measurements were carried out with a Quantasorb surface area analyzer (Model IM0325, Quantachrome Co., Syosset, NY) using Krypton gas adsorption and applying the BET equation (Brunauer et al., 1938).

Water vapor sorption Water vapor sorption experiments were carried out at 30°C by gravimetric measurements of water uptake at relative humidities of 7.6, 11.3, 21.5 and 32.4%. No crystallization from any amorphous sample of sucrose could be detected, using X-ray powder diffraction, after exposure to relative humidities at or below 32.4% over the time period of any experiment. The samples with high levels of amorphous form were stored in desiccators containing different saturated salt solutions to give the desired relative humidities (Nyqvist, 1983). For samples with high degrees of crystallinity, hence low levels of disorder, measurements were made using a vacuum assembly ($< 10^{-4}$ Torr), containing a Cahn, C2000 electrobalance (Cahn Instruments, Cerritos, CA), as previously described (Kontny et al., 1987). The balance was connected to a computer (Model 386/SX, Gateways, ND) that used a Cahn instrument software program to collect and analyze the data.

Results and Discussion

Mixtures of crystalline and amorphous samples

X-ray powder diffraction Fig. 1 shows the X-ray powder diffraction pattern for samples of sucrose used in this study taken to be completely amorphous and crystalline. The X-ray pattern of crystalline sucrose was compared to and confirmed using data from the Joint Commission for Powder Diffraction Standards, Monograph no. 74 (1977). Comparison of the peak intensities and

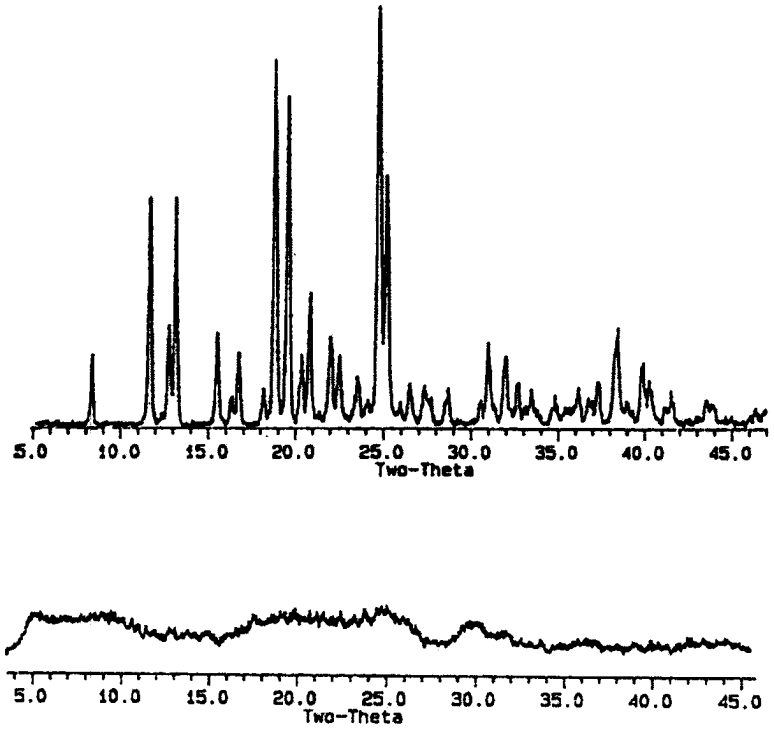


Fig. 1. X-ray powder diffraction patterns for sucrose: (top) crystalline form; (bottom) amorphous form.

the area under the largest crystalline peaks for mixtures of amorphous and crystalline samples, calculated directly from the data generated using the computer software program available (Pad V, Scintag, Scintag, CA), showed a linear relationship as a function of percent disorder. In all cases, with mixtures, the diffraction pattern was corrected point-by-point for scattering of the amorphous portion using the Pad V, Scintag computer program indicated above. The integrated peak intensity values showed better reproducibility and lower standard deviations than the peak intensities, thus only these values are reported in Fig. 2. Various peaks at different angles showed good agreement, so only the results for the crystalline peak of sucrose at 18.8° 2θ (one of the most intense peaks of sucrose) are reported. Alternatively, an internal standard (lithium fluoride was chosen since its characteristic peak does not interfere with the sucrose crystalline peaks) was added to each sample. The ratio of integrated peak intensity for the crystalline peak of sucrose at 18.8° 2θ to that of lithium fluoride at 45° 2θ was measured and plotted as a function of percent disorder as shown in Fig. 2. For sucrose, integration was carried out over a range of 18.3° 2θ to 19.0° 2θ, while for LiF the range was 44.7° 2θ to 45.2° 2θ. The angular range of integration

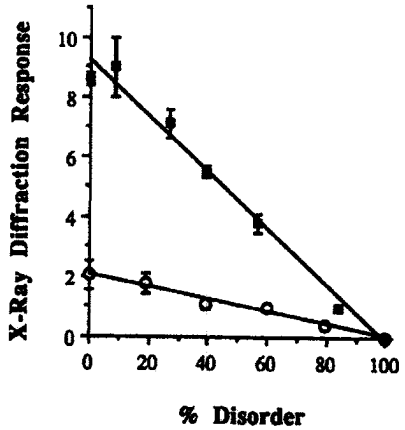


Fig. 2. X-ray powder diffraction responses vs percent disorder for mixtures of amorphous and crystalline sucrose: (■) integrated peak intensity×10^4 at 18.8°; (○) integrated peak ratio, sucrose at 18.8° and LiF at 45° as an internal standard.

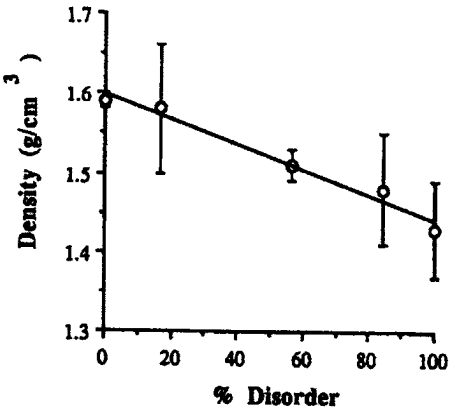


Fig. 3. Density vs percent disorder for mixtures of amorphous and crystalline sucrose.

did not change significantly as a function of percent disorder. Background subtraction for all samples was performed using the Pad V, Scintag software program. The standard curves obtained from both measurements with three independent experiments resulted in linear plots with correlation coefficients of better than 0.99. From these plots, it is apparent that X-ray powder diffraction measurements show lower standard deviations for samples with higher percentages of amorphous content; as the percentage of amorphous material decreases the sensitivity of this technique is reduced, so that one cannot distinguish between the sample that is 90% crystalline and that which is 100% crystalline for this system.

Density Fig. 3 shows the standard curve as prepared from density measurements with various mixtures. As the concentration of percent amorphous solid increases there is a decrease in density that is linear with concentration. However, due to the small change that occurs in density and the large standard deviations associated with such measurements, this technique clearly is less capable of detecting low levels of disorder than X-ray powder diffraction.

Heat of crystallization Fig. 4 shows a DSC scan of amorphous sucrose, where a distinct exotherm due to crystallization occurs above its glass transition temperature and below its melting point endotherm. The exotherm, with a peak temperature of 130°C, gave a heat of crystalliza-

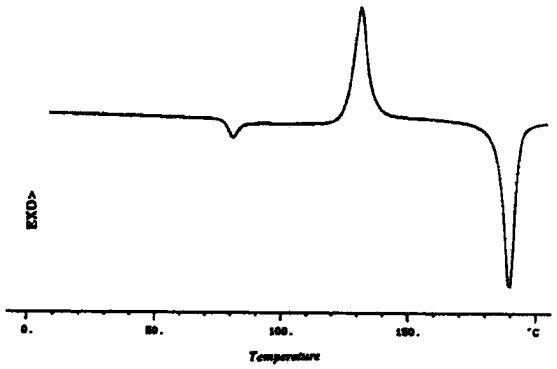


Fig. 4. Typical differential scanning calorimetry profile for sucrose showing glass transition endotherm, crystallization exotherm and fusion endotherm.

tion that was very close, but opposite in sign, to the heat of fusion, indicating that essentially complete crystallization had occurred. The heat of crystallization values for mixtures of amorphous and crystalline sucrose were calculated by estimating the area under the exothermic peak, observed to be reduced to 115°C for all mixtures. Fig. 5 shows the standard curve prepared from these values as a function of percent amorphous solid present. This technique, carried out on three independent samples, appears to be able to estimate the degree of disorder with an overall sensi-

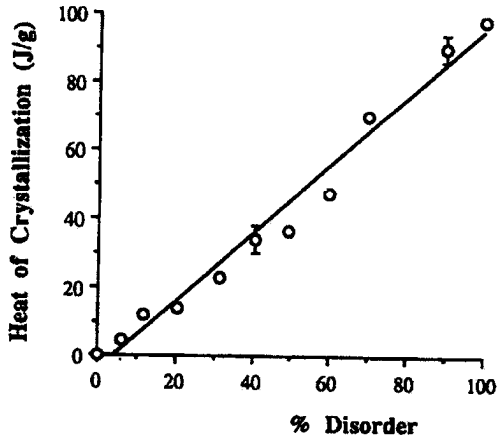


Fig. 5. Heat of crystallization vs percent disorder for mixtures of amorphous and crystalline sucrose. (Error bars represent one standard deviation for all data.)

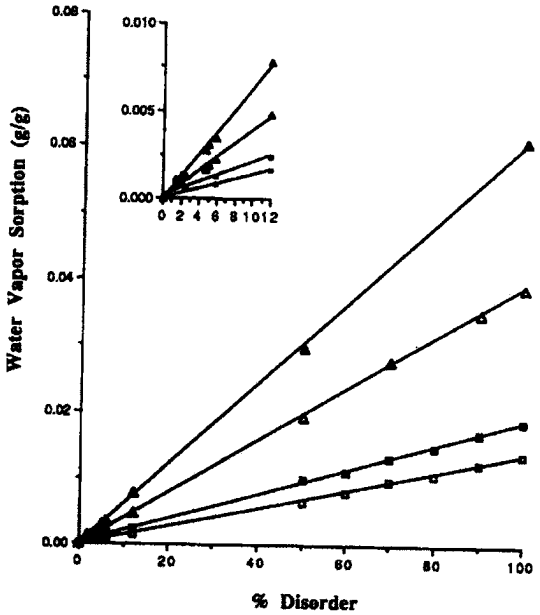


Fig. 6. Water vapor absorption at 30°C and various relative humidities vs percent disorder for mixtures of amorphous and crystalline sucrose. Inset focuses on 0–12% disorder. Relative humidities: (□) 7.6%; (■) 11.3%; (△) 21.5%; (▲) 32.4%.

tivity of ±5%. However, for highly crystalline samples, as the concentration of amorphous solid decreases below 10%, this method is not able to differentiate the low levels of disorder.

Water vapor sorption The equilibrium water vapor sorption of various samples was measured at relative humidities of 7.6, 11.3, 21.5 and 32.4%, as described above. Fig. 6 depicts the water vapor absorption vs percent amorphous sample for all four relative humidities at 30°C, showing excellent linearity over the entire range of percentages for the average of three independent measurements. The inset included shows that this is still true over the region from 0 to 12% amorphous material. Values of water vapor absorption for the completely amorphous sucrose sample at each relative humidity were in excellent agreement with those in the literature (Makower and Dyer, 1956). Measurements of water adsorption with the 100% crystalline sample indicated that such adsorption was negligible when compared to the amounts of water taken up for the amorphous

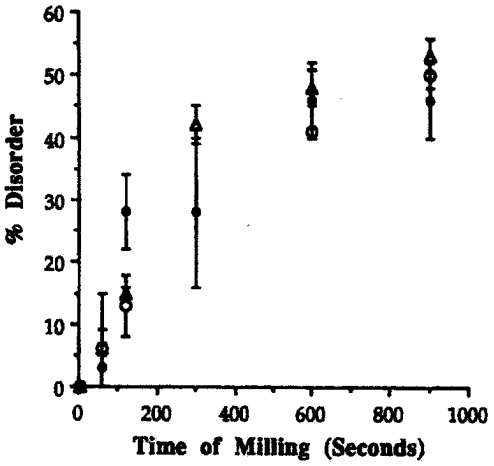


Fig. 7. Percent disorder of milled samples vs milling time estimated by: integrated X-ray peak intensity (○); integrated X-ray peak intensity ratio with LiF (△) and density (●).

samples, and thus for purposes of calculation, it was assumed that the water vapor sorption was exclusively due to the amount of amorphous solid present. From the specific surface area of the amorphous sample, estimated to be 0.71 ± 0.01 m^2/g, and the overall amounts of water vapor taken up at the various relative humidities, we also can conclude that the fraction of water adsorbed on the surface is negligible compared to that taken up into the amorphous structure. As seen in Fig. 6, excellent linearity was observed over the entire range of percent amorphous mass. Furthermore, this technique is much more sensitive than the other techniques in detecting the concentration of amorphous solid at very low values, i.e., $\approx 1.0\%$, with a higher degree of accuracy ($\pm 0.5\%$).

Percent disorder in milled samples of sucrose

X-ray powder diffraction and density Fig. 7 presents the percent disorder for samples of crystalline sucrose milled for various time periods, estimated from X-ray powder diffraction, with and without an internal standard present, and from density measurements, using the standard curves in Figs 2 and 3. As might be expected, essentially the same results were obtained by using corresponding values for the '100%' crystalline and amorphous samples in the following equation;

% disorder

$$= 100 - [(X - X_a)/(X_c - X_a)] \times 100 \qquad (1)$$

where X is the area under the curve or density value measured for the sample, and X_a and X_c denote the same values for completely amorphous and crystalline samples, respectively. From Fig. 7, it can be seen that the estimates of disorder for milled samples show good agreement for these different methods, particularly at longer milling times. However, as expected, very large standard deviations are associated with the estimates made from density measurements due to the small difference in the density values of the crystalline and amorphous sucrose samples. The results of three independent milling experiments for X-ray powder diffraction and milling measurements showed good reproducibility of percent disorder estimates at the longer milling times, but significant error at shorter milling times. It is believed that the larger error associated with shorter milling times is due to the variation in the actual milling operation over short time periods, some variation in particle size distribution produced with such short milling time, and lower levels of accuracy in the X-ray measurements in detecting the lower amounts of disorder.

Heat of crystallization

In order to translate the results of measuring the heats of crystallization of crystalline and amorphous sucrose mixtures to those of milled samples, it is necessary to take into account the fact that the temperature at which crystallization occurred varied with these two samples; the temperature of crystallization for mixtures of amorphous and crystalline samples occurred at 115 ± 5°C, whereas with the milled samples the heat of crystallization was measured at 74 ± 3°C which is the T_g for amorphous sucrose. As shown schematically in Fig. 8, the heat of crystallization from the amorphous state would be expected to increase as a function of temperature. Thus, proper corrections for the effect of temperature should be made before the heat of crystallization

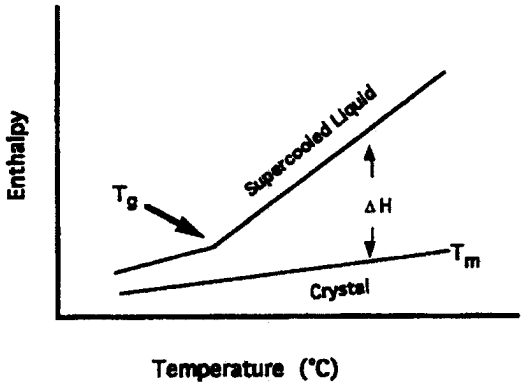


Fig. 8. Schematic representation of enthalpy vs temperature for crystalline and amorphous forms of a solid.

values measured at two different temperatures are compared on an equivalent basis for estimating the percent disorder. In 1958, Hoffman published an equation, describing the free energy change, ΔG, for crystallization at temperatures other than the melting temperature. This equation is expressed as:

$$\Delta G = (\Delta H_f \Delta T / T_m)(T / T_m) \quad (2)$$

where ΔH_f is the heat of fusion at the melting point, ΔT represents the difference between the crystallization temperature and the melting temperature, T_m is the melting temperature and T denotes the crystallization temperature. In the derivation of this equation, it was assumed that crystallization ideally occurs from the liquid state at the melting point. However, if one assumes that an amorphous solid in a supercooled liquid state behaves similarly to the liquid state, by analogy the change in the driving force of crystallization for a partially crystalline sucrose sample at a lower temperature may be expressed as follows:

$$\Delta G = (\Delta H_c \Delta T / T_c)(T / T_c) \quad (3)$$

where T_c is the crystallization temperature for mixtures of amorphous and crystalline sucrose prepared from a standard curve and ΔT denotes the temperature difference between T_c and T, the crystallization temperature for milled sucrose samples. Consequently, ΔH_f and T_m are replaced by ΔH_c and T_c, respectively. From the Gibbs-Helmholtz equation, one may express the Gibbs free energy ΔG as:

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

where ΔH is the enthalpy of crystallization at a temperature T and ΔS represents the entropy contribution due to the crystallization process. Assuming the entropy change over the small temperature range of study to be very small (Hoffman, 1958), the free energy change for the crystallization process may be assumed in such cases to be dependent primarily on the contribution from the temperature dependence of enthalpy values. Eqn 3 then, may be expressed as:

$$\Delta H = (\Delta H_c \Delta T / T_c)(T / T_c) \quad (5)$$

In the present study, this equation was utilized to correct the measured heats of crystallization for milled sucrose samples at 74°C, ΔH, to that at the higher temperature of 115°C, observed with mixed samples, ΔH_c, in the manner described with the following example. It was assumed that the entropy change, proportional to $\ln(T_2/T_1)$, was negligible over the temperature range of 388–347 K used in this analysis. For a sample milled at 900 s, the ΔH was measured to be 4.65 J/g, where ΔT is 41, T_c is 388 and T is 347. From Eqn 5, therefore, ΔH_c is estimated to be 49.2 J/g, and from the standard curve the sample is estimated to be about 52% amorphous. As shown in Fig. 9, the estimates of disorder based on the heat of crystallization values for three independent samples of milled sucrose are in very good agreement with the results obtained from X-ray powder diffraction measurements.

Water vapor sorption Fig. 9 also presents the estimated degree of disorder for sucrose samples for various time periods using data obtained from water vapor sorption at 7.6 and 32.4% relative humidity and 30°C, and from the calibration curves for predetermined mixtures obtained at these relative humidities. The relative humidities of 7.6 and 32.4% were chosen to compare the accuracy possible using the highest and lowest

possible relative humidities, and hence the highest and lowest possible amount of water sorbed. The percent disorder values estimated at these two relative humidities for three independent samples were in excellent agreement and, hence, the average obtained is given in Fig. 9. Two important observations may be made first for shorter milling times and then for the longer milling times. At shorter milling times, i.e., up to about 1 min, no statistical difference could be observed with other techniques. However, not shown on this plot was a comparison made between the sample of crystalline sucrose milled for only 5 s and one where this sample was equilibrated at 56% relative humidity, where any amorphous material formed would completely recrystallize (the '100%' crystalline sample in this study). Here, it was possible to observe that at 7.6% relative humidity, the sample milled for 5 s picked up $0.01 \pm 0.001\%$ water, equivalent to about 1% disorder, while no measurable water uptake was detected for the sample first equilibrated to 56% relative humidity to promote complete crystallization. The same percent disorder was estimated when measurements at 32.4% relative humidity were carried out with the 5-s milled sample. Thus, the water sorption technique clearly is superior as a probe of low levels of disorder in such samples. As seen in Fig. 9, however, the use of water vapor sorption for sucrose samples milled for longer than about 200 s resulted in significant differences with the other techniques, wherein estimates of disorder based on water uptake values in this region were significantly lower. The most likely explanation for such low levels of water uptake by samples milled at longer time periods would be the inaccessibility of the water vapor to a large proportion of the sample. Such inaccessibility might arise because of surface crystallization that could occur during milling (Hüttenrauch, 1978) due to the resulting increased temperature and mechanical force. Indeed, as shown in Fig. 10, sucrose samples milled up to approx. 2 min had their specific surface areas increased to a maximum value, while samples milled for longer time intervals showed a significant reduction in their specific surface area values. Agglomeration, which could cause such a decrease in specific surface area, was physically observed for these samples. Thus, it would appear most likely that enough surface crystallization of sucrose occurred during such periods of milling to significantly prevent water accessibility to the remaining amorphous portions. Although this might be seen as a disadvantage in using water vapor sorption for detecting disorder under such conditions, these experiments also illustrate the sensitivity of water vapor sorption as a diagnostic tool in detecting the formation of crystallinity on the sur-

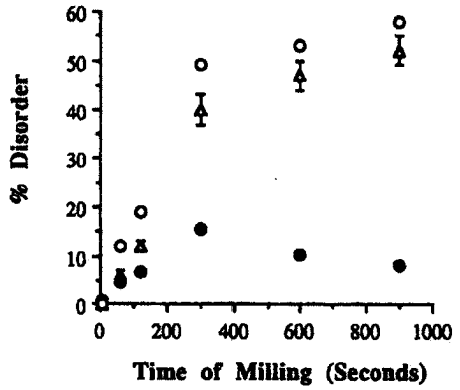


Fig. 9. Percent disorder of milled samples vs milling time estimated by: X-ray powder diffraction (△); heat of crystallization (○) and water vapor absorption (●).

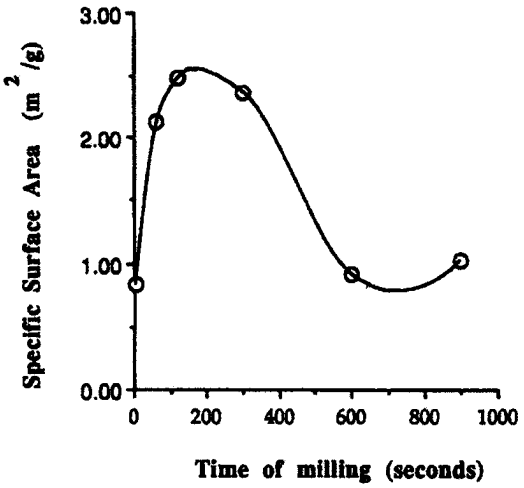


Fig. 10. Specific surface area of sucrose samples milled for various time periods. (The line is drawn simply to follow the data points.)

face of such particles during processing, where disorder might be desirable. X-ray powder diffraction detects an overall crystallinity, whereas water vapor sorption might indicate the extent to which the crystallization occurs on the available surfaces.

It also should be mentioned that the results given in Figs 9 and 10 at milling times up to 150 s allow us to assess the relative influences of increasing surface area and increasing disorder on water vapor adsorption vs absorption. For example, whereas the specific surface area increases by a factor of about 2, the amount of water taken up increases by a factor of about 10. Hence, we see an uptake of water 5-times greater than might be expected if only surface adsorption was involved because of an increase in surface area, and presumably because of the disorder introduced by the milling.

Summary and Conclusion

Comparison of X-ray powder diffraction, density, heat of crystallization and water vapor absorption measurements for mixtures of amorphous and crystalline sucrose particles reveals that a linear relationship with percent amorphous composition exists over the entire range of concentration. However, whereas the X-ray powder diffraction, density, and heat of crystallization methods could not accurately detect disorder below about a 10% level, as expected from previous reports, it was possible to detect the amorphous state down to a level of about 1%, with a high level of precision, using a Cahn microbalance vacuum assembly and measuring the specific surface area of all components. Such measurements also provided a basis for ensuring that the standard 100% crystalline samples used in this study were indeed very crystalline, relative to mildly processed samples normally thought to be highly crystalline.

Estimates of percent disorder above 10% for variously milled samples of sucrose obtained from calibration curves with predetermined mixtures of amorphous and crystalline sucrose were in very good agreement with each other when determined by the X-ray, density and heat of crystallization methods. This confirms the general validity, for this model system at least, of using predetermined mixtures of amorphous and crystalline particles, a two-state system, to assess disorder in milled samples, which most likely represent more of a one-state system.

Water vapor absorption measurements of samples milled for short times and, hence, levels of disorder below about 10% gave results that were generally consistent with the other techniques, but they were very much more precise. Results obtained with water vapor absorption at longer time periods, where extensive particle agglomeration had taken place, however, were significantly different than with the other techniques, i.e., the percent disorder detected was significantly lower. It is concluded that the agglomeration process most likely led to surface crystallization, too small to be detected by the other techniques, but great enough to block access of water to some of the amorphous structure. However, the unlikely possibility cannot presently be excluded that the milling process produced a disordered sample for which water had a different affinity, relative to an amorphous freeze-dried sample. This possibility currently is being explored with a wider range of systems and conditions.

Acknowledgments

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EXHIBIT L

CHAPTER 20

Colloidal Dispersions

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Historical Background

The term *colloid*, derived from the Greek word for glue, was applied *ca* 1850 by the British chemist Thomas Graham to polypeptides such as albumin and gelatin, to vegetable gums such as acacia, starch and dextrin, and to inorganic compounds such as gelatinous metal hydroxides and Prussian blue (ferric ferrocyanide). These compounds did not crystallize, and diffused very slowly when dissolved or dispersed in water. They could be separated from ordinary solutes such as salts and sugar, called "crystalloids," as the latter diffused through the fine pores of dialysis membranes made from animal gut which retained the "colloids." "Crystalloids" crystallized readily from solution.[1-3]

Von Weimarn was the first to identify colloidality as a state of subdivision of matter rather than as a category of substances. Many of Graham's "colloids," especially proteins, have been crystallized. Moreover, von Weimarn was able to prepare all "crystalloids" investigated in the colloidal state. Colloidal dispersions by the condensation method resulted from high relative supersaturation, which produced a large number of small nuclei.[1-4] For instance, clear, transparent solidified jellies were prepared by cooling aqueous solutions of $CaCl_2$, $Ba(SCN)_2$, and $Al_2(SO_4)_3$ and aqueous-alcoholic solutions of NaCl, KCl, NH_4Cl, KSCN, NaBr, and NH_4NO_3 which were nearly saturated at room temperature.[4]

Colloid chemistry became a science in its own right around 1906, when Wolfgang Ostwald wrote the booklet "The World of the Neglected Dimensions." In it, he focused on colloidal systems as a state of matter that has disperse phases intermediate in size between small molecules or ions in solution and large, visible particles in suspension. Otwald became the first editor of the journal *Kolloid-Zeitschrift* in 1907. The studies of colloidal systems and surface or interfacial phenomena (Chapter 19) are intimately related. The properties of colloidal dispersions are largely governed by the nature of the surface of their particles. The division of the American Chemical Society specializing in colloidal systems and interfaces is called the "Division of Colloid and Surface Chemistry," while the pertinent session of the Gordon Research Conferences is called "Chemistry at Interfaces."

Colloid and surface chemistry deals with an unusually wide variety of industrial and biological systems. A few examples are catalysts, lubricants, adhesives, latexes for paints, rubbers and plastics, soaps and detergents, clays, packaging films, cigarette smoke, liquid crystals, cell membranes, mucous secretions, and aqueous humors.

Definitions and Classifications

Colloidal Systems and Interfaces

Colloidal dispersions consist of at least two discrete phases, namely, one or more disperse, dispersed or internal phases and a continuous or external phase called the *dispersion medium* or *vehicle*. What distinguishes colloidal dispersions from solutions and coarse dispersions is the particle size of the disperse phase. Systems in the colloidal state contain one or more substances that have at least one dimension in the range of 10 to 100 Å (1 Angstrom unit = 10^{-8} cm = 10^{-10} m) or 1–10 nm (1 nanometer = 10^{-9} m) at the lower end, and a few microns (μ) or micrometers (μm) at the upper end (1 μ or 1 μm = 10^4 Å = 10^{-6} m). Thus blood, cell membranes, the thinner nerve fibers, milk, rubber latex, fog, and beer foam are colloidal systems. Some types of materials, such as many emulsions, and oral suspensions of most organic drugs, are coarser than true colloidal systems but exhibit similar behavior. Even though serum albumin, acacia and povidone form true or molecular solutions in water, the size of the individual solute molecules places such solutions in the colloidal range (particle size > 10 Å).[1,2,5,6]

The following features distinguish colloidal dispersions from coarse suspensions. Disperse particles in the colloidal range are usually too fine to be visible in a light microscope, because at least one dimension measures 1 μm or less. They are often visible in the ultramicroscope and always in the electron microscope. Coarse suspended particles are frequently visible to the naked eye and always in the light microscope. Colloidal particles, as opposed to coarse particles, pass through ordinary filter paper but are retained by dialysis or ultrafiltration membranes. Because of their small size, colloidal dispersions undergo little or no sedimentation or creaming: Brownian motion maintains the disperse particles in suspension (see below).

Except for high polymers, most soluble substances can be prepared either as low-molecular-weight solutions, or as colloidal dispersions or coarse suspensions depending on the choice of the dispersion medium and the dispersion technique.[4,7]

Because of the small size of colloidal particles, appreciable fractions of their atoms, ions or molecules are located in the boundary layer between a particle and air (surface) or between a particle and a liquid or solid (interface). The ions in the surface of a sodium chloride crystal and the water molecules in the surface of a rain drop are subjected to unbalanced forces of attraction, whereas the ions or molecules in the interior of the materials are surrounded by similar ions or molecules on all sides, with balanced force fields. Thus a surface free energy component is added to the total free energy of colloidal particles, which becomes relatively more important as the particles become smaller, ie, as greater fractions of their ions, atoms or molecules are located in their surface or interfacial region. Hence the solubility of very fine solid particles and the vapor pressure of very small liquid droplets are larger than the corresponding values of coarse particles and large drops of the same materials, respectively.

Specific Surface Area—Decreasing particle size increases the surface-to-volume ratio, which is expressed as the specific surface area A_{sp}, namely, the area A (cm^2) per unit volume V

Table I—Effect of Comminution on Specific Surface Area of a Volume of $4\pi/3$ cm³, Divided into Uniform Spheres of Radius R

Number of spheres	R	A_{sp}, cm²/cm³
1	1 cm	3
10^3	0.1 cm = 1 mm	3×10
10^6	0.1 mm	3×10^2
10^9	0.01 mm = 10 μm	3×10^3
10^{12}	1 μm	3×10^4
10^{15}	0.1 μm	3×10^5
10^{18}	0.01 μm	3×10^6
10^{21}	10 Å = 1 nm	3×10^7
10^{23}	1 Å	3×10^8

Shaded region corresponds to colloidal particle size range.

(1 cm³) or per unit mass M (1 gram). For a sphere, $A = 4\pi r^2$ and $V = 4/3\,\pi r^3$. If the density, d, of the material is expressed in g/cm³, the specific surface area is

$$A_{sp} = \frac{A}{V} = \frac{4\pi r^2}{4/3\pi r^3} = \frac{3}{r}\ \text{cm}^2/\text{cm}^3 = \frac{3}{r}\ \text{cm}^{-1}$$

or

$$A_{sp} = \frac{A}{M} = \frac{A}{Vd} = \frac{4\pi r^2}{4/3\pi r^3 d} = \frac{3}{rd}\ \text{cm}^2/\text{g}$$

Table I illustrates the effect of comminution on the specific surface area of $4\,\pi/3$ cm³ of a material consisting initially of one sphere of 1 cm radius. As the material is broken up into an increasingly larger number of smaller and smaller spheres, its specific surface area increases commensurately.

The solid adsorbents activated charcoal and kaolin have specific surface areas of about 6×10^6 cm²/g and 10^4 cm²/g, respectively. One gram of activated charcoal, because of its extensive porosity and internal voids, has an area equal to ⅙ acre.

In conclusion, colloidal systems by definition are those polyphasic systems where at least one dimension of the disperse phase measures between 10 or 100 Å and a few microns. The term "colloidal" designates a state of matter characterized by submicroscopic dimensions rather than certain substances. Any dispersed substance with the proper dimension or dimensions is in the colloidal state.

Physical States of Disperse and Continuous Phases

A useful classification of colloidal systems (systems in the colloidal particle size range) is based on the state of matter of the disperse phase and the dispersion medium, ie, whether they are solid, liquid or gaseous.[2,3,8] Table II summarizes the various combinations and lists examples. A *sol* is the colloidal dispersion of a solid in a liquid or gaseous medium. Prefixes designate the dispersion medium, such as hydrosol, alcosol, aerosol for water, alcohol and air, respectively. Sols are fluid. If the solid particles form bridged structures possessing some mechanical strength, the system is called a gel (hydrogel, alcogel, aerogel).

Interaction Between Disperse Phase and Dispersion Medium

A second useful classification of colloidal dispersions, originated by Ostwald, is based on the affinity or interaction between the disperse phase and the dispersion medium.[2,3,8] It refers mostly to solid-in-liquid dispersions. According to this classification, colloidal dispersions are divided into the two broad categories of lyophilic and lyophobic. Some soluble, low-molecular-weight substances have molecules with both tendencies, forming a third category called association colloids.

Lyophilic Dispersions—Where there is considerable attraction between the disperse phase and the liquid vehicle, ie, extensive solvation, the system is said to be *lyophilic* (solvent-loving). If the dispersion medium is water, the system is said to be *hydrophilic*. Such solids as bentonite, starch, gelatin, acacia and povidone swell, disperse or dissolve spontaneously in water.

Hydrophilic colloidal dispersions can be further subdivided as follows: (a) True solutions, formed by water-soluble polymers (acacia and povidone). (b) Gelled solutions, gels or jellies if the polymers are present at high concentrations and/or at temperatures where their water solubility is low. Examples of such hydrogels are relatively concentrated solutions of gelatin and starch, which set to gels on cooling, or of methylcellulose, which gel on heating. (c) Particulate dispersions, where the solids do not form molecular solutions but remain as discrete though minute particles. Bentonite and microcrystalline cellulose form such hydrosols.

Lipophilic or oleophilic substances have pronounced affinity for oils. Oils are nonpolar liquids consisting mainly of hydrocarbons, with few polar groups and low dielectric constants. Examples are mineral oil, benzene, carbon tetrachloride, vegetable oils (cottonseed or peanut oil) and essential oils (lemon or peppermint oil). Substances which form *oleophilic* colloidal dispersions include polymers like polystyrene and unvulcanized or gum rubber which dissolve molecularly in benzene, magnesium or aluminum stearate which dissolve or disperse in cottonseed oil, and activated charcoal which forms sols or particulate dispersions in all oils.

Because of the high affinity or attraction between the dispersion medium and the disperse phase, lyophilic dispersions

Table II—Classification of Colloidal Dispersions According to State of Matter

Disperse Phase	Dispersion Medium (Vehicle): Solid	Liquid	Gas
Solid	Zinc oxide paste (zinc oxide + starch in petrolatum). Toothpaste (dicalcium phosphate or calcium carbonate with sodium carboxymethylcellulose binder). Pigmented plastics (titanium dioxide in polyethylene).	Sols: Bentonite Magma NF. Trisulfapyrimidines Oral Suspension USP. Magnesia and Alumina Oral Suspension USP. Tetracycline Oral Suspension USP.	Solid aerosols: Smoke, dust. Epinephrine bitartrate inhalation. Isoproterenol Sulfate Aerosol.
Liquid	Absorption bases (aqueous medium in Hydrophilic Petrolatum USP). Emulsion bases (oil in Hydrophilic Ointment USP). Butter.	Emulsions: Mineral Oil Emulsion. Soybean oil in water emulsion for IV feeding. Milk. Mayonnaise.	Liquid aerosols: Mist, fog. Nasal relief sprays (naphazoline hydrochloride solution). Betamethasone Valerate Aerosol. Povidone-Iodine Aerosol.
Gas	Solid foams (foamed plastics and rubbers). Pumice.	Foams. Carbonated beverages. Effervescent salts in water.	No colloidal dispersions.

form spontaneously when the liquid vehicle is brought into contact with the solid phase. They are thermodynamically stable and reversible, ie, they are easily reconstituted even after the dispersion medium has been removed from the solid phase.[2,3,5,7]

Lyophobic Dispersions—When there is little attraction between the disperse phase and the dispersion medium, the dispersion is said to be *lyophobic* (solvent-hating). *Hydrophobic* dispersions consist of particles that are not hydrated, so that water molecules interact with or attract one another in preference to solvating the particles. They include aqueous dispersions of oleophilic materials such as polystyrene or gum rubber (latex), steroids and other organic lipophilic drugs, paraffin wax, magnesium stearate, and of cottonseed or soybean oil (emulsion). While lipophilic materials are generally hydrophobic, materials like sulfur, silver chloride and gold form hydrophobic dispersions without being lipophilic. Water-in-oil emulsions are lyophobic dispersions in lipophilic vehicles.

Because of the lack of attraction between the disperse and the continuous phase, lyophobic dispersions are intrinsically unstable and irreversible. Their large surface free energy is not lowered by solvation. The dispersion process does not take place spontaneously, and once the dispersion medium has been separated from the disperse phase, the dispersion is not easily reconstituted. The dividing line between hydrophilic and hydrophobic dispersions is not very sharp. For instance, gelatinous hydroxides of polyvalent metals such as $Al(OH)_3$ and $Mg(OH)_2$, and clays such as bentonite and kaolin, possess some characteristics of both.[2,3,5]

Association Colloids—Organic compounds which contain large hydrophobic moieties together with strongly hydrophilic groups in the same molecule are said to be amphiphilic. While the individual molecules are generally too small to bring their solutions into the colloidal size range, they tend to associate in aqueous or oil solutions into aggregates called micelles. Because micelles are large enough to qualify as colloidal particles, such compounds are called association colloids.

Lyophobic Dispersions

Most of the discussion of lyophobic dispersions deals with hydrophobic dispersions or hydrosols (hydrophobic solids or liquids dispersed in aqueous media) because water is the most widely used vehicle. They comprise aqueous dispersions of insoluble organic and inorganic compounds which usually have low degrees of hydration. Organic compounds which are preponderantly hydrocarbon in nature and possess few hydrophilic or polar groups are insoluble in water and hydrophobic.

Hydrophobic dispersions are intrinsically unstable. The most stable state of such systems contains the disperse phase coalesced into large crystals or drops, so that the specific surface area and surface free energy are reduced to a minimum. Therefore, mechanical, chemical or electrical energy must be supplied to the system to break up the disperse phase into small particles, providing for the increase in surface free energy resulting from the parallel increase in specific surface area. Furthermore, special means must be found to stabilize hydrophobic dispersions, preventing the otherwise spontaneous coalescence or coagulation of the disperse phase after it has been finely dispersed.

Preparation and Purification of Lyophobic Dispersions

Colloidal dispersions are intermediate in size between true solutions and coarse suspensions. They can be prepared by aggregation of small molecules or ions until particles of colloidal dimensions result (condensation methods), or by reducing coarse particles to colloidal dimensions through comminution or peptization (dispersion methods).

Dispersion Methods—The first method, *mechanical disintegration* of solids and liquids into small particles and their dispersion in a fluid vehicle, is frequently carried out by input of mechanical energy via shear or attrition. Equipment such as colloid and ball mills, micronizers and, for emulsions, homogenizers is described in Chapters 84 and 89 and in Ref 10. Dry grinding with inert, water-soluble diluting agents also produces colloidal dispersions. Sulfur hydrosols may be prepared by triturating the powder with urea or lactose followed by shaking with water.[9]

Ultrasonic generators provide exceptionally high concentrations of energy. Successful dispersion of solids by means of ultrasonic waves can only be achieved with comparatively soft materials such as many organic compounds, sulfur, talcum, and graphite. Where fine emulsions are mandatory, such as soybean oil-in-water emulsions used for intravenous feeding, emulsification by ultrasound waves is the method of choice.[10] The formation of aerosols is described in Chap 93.

It should be reiterated that hydrosols of hydrophobic substances are intrinsically unstable. While mechanical disintegration may break up the disperse phase into colloidal particles, the resultant dispersions tend towards separation of that phase. Recrystallization, coagulation or coalescence causes the disperse particles to become progressively coarser and fewer, ultimately resulting in the separation of a macroscopic phase. To avoid this, stabilizing agents must be added during or shortly after the dispersion process (see below). For instance, lecithin may be used to stabilize soybean oil emulsions.

Peptization is a second method for preparing colloidal dispersions. The term, coined by Graham, is defined as the breaking up of aggregates or secondary particles into smaller aggregates or into primary particles in the colloidal size range. Particles which are not formed of smaller ones are called "primary." Peptization is synonymous with *deflocculation*. It can be brought about by the removal of flocculating agents, usually electrolytes, or by the addition of deflocculating or peptizing agents, usually surfactants, water-soluble polymers or ions which are adsorbed at the particle surface.[3,5,8,9]

The mechanisms of the following examples are explained in subsequent sections. When powdered activated charcoal is added to water with stirring, the aggregated grains are broken up only incompletely and the resultant suspension is gray and translucent. The addition of 0.1% or less of sodium lauryl sulfate or octoxynol disintegrates the grains into finely dispersed particles forming a deep black and opaque dispersion. Ferric or aluminum hydroxide freshly precipitated with ammonia can be peptized with small amounts of acids which reduce the pH below the isoelectric points of the hydroxides (see below). Even washing the gelatinous precipitate of $Al(OH)_3$ with water tends to peptize it. In quantitative analysis, the precipitate is therefore washed with dilute solutions of ammonium salts that act as flocculating agents, rather than with water.

Condensation Methods—The preparation of sulfur hydrosols is employed to illustrate condensation or aggregation methods. Sulfur is insoluble in water but somewhat soluble in alcohol. When an alcoholic solution of sulfur is mixed with water, a bluish white colloidal dispersion results. In the absence of added stabilizing agents, the particles tend to agglomerate and precipitate on standing. This technique of dissolving the material in a water-miscible solvent such as alcohol or acetone and producing a hydrosol by precipitation with water is applicable to many organic compounds, and has been used to prepare hydrosols of natural resins like mastic and of stearic acid.

For sulfur, another less common physical method is to introduce a current of sulfur vapor into water. Condensation

produces colloidal particles. Alternatively, the very fine powder produced by condensing sulfur vapor on cold solid surfaces (sublimed sulfur or flowers of sulfur) can be dispersed in water by addition of a suitable surfactant to produce a hydrosol.

Chemical methods include the reaction between hydrogen sulfide and sulfur dioxide, eg, by bubbling H_2S into an aqueous SO_2 solution:

$$2\,H_2S + SO_2 \rightarrow 3\,S + 2\,H_2O$$

The same reaction occurs when aqueous solutions containing sodium sulfide and sulfite are acidified with an excess of sulfuric or hydrochloric acid. Another reaction is the decomposition of sodium thiosulfate by sulfuric acid, using either very dilute or very concentrated solutions to obtain colloidally dispersed sulfur:

$$H_2SO_4 + 3\,Na_2S_2O_3 \rightarrow 4\,S + 3\,Na_2SO_4 + H_2O$$

Both reactions also produce pentathionic acid, $H_2S_5O_6$, as a by-product. The preferential adsorption of the pentathionate anion at the surface of the sulfur particles confers a negative electric charge on the particles, stabilizing the sol (see below).[3,7-9] When powdered sulfur is boiled with a slurry of lime, it dissolves with the formation of calcium pentasulfide and thiosulfate. Subsequent acidification produces the colloidal "milk of sulfur," which on washing and drying yields Precipitated Sulfur USP (see Chapter 83).

Sols of ferric, aluminum, chromic, stannic and titanium hydroxides or hydrous oxides are produced by hydrolysis of the corresponding chlorides or nitrates:

$$AlCl_3 + 3\,H_2O \rightarrow Al(OH)_3 + 3\,HCl$$

Hydrolysis is promoted by boiling the solution and/or by adding a base to neutralize the acid formed.

Double decompositions producing insoluble salts can lead to colloidal dispersions. Examples are silver chloride and nickel sulfide:

$$NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$$

$$(NH_4)_2S + NiCl_2 \rightarrow NiS + 2\,NH_4Cl$$

Compare also the preparation of White Lotion, which contains precipitated zinc sulfide and sulfur (Chapter 65). Reducing salts of gold, silver, copper, mercury, platinum, rhodium and palladium with formaldehyde, hydrazine, hydroxylamine, hydroquinone or stannous chloride produces hydrosols of the metals. These are strongly colored, eg, red or blue.[1,3,8,9]

Radioactive Colloids—Colloidal dispersions containing radioactive isotopes find increasing diagnostic and therapeutic application in nuclear medicine. Radioactive colloids that accumulate in tumors and/or lesions or emboli, indicating their location and size, may be used as diagnostic aids. Radioactive colloids with a particle size of about 300 Å, injected intravenously, locate mainly in the reticuloendothelial systems of liver, spleen and other organs and are used in scintillation imaging. The radiation emitted by the colloids is made visible by stationary or scanning devices which show the location, size and shape of the organ being investigated, as well as any tumors within. Radiocolloids are useful in anticancer radiation therapy because of their low solubility, radiation characteristics, and their ability to accumulate and remain located in certain target organs or tumors.[11]

Colloidal gold Au 198 is made by reducing a solution of gold (^{198}Au) chloride either by treatment with ascorbic acid or by heating with an alkaline glucose solution. Gelatin is added as a protective colloid (see below). The particle size ranges from 50 to 500Å with a mean of 300 Å. The color of the sol is cherry-red in transmitted light. Violet or blue sols have excessively large particle sizes and should be discarded. Colloidal gold is used as a diagnostic and therapeutic aid (se Chapter 29). The half-life of ^{198}Au is 2.7 days.

Technetium 99m sulfur colloid is prepared by reducin sodium pertechnetate ^{99m}Tc with sodium thiosulfate. Th product, a mixture of technetium sulfide and sulfur in th colloidal particle size range, is stabilized with gelatin. It i used chiefly in liver, spleen and bone scanning. Its half-lif is 6.0 hr.

Microspheres of gelatin or human serum albumin can b prepared in fairly narrow particle-size ranges from 100–20 Å through 45–55 μm. A variety of β- and γ-emitting radi onuclides such as ^{131}I, ^{99m}Tc, ^{113m}In, or ^{51}Cr can be incorpo rated to label the microspheres. Such products have bee used to scan heart, brain, urogenital and gastrointestina tracts, liver, and in pulmonary perfusion and inhalatio studies.[11]

Organic compounds that are weak bases, such as alkaloids are usually much more soluble at lower pH values where they are ionized than at higher pH values where they exist as th free base. Increasing the pH of their aqueous solutions wel above their pKa may cause precipitation of the free base Organic compounds which are weak acids, such as barbitu rates, are usually much more soluble at higher pH value where they are ionized than at lower pH values where they ar in the un-ionized acid form. Lowering the pH of their solu tions well below their pKa may cause precipitation of th un-ionized acid. Depending on the supersaturation of th un-ionized acids or bases and on the presence of stabilizing agents, the resultant dispersions may be in the colloida range.

Kinetics of Particle Formation—When the solubility of a compound in water is exceeded, its solution becomes supersaturated and the compound may precipitate or crystallize. The rate of precipitation, the particle size (whether colloidal or coarse), and the particle size uniformity or distribution (whether a narrow distribution and nearly monodisperse or homodisperse particles, or a broad distribution and polydisperse or heterodisperse particles) depend on two successive and largely independent processes, nucleation and growth of nuclei.

When a solution of a salt or of sucrose is supercooled, or when a chemical reaction produces a salt in a concentration exceeding its solubility product, separation of the excess solid from the supersaturated solution is far from instantaneous. Clusters of ions or molecules called nuclei must exceed a critical size before they become stable and capable of growing into colloidal size crystals. These embryonic particles have much more surface for a given weight of material than large and stable crystals, resulting in higher surface free energy and greater solubility.

Whether *nucleation* takes place depends on the *relative supersaturation*. If C is the actual concentration of the solute before crystallization has set in, and C_s is its solubility limit, $C - C_s$ is the supersaturation and $(C - C_s)/C_s$ is the relative supersaturation. Von Weimarn recognized that the rate or velocity of nucleation (number of nuclei formed per liter per second) is proportional to the relative supersaturation. Nucleation seldom occurs at relative supersaturations below 3. The foregoing statement refers to homogeneous nucleation, where the nuclei are clusters of the same chemical composition as the crystallizing phase. If the solution contains solid impurities, such as dust particles in suspension, these may act as nuclei or centers of crystallization (heterogeneous nucleation).[2,4,7-9]

Once nuclei have formed, the second process, *crystallization*, begins. Nuclei grow by accretion of ions or molecules from solution forming colloidal or coarser particles until the supersaturation is relieved, ie, until $C = C_s$. The rate of crystallization or growth of nuclei is proportional to the supersaturation. The appropriate equation,

$$\frac{dm}{dt} = \frac{A_{sp}D}{\delta}(C - C_s)$$

is similar to the Noyes-Whitney equation governing the dissolution of particles (see Chapter 35) except that $C < C_s$ for the latter process, making dm/dt negative. In both equations, m is the mass of material crystallizing out in time t, D is the diffusion coefficient of the molecules or ions of the solute, δ is the length of the diffusion path or the thickness of the liquid layer adhering to the growing particles, and A_{sp} is their specific surface area. The presence of dissolved impurities may affect the rate of crystallization and even change the crystal habit, provided that these impurities are surface-active and become adsorbed on the nuclei or growing crystals.[2,3,4,7-9]

Von Weimarn found that the particle size of the crystals depends strongly on the concentration of the precipitating substance. At a very low concentration and slight relative supersaturation, diffusion is quite slow because the concentration gradient is very small. Sufficient nuclei will usually form to relieve the slight supersaturation locally. Crystal growth is limited by the small amount of excess dissolved material available to each particle. Hence, the particles cannot grow beyond colloidal dimensions. This condition is represented by points A, D, and G of the schematic plot of von Weimarn (Fig 20-1). At intermediate concentrations, the extent of nucleation is somewhat greater but much more material is available for crystal growth. Coarse crystals rather than colloidal particles result (points B, E, or H).

At high concentrations, nuclei appear so quickly and in such large numbers that supersaturation is relieved almost immediately, before appreciable diffusion occurs. The high viscosity of the medium also slows down diffusion of excess dissolved ions or molecules, retarding crystal growth without substantially affecting the rate of nucleation. A large number of very small particles results which, because of their proximity, tend to link, producing a translucent gel (points C and F). On subsequent dilution with water, such gels usually yield colloidal dispersions.

Thus, colloidal systems are usually produced at very low and high supersaturations. Intermediate values of supersaturation tend to produce coarse crystals. Low solubility is a necessary condition for producing colloidal dispersions. If the solubility of the precipitate is increased, for instance by heating the dispersion, a new family of curves will result, similar in shape to ABC, DEF, and GHI of Fig 20-1, but displaced upwards (towards larger particle sizes) and to the right (towards higher concentrations).[3,4,6,9]

Condensation methods generally produce polydisperse sols because nucleation continues while established nuclei grow. The particles in the resultant dispersion grew from nuclei formed at different times and had different growth periods. The formation of monodisperse sols requires special techniques.[2,5,6,8]

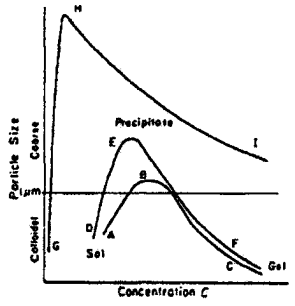


Fig 20-1. Effect of the concentration of the precipitating material and of aging on particle size.[4] Curves ABC, DEF, and GHI correspond to increasing aging. Both axes are on a logarithmic scale.

A feature of Fig 20-1 is that aging increases the particle size. Curves ABC, DEF, and GHI correspond to increasing times after mixing the reagents. Typical ages are 10–30 min, several hours, and weeks or years, respectively. This gradual increase in particle size of crystals in their mother liquor is a recrystallization process called *Ostwald ripening*. Very small particles have a higher solubility than large particles of the same substance owing to their greater specific surface area and higher surface free energy. In a saturated solution containing precipitated particles of the solute in a wide range of particle sizes, the very smallest particles dissolve spontaneously and the material deposits onto the large particles. The growth of the large crystals at the expense of the very small ones occurs because this process lowers the free energy of the dispersion. As mentioned above, the most stable system is the suspension of a few coarse crystals, whereas the colloidal dispersion of a great many fine particles of the same substance is intrinsically less stable.

The spontaneous coarsening of colloidal dispersions on aging is accelerated by a relatively high solubility of the precipitate and can be retarded by lowering the solubility or by adding traces of surface-active compounds which are adsorbed at the particle surface. For instance, barium sulfate precipitated by mixing concentrated solutions of sodium sulfate and barium chloride is largely in the colloidal range and passes through filter paper. The colloidal particles gradually grow in size by Ostwald ripening, forming large crystals which can be removed quantitatively by filtration. Heating the aqueous dispersion speeds up this recrystallization by increasing the solubility of barium sulfate in water. The addition of ethyl alcohol lowers the solubility, retarding Ostwald ripening so that the dispersion remains in the colloidal state for years.

Another process by which particles in colloidal dispersions grow in size is by agglomeration of individual particles into aggregates. This process, called coagulation, is discussed below.

Purification of Hydrosols by Dialysis and Ultrafiltration

Many hydrosols contain low molecular-weight, water-soluble impurities. Inorganic dispersions often contain salts formed by the reaction producing the disperse phase. Salts are especially objectionable in the case of hydrophobic dispersions because they tend to coagulate such dispersions. Protein solutions often contain salts added as part of the separation procedure. The blood of patients with renal insufficiency contains excessive concentrations of urea and other low-molecular-weight metabolites and salts. These dissolved impurities of small molecular size are removed from the colloidal dispersions by means of membranes with pore openings smaller than the colloidal particles.

Membranes—Conventional filter papers are permeable to colloidal particles as well as to small solute molecules. Among the early membranes capable of retaining colloidal particles but permeable to small solute molecules were pig's bladder and parchment. Most membranes in current use consist of cellulose, cellulose nitrate prepared from collodion, cellulose acetate or synthetic polymers, and are available in a variety of shapes, gauges, and pore sizes. *Gel cellophane* is most widely used. It consists of sheets or tubes of cellulose made by extruding cellulose xanthate solutions (viscose) through slit or annular dies into a sodium bisulfate/sulfuric acid bath which decomposes the xanthate, precipitating the regenerated cellulose in a highly swollen or gel state. If the cellulose film were permitted to dry after purification and washing with water, it would crystallize and shrink excessively,

losing most of its extensive pore structure and turning somewhat brittle. The film is therefore impregnated with glycerin before drying. Glycerin remains in the film rather than evaporating like water. It reduces the shrinkage and blocks crystallization. This action prevents the collapse of the porous gel structure and plasticizes the film, keeping it flexible. A typical dialysis tube made from sausage casing swells to about twice its thickness in water and has an average pore diameter of 34 Å. While the pore structure of cellophane films used in dialysis and ultrafiltration causes retention of colloidal particles but permits the passage of small solute molecules, osmotic membranes are only permeable to water and retain small solute molecules as well as colloidal particles.

Dialysis—The colloidal dispersion is placed inside a sac made of sausage casing dipping in water. The small solute molecules diffuse out into the water while the colloidal material remains trapped inside because of its size. The rate of dialysis is increased by increasing the area of the membrane, by stirring, and by maintaining a high concentration gradient across the membrane. For the latter purpose, the water is replenished continuously or at least frequently. A membrane configuration which provides a particularly extensive transfer area for a given volume of dispersion is the hollow fiber. A typical fiber measures 175 μm inside diameter and 225 μm outside diameter. The dispersion to be dialyzed is circulated inside a bundle of parallel fibers while water is circulated outside the fibers throughout the bundle. Dialysis of the diffusing species takes place across the thin fiber wall. Dialysis is used in the laboratory to purify sols and to study binding of drugs by proteins, as well as in some manufacturing processes.

Electrodialysis—If the low-molecular-weight impurities to be removed are electrolytes, the dialysis can be speeded up by applying an electric potential to the sol which produces electrolysis. An electrodialyzer (Fig 20-2) is divided into three compartments by two dialysis membranes supported by screens. The two outer compartments, in which the two electrodes are placed, are filled with water while the sol is placed into the center compartment. Under the influence of the applied potential, the anions migrate from the sol into the anode (right) compartment while the cations migrate into the cathode compartment. Low-molecular-weight nonelectrolyte solutes diffuse into either compartment.

Colloidal particles are usually charged and therefore tend to migrate towards the membrane sealing off the compartment with the electrode of opposite charge. The combination of electrophoresis (see below) and gravitational sedimentation produces the accumulation of negatively charged sol particles shown in Fig 20-2. Hence the supernatant liquid can be changed by decantation. This process, which may be used to speed up electrodialysis, is called *electrodecantation*.[1,3,9]

Ultrafiltration—When a sol is placed in a compartment closed by a dialysis membrane and pressure is applied, the liquid and the small solute molecules are forced through the membrane while the colloidal particles are retained. This process, called ultrafiltration, is based on a sieving mechanism in which all components smaller than the pore size of the filter membrane pass through it. The pressure difference required to push the dispersion medium through the ultrafilter is provided by gas pressure applied on the sol side or by suction on the filtrate side. The membrane is usually supported on a fine wire screen.[3,9]

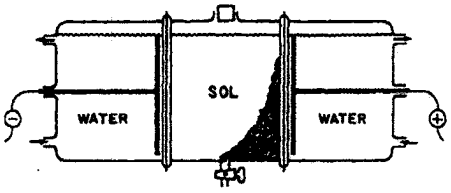


Fig 20-2. Electrodialyzer showing electrodecantation.

As ultrafiltrate is being removed, the sol becomes more concentrated because a constant amount of disperse particles is confined to a decreasing volume of liquid. Some dissolved small molecules or ions are left in the sol together with the residual water. To avoid the increase in concentration of the colloidal particles and remove the dissolved impurities completely, the ultrafiltrate squeezed from the sol is replenished continuously or intermittently with an equal volume of water. During ultrafiltration, solids tend to accumulate on and near the membrane. To prevent this buildup and maintain uniform composition throughout the sol, it is stirred.

Bundles of hollow fibers are used for ultrafiltration in the laboratory and on large scale. To withstand higher pressures, the wall thickness of the fibers used in ultrafiltration is usually greater than that of fibers used exclusively for dialysis. When hollow fibers are fouled by excessive accumulation of solids on the inner wall, they are cleaned by backflushing with water or ultrafiltrate.

Hemodialysis—The blood of uremic patients is dialyzed periodically in "artificial kidney" dialyzers to remove urea, creatinine, uric acid, phosphate and other metabolites, and excess sodium and potassium chloride. The dialyzing fluid contains sodium, potassium, calcium, chloride and acetate ions (the latter are converted in the body to bicarbonate), dextrose and other constituents in the same concentration as normal plasma. Since it contains no urea, creatinine, uric acid, phosphate nor any of the other metabolites normally eliminated by the kidneys, these compounds diffuse from the patient's blood into the dialyzing fluid until their concentration is the same in blood and fluid. Sodium and potassium chloride diffuse from blood to fluid because of their higher initial concentration in the blood, and continue to diffuse until the concentration is equalized. The volume of dialyzing fluid is much greater than that of blood. The great disparity in volume and the replenishment of dialyzate with fresh fluid ensure that the metabolites and the excess of electrolytes are removed almost completely from the blood. Hemodialysis is also employed in acute poisoning cases.

Plasma proteins and blood cells cannot pass through the dialysis membrane because of their size. Edema resulting from water retention can be relieved by ultrafiltration through the application of a slight pressure on the blood side or a partial vacuum on the fluid side.

The three geometries used to circulate the blood and the dialyzing fluid in a countercurrent fashion are a coil of flattened cellulose tubing wound concentrically with a supporting mesh screen around a core, a stack of flat cellulose sheets separated by ridged or grooved plates, and hollow fibers. The regenerated cellulose used in the former two is precipitated from a cuprammonium solution. The hollow cellulose acetate fibers have an outside diameter of about 270 μm and a wall thickness of 30 μm.[12] The advantage of hollow fibers is their compactness. A bundle of 10,000 fibers 18 cm long has a surface area of 1.4 m^2.

Particle Shape, Optical, and Transport Properties of Lyophobic Dispersions

Hydrophobic materials handled by pharmacists in aqueous dispersion range from metallic conductors to inorganic precipitates to organic solids and liquids which are electric insulators. Despite the great diversity of the hydrophobic disperse phase, their hydrosols have certain common characteristics.

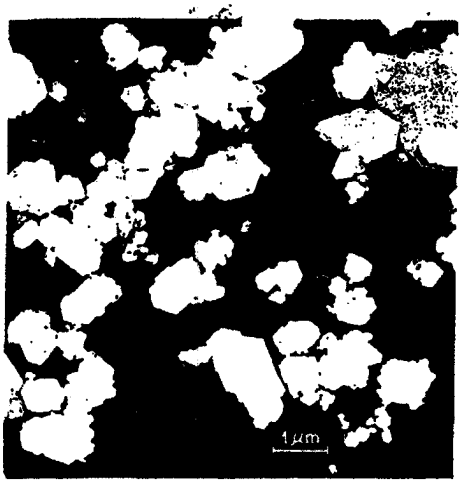


Fig 20-3. Transmission electron micrograph of a well crystallized, fine-particle kaolin. Note hexagonal shape of the clay platelets (courtesy, John L. Brown, Engineering Experiment Station, Georgia Institute of Technology).

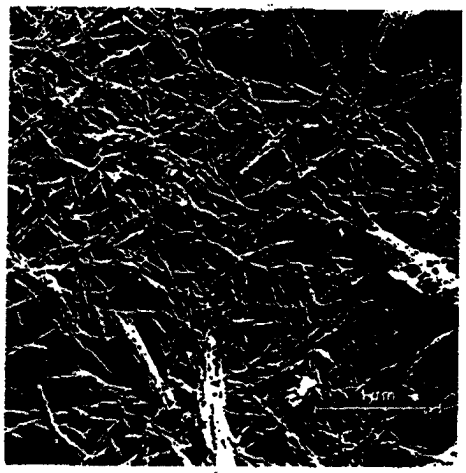


Fig 20-4. Transmission electron micrograph of Avicel RC-591 thickening grade microcrystalline cellulose. The needles are individual cellulose crystallites; some are aggregated into bundles (courtesy, FMC Corporation; Avicel is a registered trademark of FMC Corporation).

Particle Shape and Particle Size Distribution—Both of these properties depend on the chemical and physical nature of the disperse phase and on the method employed to prepare the dispersion. Primary particles exist in a great variety of shapes. Their aggregation produces an even greater variety of shapes and structures. Precipitation and mechanical comminution generally produce randomly shaped particles unless the precipitating solids possess pronounced crystallization habits or the solids being ground possess strongly developed cleavage planes. Precipitated aluminum hydroxide gels and micronized particles of sulfonamides and other organic powders have typical irregular random shapes. An exception is bismuth subnitrate. Even though its particles are precipitated by hydrolyzing bismuth nitrate solutions with sodium carbonate, its particles are lath-shaped. Precipitated silver chloride particles have a cubic habit which is apparent under the electron microscope. Lamellar or plate-like solids in which the molecular cohesion between layers is much weaker than within layers frequently preserve their lamellar shape during mechanical comminution, because milling and micronization break up stacks of thin plates in addition to fragmenting plates in the lateral dimensions. Examples are graphite, mica, and kaolin. Fig 20-5 shows a Georgia crude clay as mined. Processing yields the refined, fine-particle kaolinite of Fig 20-3. Similarly, macroscopic asbestos and cellulose fibers consist of bundles of microscopic and submicroscopic fibrils. Mechanical comminution or beating splits these bundles into the component fibrils of very small diameters as well as cutting them shorter.

Microcrystalline cellulose is a fibrous thickening agent and tablet additive made by selective hydrolysis of cellulose. Native cellulose consists of crystalline regions where the polymer chains are well aligned and in registry, with maximum interchain attraction by secondary valence forces, called crystallites, and of more disordered regions having lower density and reduced interchain attraction and crystallinity, the so-called "amorphous" regions. During treatment with dilute mineral acid, the acid penetrates the amorphous regions relatively fast and hydrolyzes the polymer chains into water-soluble fragments. If the acid is washed out before it penetrates the crystalline regions appreciably, the crystallites remain intact. Wet milling and spray-drying the aqueous suspension produces spongy and porous aggregates of rod-shaped or fibrillar bundles shown in Fig 20-6. These aggregates, averaging 100 μm in size, were embrittled by the acid treatment and lost the elasticity of the native cellulose. They are well compressible and capable of undergoing plastic deformation, a property important in tableting. Their porosity permits the aggregates to absorb liquid ingredients while still remaining a free-flowing powder, thus preventing these liquids from reducing the flowability of the granulation or direct-



Fig 20-5. Scanning electron micrograph of a crude kaolin clay as mined. Processing yields the fine particle material of Fig 20-3 (courtesy, John L. Brown, Engineering Experiment Station, Georgia Institute of Technology).



Fig 20-6. Scanning electron micrograph of Avicel PH-102 tableting grade microcrystalline cellulose. The aggregates of fiber bundles are porous and compressible (courtesy, FMC Corporation; Avicel is a registered trademark of FMC Corporation).

compression mass during tableting. The swelling of the cellulosic particles in water speeds up the disintegration of the ingested tablets.

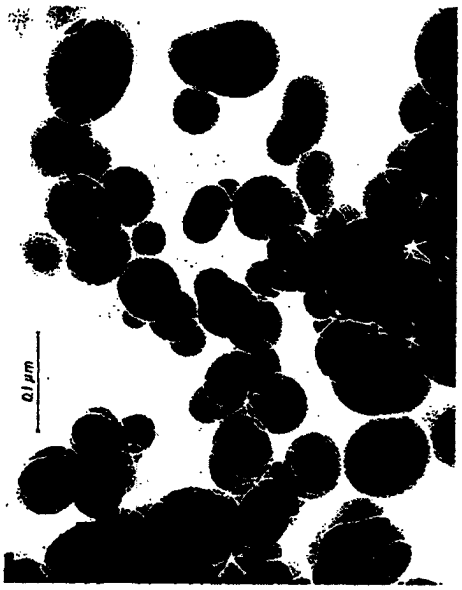


Fig 20-7. Transmission electron micrograph of Aerosil OX 50, ground and dusted on. The spheres are translucent to the electron beam, causing overlapping portions to be darker owing to increased thickness (courtesy, Degussa AG of Hanau, West Germany; Aerosil is a registered trademark of Degussa). The suffix 50 indicates the specific surface area in m^2/g.

Additional shear breaks up the aggregated bundles into the individual, needle- or rod-shaped cellulose crystallites shown in Fig 20-4. The latter, which average 0.3 μm in length and 0.02 μm in width, are of colloidal dimensions. These primary particles act as suspending agents in water, producing thixotropic structured vehicles. At concentrations above 10%, e g 14 or 15%, the cellulose microcrystals gel water to ointment consistency by swelling and producing a continuous network of rods extending throughout the entire vehicle. Attraction between the elongated particles is presumably due to flocculation in the secondary minimum (see below). Treatment of the microcrystalline mass with sodium carboxymethylcellulose facilitates its disintegration into the primary needle-shaped particles and enhances their thickening action.

While in the special cases of certain clays and cellulose, comminution produces lamellar and fibrillar particles, respectively, as a rule regular particle shapes are produced by condensation rather than by disintegration methods. *Colloidal silicon dioxide* is called fumed or pyrogenic silica because it is manufactured by high-temperature, vapor-phase hydrolysis of silicon tetrachloride in an oxy-hydrogen flame, ie, a flame produced by burning hydrogen in a stream of oxygen. The resultant white powder consists of submicroscopic spherical particles of rather uniform size (narrow particle size distribution). Different grades are produced by different reaction conditions. Relatively large, single spherical particles are shown in Fig 20-7. Their average diameter is 50 nm (500 Å), corresponding to the comparatively small specific surface area of 50 m^2/g. Smaller spherical particles have correspondingly larger specific surface areas; the grade with the smallest average diameter, 5 nm, has a specific surface area of 380 m^2/g. During the manufacturing process, the finer-grade particles tend to sinter or grow together into chain-like aggregates resembling pearl necklaces or streptococci (see Fig 20-8).

Since fumed silica is amorphous, its inhaled dust causes no silicosis. The spheres of colloidal silicon dioxide are nonporous. While the density of the spherical particles is 2.13 g/cm^3, the bulk density of their powder is a mere 0.05 g/cm^3; the powder is extremely light. This results in two pharmaceutical and cosmetic applications for colloidal silicon dioxide. It is used to increase the fluffiness or bulk volume of powders. Even more than microcrystalline cellulose, its high porosity enables it to absorb a variety of liquids from fluid fragrances to viscous tars, transforming them into free-flowing powders that can be incorporated into tablets or capsules. The porosity in colloidal silicon dioxide is due entirely to the enormous void space between the particles, which themselves are solid.

When these ultrafine particles are incorporated at levels as low as 0.1–0.5% into a powder consisting of coarse particles or granules, they coat the surface of the latter and act as tiny ball bearings and spacers, improving the flowability of the powder and eliminating caking. This action is important in tableting. Moreover, colloidal silicon dioxide improves tablet disintegration.

The surface of the particles contains siloxane (Si—O—Si) and silanol (Si—OH) groups. When colloidal silicon dioxide powder is dispersed in nonpolar liquids, the particles tend to adhere to one another by hydrogen bonds between their surface groups. With finer grades of colloidal silicon dioxide, the spherical particles are linked together into short chain-like aggregates as shown in Fig 20-8, thus agglomerating into loose three-dimensional networks which increase the viscosity of the liquid vehicles very effectively at levels as low as a few percent. These hydrogen-bonded structures are torn apart by stirring but rebuilt while at rest, conferring thixotropy to the thickened liquids.

The grades which consist of relatively large and unattached spherical particles, such as those of Fig 20-7, are less efficient

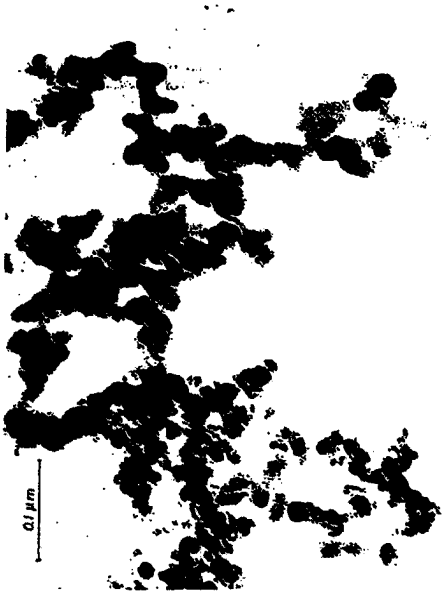


Fig 20-6. Transmission electron micrograph of Aerosil 130, ground and dusted on. The spheres are fused together into chain-like aggregates (courtesy, Degussa AG of Hanau, West Germany; Aerosil is a registered trademark of Degussa). The suffix 130 gives the specific surface area in m^2/g.

thickening agents as they lack the high specific surface area and the asymmetry of the finer grades, which consist of short chains of fused spherical particles. In the latter category is Aerosil 200, the grade most widely used as a pharmaceutical adjuvant, whose primary spheres, which are extensively sintered together, have an average diameter of 12 nm. At levels of 8–10%, it thickens liquids of low polarity such as vegetable and mineral oils to the consistency of ointments, imparting considerable yield values to them. The consistency of ointments thickened with colloidal silicon dioxide is not appreciably reduced at higher temperatures. Incorporation of colloidal silicon dioxide into ointments and pastes, such as those of zinc oxide, also reduces the syneresis or *bleeding* of the liquid vehicles.

Hydrogen-bonding liquids like alcohols and water solvate the silica spheres, reducing the hydrogen bonding between particles. These solvents are gelled at silica levels of 12–18% or higher.

Latexes of polymers are aqueous dispersions prepared by emulsion polymerization. Their particles are spherical because polymerization of solubilized liquid monomer takes place inside spherical surfactant micelles which swell because additional monomer keeps diffusing into the micelles. Examples include latex-based paints. Some *clays* grow as plate-like particles possessing straight edges and hexagonal angles, eg bentonite and kaolin (see Fig 20-3). Other clays have lath-shaped (nontronite) or needle-shaped particles (attapulgite).

Emulsification produces spherical droplets to minimize the oil-water interfacial area. Cooling the emulsion below the melting point of the disperse phase freezes it in the spherical shape. For instance, paraffin can be emulsified in 80° water; cooling to room temperature produces a hydrosol with spherical particles.

Sols of viruses and globular proteins, which are hydrophilic, contain compact particles possessing definite geometric shapes. Poliomyelitis virus is spherical, tobacco mosaic virus is rod-shaped, while serum albumin and the serum globulins are prolate ellipsoids of revolution (football-shaped).

Dispersion methods produce sols with wide particle size distributions. Condensation methods *may* produce essentially monodisperse sols provided specialized techniques are employed. Monodisperse polystyrene latexes are available for calibration of electron micrographs (see Fig 20-3). Biologic hydrophilic polymers, such as nucleic acids and proteins, form largely monodisperse particles, as do more highly organized structures such as lipoproteins and viruses.

Electron Microscopy—Because of their size, particles in the middle and lower colloidal size range are too small for direct microscopic observation. The ordinary *light microscope* has a resolving power of about 2000 Å (0.2 μm), ie, it cannot reveal or distinguish structural details or particles which are separated by smaller distances. Two particles which are closer together would appear as one.

The resolving power is directly proportional to the wavelength of the radiation. The *electron microscope*, developed in the late 1930s, employs electrons with a wavelength of about 0.1 Å and has a resolving power of about 5 Å. This value is limited by lens aberrations rather than by the wavelength. The "lenses" used to focus the electron beam are magnetic fields. The electrons transmitted through the object produce an image that is focused and projected with the aid of additional electromagnetic lenses onto a fluorescent screen or photographic plate. Electrons can travel unhindered only in high vacuum. Therefore, only specimens with negligible vapor pressure can be observed. Hydrosols must be thoroughly dried beforehand. If the sample appears flocculated, flocculation may have existed in the original sol or it may have been caused by drying. The electron beam may also alter the sample.

Another shortcoming of the *transmission electron microscope* is that the image is due to differences in transparency to electrons. Transparency depends on the thickness and on the atomic number of the atoms forming the specimen. Organic materials containing only atoms of low atomic numbers, namely, hydrogen, carbon, oxygen and nitrogen, are almost equally and highly transparent. Their pictures show little contrast. Materials containing heavy metal atoms produce better images. Larger objects are cut into very thin slices by means of microtomes for examination with a transmission electron microscope.

The *shadow-casting* technique is used to give a three-dimensional effect to transmission electron micrographs and to improve the contrast. Chromium, gold or other metals are evaporated in vacuum at a small and known angle to the specimen. The shadows cast by salient features of the specimen, ie, the areas behind those features which are not covered by chromium, provide the electron micrograph with relief (see Figs 20-3 and 20-4). From the length of the shadow and from the angle of vaporization, it is possible to calculate the height of the particle. For instance, the sample of Fig 20-3 was shadowed at an 18° angle. Tan 18° = ⅓ is the ratio of particle thickness to length of shadow. The few spherical particles with diameters of 0.25 μm belong to a monodisperse polystyrene latex which was added to the sample to verify magnification and shadow angle.

Replication is a technique for studying the surface structure of a specimen. A thin, uniform carbon film is deposited onto the sample by vacuum evaporation. This replica film is then separated from the sample and examined with the electron microscope.[5,6]

The *scanning electron microscope*, developed during the late 1960s, uses an electron source and electromagnetic lenses to condense and focus the electron beam, just like the trans-

mission electron microscope. A narrow, finely focused, high-energy primary electron beam is made to impinge on the specimen surface. Back-scattered electrons, as well as emitted secondary electrons, photons and X-rays produced by impact ionization, are collected by a scintillation detector which produces an emission current. The current is amplified and regulates the intensity of a spot of light on a cathode-ray tube display. The incident beam is moved across the specimen surface by scanning coils while the display spot moves in synchronism across the display tube in TV fashion. The surface of the specimen is scanned by a raster or series of lines, each of which corresponds to a narrow surface strip. The brightness and contrast of each point observed on the display tube is related to a surface detail of the specimen and depends on the atomic number of the material being scanned, the angle which the surface element makes with respect to the incident beam, and on the shape, thickness and position of the surface element. Protrusions, holes and edges show up more distinctly than flat surfaces. The surface structure displayed on the cathode ray tube is recorded by photographing its screen. The details of surface structure and the unusually great depth of focus of scanning electron micrographs are illustrated by Figs 20-5 and 20-6.

The magnifying power of the scanning electron microscope ranges approximately from 40 to 100,000. The magnification is easily adjusted by changing the length of the scan line on the specimen relative to the length of the display line on the screen, which remains constant. For instance, if the beam scans a 1 mm × 1 mm specimen area and the image is displayed on a 10 cm × 10 cm screen, the magnification is 100. Reducing the area scanned to 0.1 mm × 0.1 mm increases the magnification to 1,000.

Unlike the transmission electron microscope, the scanning electron microscope examines surfaces regardless of the thickness of the specimens. It requires none of the lengthy specimen preparation techniques used with the former.[13]

Light Scattering by Colloidal Particles—The optical properties of a medium are determined by its refractive index. When the refractive index is uniform throughout, light will pass the medium undeflected. Whenever there are discrete variations in the refractive index caused by the presence of particles or by small-scale density fluctuations, part of the light will be scattered in all directions. An optical property characteristic of colloidal systems, called the *Tyndall beam*, is familiar to everyone in the case of aerosols. When a narrow beam of sunlight is admitted through a small hole into a darkened room, the presence of the minute dust particles suspended in air is revealed by bright flashing points.

A beam of light striking a particle polarizes the atoms and molecules of that particle, inducing dipoles which act as secondary sources and reemit weak light of the same wavelength as the incident light. This phenomenon is called *light scattering*. The scattered radiation propagates in all directions away from the particle. In a bright room, the light scattered by the dust particles is too weak to be noticeable.

Colloidal particles suspended in a liquid also scatter light. When an intense, narrowly defined beam of light is passed through a suspension, its path becomes visible because of the scattering of light by the particles in the beam. This Tyndall beam becomes most visible when viewed against a dark background in a direction perpendicular to the incident beam. The magnitude of the turbidity or opalescence depends on the nature, size and concentration of the particles. When clear mineral oil is dispersed in an equal volume of a clear aqueous surfactant solution, the resultant emulsion is milky white and opaque due to light scattering. Microemulsions, where the emulsified droplets are about 400 Å in diameter, ie, much smaller than the wavelength of visible light, are transparent and clear to the naked eye.

The *dark-field microscope* or *ultramicroscope*, which permits observation of particles much smaller than the wavelength of light, was the only means of detecting submicroscopic particles before the advent of electron microscopy. A special cardioid condenser produces a hollow cylinder of light and converges it into a hollow cone focused on the sample. The sample is at the apex of the cone, where the light intensity is high. After passing through the sample, the cone of light diverges and passes outside of the microscope objective. A homogeneous sample thus gives a dark field. A similar effect can be produced with a regular Abbe condenser outfitted with a central stop and a strong light source. Colloidal particles scatter light in all directions. Some of the scattered light enters the objective and shows up the particles as bright spots. Thus, even particles smaller than the wavelength of light can be detected, provided their refractive index differs sufficiently from that of the medium. Dissolved polymer molecules and highly solvated gel particles do not scatter enough light to become visible. Asymmetric particles like flat bentonite platelets give flashing effects as they rotate in Brownian motion, because they scatter more light with their basal plane perpendicular to the light beam than edgewise. Brownian motion, sedimentation, electrophoretic mobility, and the progress of flocculation can be studied with the dark-field microscope. Polydispersity can be estimated qualitatively because larger particles scatter more light and appear brighter. The resolving power of the ultramicroscope is no greater than that of the ordinary light microscope. Particles closer together than 0.2 μm appear as a single blur.

Turbidity may be used to measure the concentration of dispersed particles in two ways. In *turbidimetry*, a spectrophotometer or photoelectric colorimeter is used to measure the intensity of the light transmitted in the incident direction. Turbidity τ is defined by an equation analogous to Beer's law for the absorption of light (see Chapter 34),[5,6,8] namely,

$$\tau = \frac{1}{l} \ln \frac{I_0}{I_t}$$

where I_0 and I_t are the intensities of the incident and transmitted light beams, and l is the length of the dispersion through which the light passes.

If the dispersion is less turbid, the intensity of light scattered at 90° to the incident beam is measured with a *nephelometer*. Both methods require careful standardization with suspensions containing known amounts of particles similar to those to be measured. The concentration of colloidal dispersions of inorganic and organic compounds and of bacterial suspensions can thus be measured by their turbidity.

The turbidity or Tyndall effect of hydrophilic colloidal systems like aqueous solutions of gums, proteins and other polymers is far weaker than that of lyophobic dispersions. These solutions appear clear to the naked eye. Their turbidity can be measured with a photoelectric cell/photomultiplier tube and serves to determine the molecular weight of the solute.

The theory of light scattering was developed in detail by Lord Rayleigh. For white nonabsorbing nonconductors or dielectrics like sulfur and insoluble organic compounds, the equation obtained for spherical particles whose radius is small compared to the wavelength of light λ is[5-8]

$$I_s = I_0 \frac{4\pi^2 n_0^2 (n_1 - n_0)^2}{\lambda^4 d^2 c} (1 + \cos^2 \theta)$$

I_0 is the intensity of the unpolarized incident light; I_s is the intensity of light scattered in a direction making an angle θ

with the incident beam and measured at a distance d. The scattered light is largely polarized. The concentration c is expressed as the number of particles per unit volume. The refractive indices n_1 and n_0 refer to the dispersion and the solvent, respectively.

Since the intensity of scattered light is inversely proportional to the fourth power of the wavelength, blue light ($\lambda \simeq 4500$ Å) is scattered much more strongly than red light ($\lambda \simeq 6500$ Å). With incident white light, colloidal dispersions of colorless particles appear blue when viewed in scattered light, i.e., in lateral directions such as 90° to the incident beam. Loss of the blue rays due to preferential scattering leaves the transmitted light yellow or red. Preferential scattering of blue radiation sideways accounts for the blue color of the sky, sea, cigarette smoke, and diluted milk and for the yellow-red color of the rising and setting sun viewed head-on.

The particles in pharmaceutical suspensions, emulsions and lotions are generally larger than the wavelength of light λ. When the particle size exceeds $\lambda/20$, destructive interference between light scattered by different portions of the same particle lowers the intensity of scattered light and changes its angular dependence. Rayleigh's theory was extended to large and to strongly absorbing and conducting particles by Mie and to nonspherical particles by Gans.[1,3,5-8] By using appropriate precautions in experimental techniques and in interpretation, it is possible to determine an average particle size and even the particle size distribution of colloidal dispersions and coarser suspensions by means of turbidity measurements.[12]

Diffusion and Sedimentation—The molecules of a gas or liquid are engaged in a perpetual, random thermal motion which causes them to collide with one another and with the container wall billions of times per second. Each collision changes the direction and the velocity of the molecules involved. Dissolved molecules and suspended colloidal particles are continuously and randomly buffeted by the molecules of the suspending medium. This random bombardment imparts to solutes and particles an equally unceasing and erratic movement called *Brownian motion*, after the botanist Robert Brown who first observed it under the microscope with an aqueous pollen suspension. The Brownian motion of colloidal particles mirrors on a magnified scale the random movement of the molecules of the liquid or gaseous suspending medium, and represents a three-dimensional random walk.

Solute molecules and suspended colloidal particles undergo rotational and translational Brownian movement. For the latter, Einstein derived the equation

$$\bar{x} = \sqrt{2Dt}$$

where $\bar{x}$ is the mean displacement in the x-direction in time t and D is the *diffusion coefficient*. Einstein also showed that for spherical particles of radius r under conditions specified in Chapter 22 for the validity of Stokes' law and Einstein's law of viscosity,

$$D = \frac{RT}{6\pi\eta r N}$$

where R is the gas constant, T the absolute temperature, N Avogadro's number, and η the viscosity of the suspending medium.

The diffusion coefficient is a measure of the mobility of a dissolved molecule or suspended particle in a liquid medium. Representative values at room temperature, in cm^2/sec, are 4.7×10^{-6} for sucrose and 6.1×10^{-7} for serum albumin in water. With a diffusion coefficient of 1×10^{-7} cm^2/sec, Brownian motion causes a particle to move by an average distance of 1 cm in one direction in 58 days, by 1 mm in 14 hrs, and by 1 μm in 0.05 sec. Smaller molecules diffuse faster in a given medium. Assuming spherical shape, the radius of a serum albumin molecule is 35 Å and that of a sucrose molecule 4.4 Å. The ratio of the radii of the two molecules 35/4.4 = 7.9, is nearly identical with the inverse ratio of their diffusion coefficients in water, $4.7 \times 10^{-6}/6.1 \times 10^{-7} = 7.7$, in agreement with the above equation. Diffusion coefficients of steroids and other molecules of similar size dissolved in absorption bases based on petrolatum are generally in the 10^{-10} to 10^{-8} cm^2/sec range. Steroids have only slightly higher molecular weights than sucrose. Their much smaller diffusion coefficients are due to the much higher viscosity of the vehicle.

Brownian motion and convection currents maintain dissolved molecules and small colloidal particles in suspension indefinitely. As the particle size and r increase, the Brownian motion decreases; $\bar{x}$ is proportional to $r^{-1/2}$. Provided that the density of the particle d_P and of the liquid vehicle d_L are sufficiently different, larger particles have a greater tendency to settle out when $d_P > d_L$ or to rise to the top of the suspension when $d_P < d_L$ than smaller particles of the same material.

The rate of *sedimentation* is expressed by the Stokes' equation given in Chapter 22, which can be rewritten as

$$h = \frac{2(d_P - d_L)r^2 g t}{9\eta}$$

where h is the height through which a spherical particle settles in time t and g is the acceleration of gravity. The rate of sedimentation is proportional to r^2. Thus, with increasing particle size, the Brownian motion diminishes while the tendency to sediment increases. The two become equal for a critical radius when the distance h through which the particle settles equals the mean displacement $\bar{x}$ due to Brownian motion in the same time interval t.[15] In most pharmaceutical suspensions, sedimentation prevails. Intravenous vegetable oil emulsions do not tend to cream because the mean droplet size, *ca* 0.5 μm, is smaller than the critical radius.

Passive diffusion caused by a concentration gradient and carried out through Brownian motion is important in the release of drugs from topical preparations (see Chapter 88) and in the gastrointestinal absorption of drugs (see Chapter 37).

Viscosity—Most lyophobic dispersions have viscosities not much greater than that of the liquid vehicle. This holds true even at comparatively high volume fractions of the disperse phase unless the particles form continuous network aggregates throughout the vehicle, in which case yield values are observed. Most O/W and W/O emulsions have specific viscosities not much greater than those predicted by Einstein's modified law of viscosity (see Eq 11 of Chapter 22 and text). For instance, emulsions containing 40% v/v of the internal phase generally have viscosities only three to five times higher than that of the continuous phase. By contrast, the apparent viscosities of lyophilic dispersions, especially of polymer solutions, are several orders of magnitude greater than the viscosity of the solvent or vehicle even at concentrations of only a few percent solids. Lyophilic dispersions are also generally much more pseudoplastic or shear-thinning than lyophobic dispersions (see Chapter 22).

Electric Properties and Stability of Lyophobic Dispersions

Difference between Lyophilic and Lyophobic Dispersions—*Lyophilic* or solvent-loving solids are called hydrophilic if the solvent is water. Owing to the presence of high concentrations of hydrophilic groups, they dissolve or disperse spontaneously in water as far as is possible without breaking covalent bonds. Among hydrophilic groups are ionized ones which dissociate into highly hydrated ions like carboxylate, sulfonate or alkylammonium ions, and organic functional

groups like hydroxyl, carbonyl, amino, and imino which bind water through hydrogen bonding.

The free energy of dissolution or dispersion, ΔG_s, of hydrophilic solids includes a large negative (exothermic) heat or enthalpy of solvation, ΔH_s, and a large increase in entropy, ΔS_s. Since $\Delta G_s = \Delta H_s - T\Delta S_s$, ΔG_s has a large negative value: the dissolution of hydrophilic macromolecules and the dispersion of hydrophilic particulate solids in water occur spontaneously (see Chapter 16), overcoming the parallel increases in surface area and surface free energy. Dissolution and dispersion take place so that water can come into contact and interact with the hydrophilic groups of the solids (enthalpy of solvation), and to increase the number of available configurations of the macromolecules and particles (entropy increase).

The van der Waals energies of attraction between dissolved macromolecules or dispersed hydrophilic solid particles are smaller than ΔG_s and are, therefore, insufficient to cause separation of a solid polymer phase or agglomeration through flocculation or coagulation of the dispersed particles. Furthermore, the hydration layer surrounding dissolved macromolecules and dispersed particles forms a barrier preventing their close approach.

Hydrophobic solids and liquids such as organic compounds consisting largely of hydrocarbon portions with few if any hydrophilic functional groups, like cholesterol and other steroids, and some nonionized inorganic substances like sulfur, are hydrated slightly or not at all. Hence they do not disperse or dissolve spontaneously in water: ΔG_s is positive because of a positive (endothermic) ΔH_s term, making the reverse process (agglomeration) the spontaneous one. Aqueous dispersions of such hydrophobic solids or liquids can be prepared by physical means which supply the appropriate energy to the system (see above). They are unstable, however. The van der Waals attractive forces between the particles cause them to aggregate, since the solvation forces which promote dispersal in water are weak. If aqueous dispersions of hydrophobic solids are to resist reaggregation (coagulation and flocculation), they must be stabilized. Stabilizing factors include electric charges at the particle surface (due to dissociation of ionogenic groups of the solid or pertaining to adsorbed ions such as ionic surfactants) and the presence of adsorbed macromolecules or nonionic surfactants. These stabilizing factors do not alter the intrinsic thermodynamic instability of lyophobic dispersions: ΔG_s is still positive so that the reverse process of phase separation or aggregation is energetically favored over dispersal. They establish kinetic barriers which delay the aggregation processes almost indefinitely; the dispersed particles cannot come together close enough for the van der Waals attractive forces to produce coagulation.[5,8] These stabilization mechanisms are discussed below.

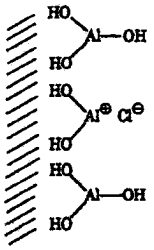
The reductions in surface area and surface free energy accompanying flocculation or coagulation are small because irregular solid particles, being rigid, touch only at a few points upon aggregation. The loose initial contacts may grow with time by sintering or recrystallization. Sintering consists of the "fusion" of primary particles into larger primary particles which propagates from initial small areas of contact. This recrystallization process is spontaneous because it decreases the specific surface area of the disperse solid and the surface free energy of the dispersion. Sintering is analogous to Ostwald ripening, the recrystallization process of transferring solid from colloidal to coarse particles discussed above. Low solubility and the presence of adsorbed surface-active substances retard both processes.

Origin of Electric Charges—Particles can acquire charges from several sources. In *proteins*, one end group of the polypeptide chain and aspartic and glutamic acid units contribute carboxylic acid groups, which are ionized into carboxylate ions in neutral to alkaline media. The other chain end group and lysine units contribute amino groups, arginine units contribute guanidine groups, and histidine units contribute imidazole groups. The nitrogen atoms of these groups become protonated in neutral to acid media. For electroneutrality, these cationic groups require anions, such as Cl^- if hydrochloric acid was used to make the medium acid and to supply the protons. The neutralizing ions, called counterions, dissociate from the ionogenic basic functional groups and can be replaced by other ions of like charge: they are not an integral part of the protein particle but are located in its immediate vicinity. The alkylammonium, guanidinium and imidazolium ions, which are attached to the protein molecule by covalent bonds, confer a positive charge to it. In neutral and alkaline media, Na^+, K^+, Ca^{2+}, and Mg^{2+} are among the counterions neutralizing the negative charges of the carboxylate groups. The latter are covalently attached to and constitute an integral part of the protein particle, conferring a negative charge to it.

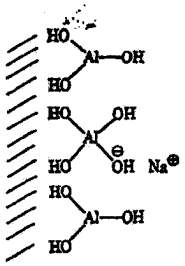
At an intermediate pH value, which ranges from 4.5 to 7 for the various proteins, the carboxylate anions and the alkylammonium, guanidinium, and imidazolium cations neutralize each other exactly. There is no need for counterions since the ionized functional groups which are an integral part of the protein molecule are in exact balance. At this pH value, called the *isoelectric point*, the protein particle or molecule is neutral: its electric charge is neither negative nor positive but zero.[5]

Many other organic polymers contain ionic groups and are, therefore, called *polyelectrolytes* (polymeric electrolytes or salts). Natural polysaccharides of vegetable origin such as acacia, tragacanth, alginic acid and pectin contain carboxylic acid groups, which are ionized in neutral to alkaline media. Agar and carrageen, as well as the animal polysaccharides heparin and chondroitin sulfate, contain sulfuric acid hemiester groups, which are strongly acidic and ionize even in acid media. Cellulosic polyelectrolytes include *sodium carboxymethylcellulose*, while synthetic carboxylated polymers include *carbomer*, a copolymer of acrylic acid.

Aluminum hydroxide, $Al(OH)_3$, is dissolved by acids and alkalis forming aluminum ions, Al^{3+}, and aluminate ions, $[Al(OH)_4]^-$, respectively. In neutral or weakly acid media, at acid concentrations too low to cause dissolution, an aluminum hydroxide particle has some positive charges attributable to incompletely neutralized positive Al^{3+} valences. The portion of the surface of an aluminum hydroxide particle represented schematically below has one such positive charge neutralized by a Cl^- counterion:



In weakly alkaline media, at base concentrations too low to transform the aluminum hydroxide particles completely into aluminate and dissolve them, they bear some negative charges due to the presence of a few aluminate groups. The portion of the particle surface represented schematically below has one such negative group neutralized by a Na^+ counterion:



At a pH of 8.5–9.1,[16,17] there are neither $[Al(OH)_2]^+$ nor $[Al(OH)_4]^-$ ions in the particle surface but only neutral $Al(OH)_3$ molecules. The particles have zero charge and therefore need no counterions for charge neutralization. This pH is the isoelectric point. In the case of inorganic particulate compounds such as aluminum hydroxide, it is also called zero point of charge.

Bentonite clay is a lamellar aluminum silicate. Each lattice layer consists of a sheet of hydrated alumina sandwiched between two silica sheets. Isomorphous replacement of Al^{3+} by Mg^{2+} or of Si^{4+} by Al^{3+} confers net negative charges to the thin clay lamellas in the form of cation-exchange sites resembling silicate ions built into the lattice. The counterions producing electroneutrality are usually Na^+ (sodium bentonite) or Ca^{2+} (calcium bentonite). The zero point of charge is probably close to that of quartz, silica gel and other silicates, namely, at a pH of about 1.5–2.

Silver iodide sols can be prepared by the reaction

$$AgNO_3 + NaI \rightarrow AgI(s) + NaNO_3$$

In the bulk of the silver iodide particles, there is a 1:1 stoichiometric ratio of Ag^+ to I^- ions. If the reaction is carried out with an excess silver nitrate, there will be more Ag^+ than I^- ions in the surface of the particles. The particles will thus be positively charged and the counterions surrounding them will be NO_3^-. If the reaction is carried out using an exact stoichiometric 1:1 ratio of silver nitrate to sodium iodide or with an excess sodium iodide, the surface of the particles will contain an excess I^- over Ag^+ ions.[5,6,8] The particles will be negatively charged, and Na^+ will be the counterions surrounding the particles and neutralizing their charges.

An additional mechanism through which particles acquire electric charges is by the adsorption of ions,[5–8] including ionic surfactants.

Electric Double Layers—The surface layer of a silver iodide particle prepared with an excess of sodium iodide contains more I^- than Ag^+ ions, whereas its bulk contains the two ions in exactly equimolar proportion. The aqueous solution in which this particle is suspended contains relatively high concentrations of Na^+ and NO_3^-, a lower concentration of I^-, and traces of H^+, OH^-, and Ag^+.

The negatively charged particle surface attracts positive ions from the solution and repels negative ions: the solution in the vicinity of the surface contains a much higher concentration of Na^+, which are the counterions, and a much lower concentration of NO_3^- ions than the bulk of the solution. A number of Na^+ ions equal to the number of excess I^- ions in the surface (ie, the number of I^- ions in the surface layer minus the number of Ag^+ ions in the surface layer) and equivalent to the net negative surface charge of a particle are pulled towards its surface. These counterions tend to stick to the surface, approaching it as closely as their hydration spheres permit (Helmholtz double layer), but the thermal agitation of the water molecules tends to disperse them throughout the solution. As a result, the layer of counterions surrounding the particle is spread out. The Na^+ concentration is highest in the immediate vicinity of the negative surface, where they form a compact layer called the Stern layer, and decreases with distance from the surface, throughout a diffuse layer called the Gouy-Chapman layer: the sharply defined negatively charged surface is surrounded by a cloud of Na^+ counterions required for electroneutrality. The combination of the two layers of oppositely charged ions constitutes an electric double layer. It is illustrated in the top part of Fig 20-9. The horizontal axis represents the distance from the particle surface in both the top and bottom parts.

The electric potential of a plane is equal to the work against electrostatic forces required to bring a unit electric charge from infinity (in this case, from the bulk of the solution) to that plane. If the plane is the surface of the particle, the potential is called surface or ψ_0 potential, which measures the total potential of the double layer. This is the thermodynamic potential which operates in galvanic cells. On moving away from the particle surface towards the bulk solution in the direction of the horizontal axis, the potential drops rapidly across the Stern layer because the Na^+ ions in the immediate vicinity of the surface screen Na^+ ions farther removed, in the diffuse part of the double layer, from the effect of the negative surface charge. The decrease in potential across the Gouy-Chapman layer is more gradual. The diffuse double layer gradually comes to an end as the composition approaches that of the bulk liquid where the anion concentration equals the cation concentration, and the potential approaches zero asymptotically. In view of the indefinite end point, the thickness δ of the diffuse double layer is arbitrarily assigned the value of the distance over which the potential at the boundary between the Stern and Gouy-Chapman layers drops to $1/e = 0.37$ of its value.[5–8] The thickness of double layers usually ranges from 10 to 1000 Å. It decreases as the concentration of electrolytes in solution increases, more rapidly for counterions of higher valence. δ is approximately equal to the reciprocal of the Debye-Hückel theory parameter κ discussed in Chapters 17 and 21.

Of practical importance, because it can be measured experimentally, is the electrokinetic or ζ (zeta) potential. In aqueous dispersion, even relatively hydrophobic inorganic particles and organic particles containing polar functional groups are surrounded by a layer of water of hydration attached to them by ion-dipole and dipole-dipole interaction. When a particle moves, this shell of bound water and all ions located inside it move along with the particle. Conversely, if water or a solution flows through a fixed bed of these solid particles, the hydration layer surrounding each particle remains stationary and attached to it. The electric potential at the plane of shear or slip separating the bound water from the free water is the ζ potential. It does not include the Stern layer and only that part of the Gouy-Chapman layer which lies outside the hydration shell. The various potentials are shown on the bottom part of Fig 20-9.

Stabilization by Electrostatic Repulsion—When two uncharged hydrophobic particles are in close proximity, they attract each other by van der Waals secondary valences, mainly by London dispersion forces. For individual atoms and molecules, these forces decrease with the seventh power of the distance between them. In the case of two particles, every atom of one attracts every atom of the other particle. Because the attractive forces are nearly additive, they decay much less rapidly with the interparticle distance as a result of this summation, approximately with the second or third power (see Chapter 21, particularly Eqs 13–16). Since energies of attraction are equal to force x distance, they decrease approximately with the first or second power of the distance. Therefore, whenever two particles approach each other closely, the attractive forces take over and cause them to adhere. Coagulation occurs as the primary particles aggregate into increasingly larger secondary particles or flocs.

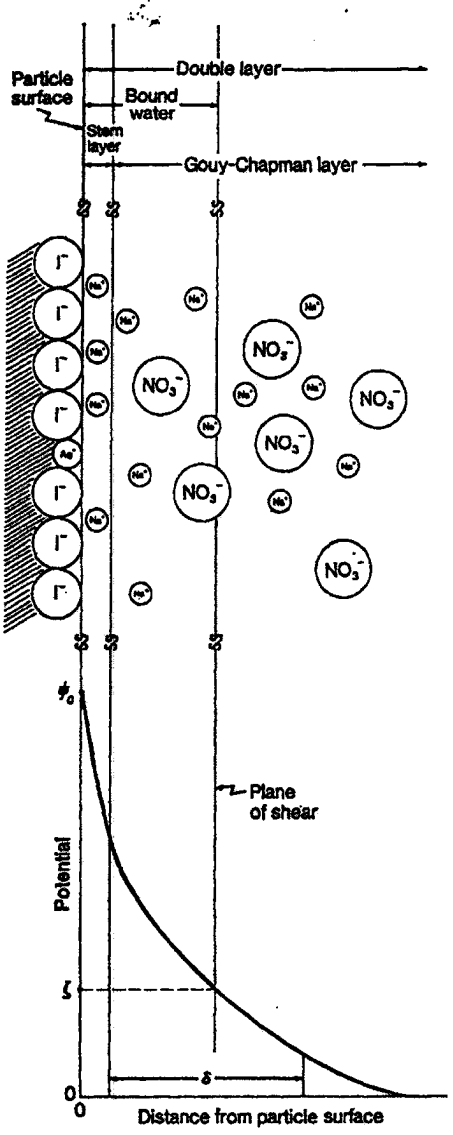


Fig 20-9. Electric double layer at the surface of a silver iodide particle (upper part) and the corresponding potentials (lower part). The distance from the particle surface, plotted on the horizontal axis, refers to both the upper and lower parts.

If the dispersion consists of two kinds of particles with positive and negative charges, respectively, the electrostatic attraction between oppositely charged particles is superimposed on the attraction by van der Waals forces, and coagulation is accelerated. If the dispersion contains only one kind, as is customary, all particles have surface charges of the same sign and density. In that case, electrostatic repulsion tends

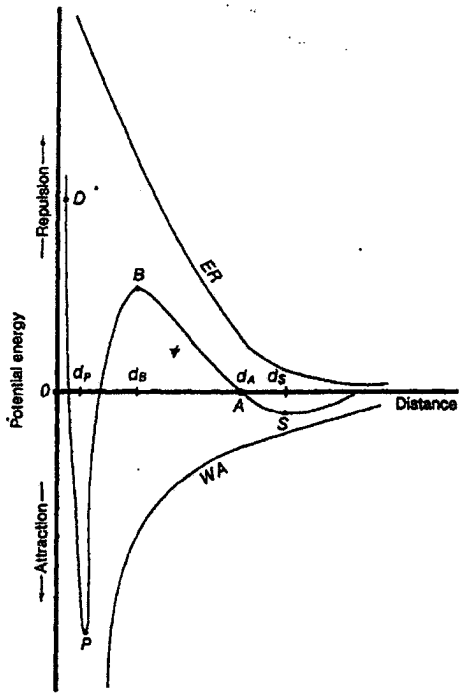


Fig 20-10. Curves representing the van der Waals energy of attraction (*WA*), the energy of electrostatic repulsion (*ER*), and the net energy of interaction (*DPBAS*) between two identical charged particles, as a function of the interparticle distance.

to prevent the particles from approaching closely enough to come within effective range of each other's van der Waals attractive forces, thus stabilizing the dispersion against interparticle attachments or coagulation. The electrostatic repulsive energy has a range of the order of δ.

A quantitative theory of the interaction between lyophobic disperse particles was worked out independently by Derjaguin and Landau in the USSR and by Verwey and Overbeek in the Netherlands in the early 1940s.[1,5–8,18] The so-called DLVO theory predicts and explains many but not all experimental data. Its refinement to account for discrepancies is still continuing.

The DLVO theory is summarized in Fig 20-10, where curve *WA* represents the van der Waals attractive energy which decreases approximately with the second power of the interparticle distance, and curve *ER* represents the electrostatic repulsive energy which decreases exponentially with distance. Because of the combination of these two opposing effects, attraction predominates at small and large distances whereas repulsion may predominate at intermediate distances. Negative energy values indicate attraction, and positive values repulsion. The resultant curve *DPBA*, obtained by algebraic addition of curves *WA* and *ER*, gives the total, net energy of interaction between two particles.

The interparticle attraction depends mainly on the chemical nature and particle size of the material to be dispersed. Once these have been selected, the attractive energy is fixed and cannot readily be altered. The electrostatic repulsion

depends on ψ_0 or the density of the surface charge and on the thickness of the double layer, both of which govern the magnitude of the ζ potential. Thus, stability correlates to some extent with this potential.[5] The ζ potential can be adjusted within wide limits by additives, especially ionic surfactants, water-miscible solvents, and electrolytes (see below). If the absolute value of the ζ potential is small, the resultant potential energy is negative and van der Waals attraction predominates over electrostatic repulsion at all distances. Such sols coagulate rapidly.

The two identical particles whose interaction is depicted in Fig 20-10 have a large (positive or negative) ζ potential resulting in an appreciable positive or repulsive potential energy at intermediate distances. They are on a collision course because of Brownian motion, convection currents, sedimentation, or because the dispersion is being stirred.

As the two particles approach each other, the two atmospheres of counterions surrounding them begin to interpenetrate or overlap at point A corresponding to the distance d_A. This produces a net repulsive (positive) energy because of the work involved in distorting the diffuse double layers and in pushing water molecules and counterions aside, which increases if the particles approach further. If the particles continue to approach each other even after most of the intervening solution of the counterions between them has been displaced, the repulsion between their surface charges increases the net potential energy of interaction to its maximum positive value at B. If the height of the potential energy barrier B exceeds the kinetic energy of the approaching particles, they will not come any closer than the distance d_B but move away from each other. A net positive potential energy of about 25 kT units usually suffices to keep them apart, rendering the dispersion permanently stable; k is the Boltzmann constant and T is the absolute temperature. At $T = 298°K$, this corresponds to 1×10^{-12} erg. The kinetic energy of a particle is of the order of kT.

On the other hand, if their kinetic energy exceeds the potential energy barrier B, the particles continue to approach each other past d_B, where the van der Waals attraction becomes increasingly more important compared to the electrostatic repulsion. Therefore, the net potential energy of interaction decreases to zero and then becomes negative, pulling the particles still closer together. When the particles touch, at a distance d_P, the net energy has acquired the large negative value P. This deep minimum in potential energy corresponds to a very stable situation in which the particles adhere. Since it is unlikely that enough kinetic energy can be supplied to the particles or that their ζ potential can be increased sufficiently to cause them to climb out of the potential energy well P, they are attached permanently to each other. When most or all of the primary particles agglomerate into secondary particles by such a process, the sol coagulates.

Any closer approach of two particles than the touching distance d_P is met with a very rapid rise in potential energy along PD because the solid particles would interpenetrate each other, causing atomic orbitals to overlap (Born repulsion).

Coagulation of Hydrophobic Dispersions—The height of the potential energy barrier and the range over which the electrostatic repulsion is effective (or the thickness of the double layer) determine the stability of hydrophobic dispersions. Both factors are reduced by the addition of electrolytes. The transition between a coagulating and a stable sol is gradual and depends on the time of observation. By using standard conditions, however, it is possible to classify a sol as either coagulated or coagulating, or as stable or fully dispersed.

To determine the value of the coagulating concentration of a given electrolyte for a given sol, a series of test tubes is filled with equal portions of the sol. Identical volumes of solutions of the electrolyte, of increasing concentration, are added with vigorous stirring. After some time at rest (eg, 2 hrs), the mixtures are agitated again. After an additional, shorter rest period (eg, ½ hr), they are inspected for signs of coagulation. The tubes can be classified into two groups, one showing no signs of coagulation and the other showing at least some signs, eg, visible flocs. Alternatively, they can be classified into one group showing complete coagulation and the other containing at least some deflocculated colloid left in the supernatant. In either case, the separation between the two classes is quite sharp. The intermediate agitation breaks the weakest interparticle bonds and brings small particles in contact with larger ones, thus increasing the sharpness of separation between coagulation and stability. After repeating the experiment with a narrower range of electrolyte concentrations, the coagulation value c_{CV} of the electrolyte, ie, the lowest concentration at which it coagulates the sol, is established with good reproducibility.[2,6,8]

Typical c_{CV} data for a silver iodide sol prepared with an excess of iodide are listed in Table III. The following conclusions can be drawn from the left half of Table III:

1. The c_{CV} does not depend on the valence of the anion, since nitrate and sulfate of the same metal have nearly identical values.
2. The differences among the c_{CV}'s of cations with the same valence are relatively minor. However, there is a slight but significant trend of decreasing c_{CV} with increasing atomic number in the alkali and in the alkaline earth metal groups. Arranging these cations in the order of decreasing c_{CV} produces the *Hofmeister* or *lyotropic series*. It governs many other colloidal phenomena, including the effect of salts on the temperature of gelation and the swelling of aqueous gels and on the viscosity of hydrosols, the salting out of hydrophilic colloids, the cation exchange on ion-exchange resins, and the permeability of membranes towards salts. The series is also observed in many phenomena involving only small atoms or ions and true solutions, including the ionization potential and electronegativity of metals, the heats of hydration of cations, the size of the hydrated cations, the viscosity, surface tension, and infrared spectra of salt solutions, and the solubility of gases therein. For monovalent cations, the lyotropic series is

$$Li^+ > Na^+ > K^+ > NH_4^+ > Rb^+ > Cs^+$$

A similar lyotropic series exists for anions.[2,5-7]

The lithium ion has a higher c_{CV} than the cesium ion because it is more extensively hydrated, so that Li^+ (aq), including the hydration shell, is larger than Cs^+ (aq). Owing to its smaller size, the hydrated cesium ion can approach the negative particle surface more closely than the hydrated lithium ion. Moreover, because of its greater electron cloud, the Cs^+ ion is more polarizable than the Li^+ ion. Therefore, it is more strongly adsorbed in the Stern layer, which makes it a more effective coagulating agent.

Table III—Coagulation Values for Negative Silver Iodide Sol[a]

Electrolyte	c_{CV}, mM/L	Electrolyte	c_{CV}, mM/L
$LiNO_3$	165	$AgNO_3$	0.01
$NaNO_3$	140	½ $(C_{12}H_{25}NH_3)_2SO_4$	0.7
½ Na_2SO_4	141	Strychnine nitrate	1.7
KNO_3	136	½ Morphine sulfate	2.5
½ K_2SO_4	138		
$RbNO_3$	126		
Mean	141		
$Mg(NO_3)_2$	2.60	Quinine sulfate	0.7
$MgSO_4$	2.57		
$Ca(NO_3)_2$	2.40		
$Sr(NO_3)_2$	2.38		
$Ba(NO_3)_2$	2.26		
$Zn(NO_3)_2$	2.50		
$Pb(NO_3)_2$	2.43		
Mean	2.45		
$Al(NO_3)_3$	0.067		
$La(NO_3)_3$	0.069		
$Ce(NO_3)_3$	0.069		
Mean	0.068		

[a] From Ref 1 and unpublished data.

3. The coagulation values depend primarily on the valence of the counterions, decreasing by one to two orders of magnitude for each increase of one in their valence (Schulze-Hardy rule). According to the DLVO theory, the coagulation values vary inversely with the sixth power of the valence of the counterions. For mono-, di-, and trivalent counterions, they should be in the ratio

$$\frac{1}{1^6}:\frac{1}{2^6}:\frac{1}{3^6} \quad \text{or} \quad 100:1.6:0.14$$

The mean c_{CV}'s of Table III are 141 : 2.45 : 0.068, or 100 : 1.7 : 0.05, in satisfactory agreement with the DLVO theory.

The following conclusion can be drawn from the right half of Table III:

4. The cations on the right side of Table III constitute obvious exceptions to the preceding. Ag^+ is the potential-determining counterion. *Potential-determining ions* are those whose concentration determines the surface potential. When silver nitrate is added to the negative silver iodide dispersion, some of its silver ions are incorporated into the negatively charged surface of the particles and lower the magnitude of their charge by reducing the excess of I^- ions in the surface. Thus, silver salts are exceptionally effective coagulating agents because they reduce the magnitude of the ψ_0 as well as of the ζ potential. *Indifferent salts*, which reduce only the latter, require much higher salt concentrations for comparable reductions in the ζ potential. The other potential-determining ion of silver iodide is I^-. Alkali iodides have higher c_{CV}'s than 141 millimole/liter because they supply iodide ions which enter the surface layer of the silver iodide particles and increase its excess of I^- over Ag^+ ions, thereby making ψ_0 more negative. Bromide and chloride ions act similarly but less effectively.

The principal potential-determining ion for proteins is H^+; those for aluminum hydroxide are OH^- (and hence H^+) and Al^{3+}, but also Fe^{3+} and Cr^{3+} which form mixed hydroxides with Al^{3+}.

5. The cationic surfactant in Table III and the alkaloidal salts, which also behave as such, constitute the second exception to the Schulze-Hardy rule. Surface-active compounds contain hydrophilic and hydrophobic moieties in the same molecule, the latter being hydrocarbon portions which by themselves are water-insoluble. Their dual nature causes these compounds to accumulate in interfaces. Dodecylammonium and alkaloidal cations displace inorganic monovalent cations from the Stern layer of a negatively charged silver iodide particle because they are attracted to it not only by electrostatic forces like sodium ions but also by van der Waals forces between their hydrocarbon moieties (dodecyl chains in the case of the dodecylammonium ions) and the solid. Because they are strongly adsorbed from solution onto the surface and do not tend to dissociate from it, surface-active cations are very effective in reducing the ζ potential of the negative silver iodide particles, ie, they have lower c_{CV} than purely inorganic cations of the same valence.

6. Anionic surfactants like those containing lauryl sulfate ions also have a tendency to be adsorbed at solid-liquid interfaces. However, because of electrostatic repulsion between the negatively charged surface of silver iodide particles whose surface layer contains an excess iodide ions and the surface-active anions, adsorption usually does not occur below the critical micelle concentration (see below). If such adsorption does occur, it increases the density of negative charges in the particle surface, raising the c_{CV} of anionic surfactants above that corresponding to their valence.

Ionic solids with surface layers containing the ionic species in near proper stoichiometric balance, and most water-insoluble organic compounds have relatively low surface charge densities. They adsorb ionic surfactants of like charge from solution even at low concentrations, which increases their surface charge densities and the magnitude of their ζ potentials, stabilizing their aqueous dispersions.

The addition of water-miscible solvents such as alcohol, glycerin, propylene glycol or polyethylene glycols to aqueous dispersions lowers the dielectric constant of the medium. This reduces the thickness of the double layer and, therefore, the range over which electrostatic repulsion is effective, and lowers the size of the potential energy barrier. Addition of solvents to aqueous dispersions tends to coagulate them. At concentrations too low to cause coagulation by themselves, solvents make the dispersions more sensitive to coagulation by added electrolytes, ie, they lower the c_{CV}'s.

Progressive addition of the salt of a counterion of high valence reduces the ζ potential of colloidal particles gradually to zero. Eventually, the sign of the ζ potential may be inverted and its magnitude may increase again, but in the opposite direction. The ψ_0 and ζ potentials of aqueous sulfamerazine suspensions are negative above their isoelectric points; those of bismuth subnitrate are positive. As discussed in Chapter 21, the addition of Al^{3+} to the former and of PO_4^{3-} to the latter in large enough amounts inverts the sign of their ζ potentials; their ψ_0 potentials remain unchanged. Surface-active ions of opposite charge may also produce such charge inversion.

The superposition of the van der Waals attractive energy with its long-range effectiveness and the electrostatic repulsive energy with its intermediate-range effectiveness frequently produces a shallow minimum (designated S in Fig 20-10) in the resultant energy-distance curve at interparticle distances d_S several times greater than δ. If this minimum in potential energy is small compared to kT, Brownian motion prevents aggregation. For large particles such as those of many pharmaceutical suspensions and for particles which are large in one or two dimensions (rods and plates), the *secondary minimum* may be deep enough to trap them at distances d_S from each other. This requires a depth of several kT units. Such fairly long-range and weak attraction produces loose aggregates or flocs which can be dispersed by agitation or by removal or reduction in the concentration of flocculating electrolytes.[1,6–8,18] This reversible aggregation process involving the secondary minimum is called *flocculation*. By contrast, aggregation in the deep primary minimum P, called *coagulation*, is irreversible.

Stabilization by Adsorbed Surfactants—As discussed in Chapter 19, surfactants tend to accumulate at interfaces because of their amphiphilic nature. This process is an *oriented physical adsorption*. Surfactant molecules arrange themselves at the interface between water and an organic solid or liquid of low polarity in such a way that the hydrocarbon chain is in contact with the surface of the solid particle or sticks inside the oil droplet while the polar headgroup is oriented towards the water phase. This orientation removes the hydrophobic hydrocarbon chain from the bulk of the water, where it is unwelcome because it interferes with the hydrogen bonding among the water molecules, while leaving the polar headgroup in contact with water so that it can be hydrated.

Fig 20-11*A* shows schematically that at low surfactant concentration and low surface coverage, the hydrocarbon chains of the adsorbed surfactant molecules lie flat against the solid surface. At higher surfactant concentrations, the surfactant molecules are adsorbed in the upright position to permit the adsorption of more surfactant per unit surface area. Fig 20-11*B* shows a nearly close-packed monolayer of adsorbed surfactant molecules. The terminal methyl groups of their hydrocarbon tails are in contact with the hydrophobic surface and the hydrocarbon tails are in lateral contact with each other. London dispersion forces promote attraction between both types of adjoining groups. The polar headgroups protrude into the water and are hydrated.

The adsorption of ionic surfactants increases the charge density and the ζ potential of the disperse particles. These two parameters are low for organic substances lacking ionic or strongly polar groups. The increase in electrostatic repulsion among the nonpolar organic particles due to adsorption of surface-active ions stabilizes the dispersion against coagulation. This "charge stabilization" is described by the DLVO theory.

Most water-soluble nonionic surfactants are polyoxyethylated (see Chapter 19): Each molecule consists of a hydrophobic hydrocarbon chain combined with a hydrophilic polyethylene glycol chain, eg $CH_3(CH_2)_{15}(OCH_2CH_2)_{10}OH$. Hydration of the 10 ether groups and of the terminal hydroxyl group renders the surfactant molecule water-soluble. It adsorbs at the interface between a hydrophobic solid and water, with the hydrocarbon moiety adhering to the solid surface and the polyethylene glycol moiety protruding into the water, where it is hydrated. The particle surface is thus surrounded

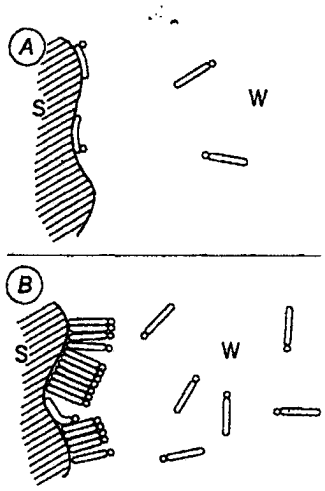


Fig 20-11. Schematic representation of the physical adsorption of surfactant molecules at a hydrophobic solid (S)/water (W) interface. Cylindrical portions and spheres represent hydrocarbon chains and polar headgroups of the surfactant molecules, respectively. (A) low surfactant concentration/low surface coverage; (B) near critical micelle concentration/surface coverage near saturation.

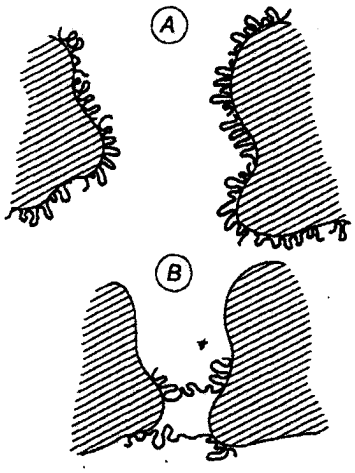


Fig 20-12. Protective action (A) and sensitization (B) of sols of hydrophobic particles by adsorbed polymer chains.

by a thin layer of hydrated polyethylene glycol chains. This hydrophilic shell forms a steric barrier which prevents close contact between particles and, hence, coagulation ("steric stabilization"). Nonionic surfactants also reduce the sensitivity of hydrophobic dispersions towards coagulation by salts, ie, they increase the coagulation values.[19]

In a flocculated dispersion, groups of several particles are agglomerated into flocs. Frequently, the particles of a floc are in physical contact. When a surfactant is added to a flocculated sol, the dissolved surfactant molecules become adsorbed at the surface of the particles. Surfactant molecules tend to pry apart flocs by wedging themselves between the particles at their areas of contact. This action opens up for surfactant adsorption additional surface area that was previously blocked by adhesion of another solid surface. The breaking up of flocs or secondary particles is defined above as deflocculation or peptization.

Ophthalmic suspensions should be deflocculated because the large particle size of flocs causes eye irritation. Parenteral suspensions should be deflocculated to prevent flocs from blocking capillary blood vessels and hypodermic syringes, and to reduce tissue irritation. Deflocculated suspensions tend to cake, however, ie, the sediment formed by gravitational settling is compact and may be hard to disperse by shaking. Caking in oral suspensions is prevented by controlled flocculation as discussed in Chapter 21.

Stabilization by Adsorbed Polymers—Water-soluble polymers are adsorbed at the interface between water and a hydrophobic solid if they have some hydrophobic groups that limit their water solubility and render them amphiphilic and, hence, surface-active. Such polymers also tend to accumulate at the air-water interface and lower the surface tension of the aqueous phase. A high concentration of ionic groups in polyelectrolytes tends to eliminate surface activity and the tendency to adsorb at interfaces, because the polymer is excessively water-soluble. An example is *sodium carboxymethylcellulose*. *Polyvinyl alcohol* is very water-soluble due to the high concentration of hydroxyl groups and does not adsorb extensively at interfaces. Polyvinyl alcohol is manufactured by the hydrolysis of polyvinyl acetate, which is water-insoluble. Incomplete hydrolysis of, say, only 85% of the acetyl groups produces a copolymer which is water-soluble but surface-active as well. Other surface-active polymers include methylcellulose, hydroxypropyl cellulose, high-molecular-weight polyethylene glycols (polyethylene oxides), and proteins. The surface activity of proteins is due to the presence of hydrophobic groups in the side chains at concentrations too low to cause insolubility in water. Proteins are denatured upon adsorption at air-water and solid-water interfaces.

The long, chain-like polymer molecules are adsorbed from solution onto solid surfaces in the form of loops projecting into the aqueous phase, as shown in Fig 20-12*A*, rather than lying flat against the solid substrate. Only a small portion of the chain segments of an adsorbed macromolecule is actually in contact with and adheres directly to the surface. Because of its great length, however, there are enough of such areas of contact to anchor the adsorbed macromolecule firmly onto the solid. Fig 20-11 is drawn on a much more expanded scale than Fig 20-12.

The sol particles are surrounded by a layer consisting of the adsorbed polymer chains, the water of hydration associated with them, and water trapped mechanically inside the chain loops. This sheath is an integral part of the particle surface. The layers of adsorbed polymer prevent the particles from approaching each other closely enough for the interparticle attraction by London dispersion forces to produce coagulation. These forces are effective only over very small interparticle distances of less than twice the thickness of the adsorbed polymer layer.

The mechanisms of *steric stabilization* by which adsorbed nonionic macromolecules prevent coagulation of hydrophobic sols (*protective action*) are also operative in the stabilization of sols by nonionic surfactants. The difference between adsorbed nonionic surfactants and adsorbed polymers is that the hydrophilic polyethylene glycol moieties of the adsorbed surfactant molecules protruding into water resemble the chain ends of the adsorbed macromolecules rather than their looped

segments. The following protective mechanisms are operative:

1. The layer of adsorbed polymer and enmeshed water surrounding the particles forms a *mechanical* or *steric barrier* between them that prevents the close interparticle approach necessary for coagulation. At dense surface coverage, these layers are somewhat elastic. They may be dented by a collision between two particles but tend to spring back.
2. When two particles approach so closely that their adsorbed polymer layers overlap, the chain loops of the two opposing layers compress and mix with or interpenetrate each other. The resulting restriction to the freedom of motion of the chain segments in the overlap region produces a negative entropy change which tends to make the free energy change for the reduction in interparticle distance required for coagulation positive. The reverse process of disentanglement of the two opposing adsorbed polymer layers resulting from separation of the particles occurs because it is energetically more favorable. The particles are thus prevented from coagulation by *entropic repulsion* through the mechanism of *entropic stabilization* of the sol. This mechanism predominates when the concentration of polymer in the adsorbed layer is low.
3. As the polymer layers adsorbed on two approaching particles overlap and compress or interpenetrate each other, more polymer segments become crowded into a given volume of the aqueous region between the particles. The increased polymer concentration in the overlap region causes a local increase in osmotic pressure, which is relieved by an influx of water. This influx to dilute the polymer loops pushes the two particles apart, preventing coagulation.
4. If the adsorbed polymer has some ionic groups, stabilization by electrostatic repulsion or charge stabilization described above is added to the three steric stabilization mechanisms to prevent a close interparticle approach and, hence, coagulation.
5. The adsorption of water-soluble polymers changes the nature of the surface of the hydrophobic particles to hydrophilic, resulting in an increased resistance of the sol to coagulation by salts.[20]

The water-soluble polymers whose adsorption stabilizes hydrophobic sols and protects them against coagulation are called *protective colloids*. *Gelatin* and *serum albumin* are the preferred protective colloids for stabilizing parenteral suspensions because of their biocompatibility. These two polymers, as well as casein (milk protein), dextrin (partially hydrolyzed starch) and vegetable gums like acacia and tragacanth are metabolized in the human body. Cellulose derivatives and most synthetic protective colloids such as *povidone* are not biotransformed. Because of this and because of their large molecular size, polymers pertaining to the last two categories are not absorbed but excreted intact when they are administered in an oral dosage form.

A semiquantitative assessment of the stabilizing efficiency of protective colloids is the *gold number*, developed by Zsigmondy. It is the largest number of milligrams of a protective colloid which, when added to 10 mL of a special standardized gold sol, just fails to prevent the change in color from red to blue on addition of 1 mL of 10% NaCl solution. The gold sol contains 0.0058% gold with a particle size of about 250 Å. Coagulation by sodium chloride causes the color change. Representative gold numbers are 0.005–0.01 for gelatin, 0.01 for casein, 0.02–0.5 for egg albumin, 0.15–0.5 for acacia, and 1–7 for dextrin.[8,9] Gelatin is a more effective protective colloid than acacia and dextrin because the presence of some hydrophobic side groups makes it more surface active and causes more extensive adsorption from solution. Other protective numbers are based on different hydrophobic disperse solids, eg, silver, Prussian blue, sulfur, ferric oxide. The ranking of different protective colloids depends somewhat on the substrate. When formulating a disperse dosage form, one should measure the protective action on the actual solid hydrophobic phase to be dispersed as a sol.

Sensitization is the opposite of protective action, namely, a decrease in the stability of hydrophobic sols. It is brought about by some protective colloids, at concentrations well below those at which they exert a protective action. A protective colloid may, at very low concentrations, flocculate a sol in the absence of added salts and/or lower the coagulation values of the sol.

In the case of nonionic polymers or of polyelectrolytes with charges of the same sign as the sol, flocculation is the result of the bridging mechanism illustrated in Fig 20-12*B*. At very low polymer concentrations, there are not nearly enough polymer molecules present to cover each sol particle completely. Since the particle surfaces are largely bare, a single macromolecule may be adsorbed on two particles, bridging the gap between them and pulling them close together. Flocs of several particles are formed when one particle is bridged or connected to two or more other particles by two or more polymer molecules adsorbed jointly on two or possibly even three particles. Such flocculation usually occurs over a narrow range and at very low values of polymer concentrations. At higher concentrations, when enough polymer is available to cover the surface of all particles completely, bridging is unlikely to occur and the adsorbed polymer stabilizes or peptizes the sol.[20]

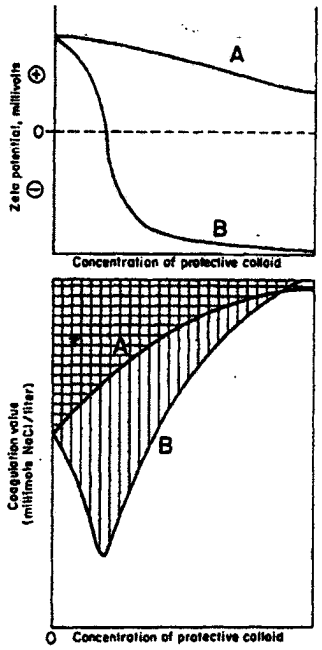


Fig 20-13. Protective action and sensitization: Polymer A exerts protective action at all concentrations while Polymer B sensitizes at low concentrations and stabilizes at high concentrations. Horizontal and vertical hatching indicates region of flocculation for a sol treated with various concentrations of Polymers A and B, respectively. Clear region underneath indicates sol is deflocculated.

The nonionic Polymer A of Fig 20-13 stabilizes the sol at all concentrations. Neither sensitization by bridging nor by charge neutralization is observed. The reason that Polymer A lowers the positive ζ potential of the sol slightly is that increasing amounts of adsorbed polymer chains gradually shift the plane of shear outwards, away from the positively charged surface. If Polymer A were a cationic polyelectrolyte, the ζ potential–protective colloid concentration plot would gradually rise with increasing polymer adsorption rather than drop.

If the polymer has ionic groups of charge opposite to the charge of the sol particles, limited adsorption neutralizes the charge of the particles, reducing their ζ potential to near zero.

With stabilization by electrostatic repulsion thus inoperative, and steric stabilization ineffective because of low surface coverage with adsorbed polymer, the sol either coagulates by itself or is coagulated by very small amounts of sodium chloride. At higher polymer concentrations and more extensive adsorption, charge reversal of the particles to the sign of the charge of the polyelectrolyte reactivates charge stabilization and adds steric stabilization, increasing the coagulation value of the sol well above the initial value before polymer addition.

For example, a partly hydrolyzed polyacrylamide with about 20% of ammonium acrylate repeat units is an anionic polyelectrolyte. At the ppm level, the polymer flocculates aluminum hydroxide sols at a pH of 6–7, where the sols are positively charged and the polyelectrolyte is fully ionized. At a polymer concentration of 1:10,000, the sol becomes negatively charged because extensive polymer adsorption introduces an excess of $-COO^-$ groups over $\equiv Al^+$ ions into the particle surface. Steric stabilization plus electrostatic repulsion make the sol more stable against flocculation by salts than it was before the polyacrylamide addition.

Polymer B in Fig 20-13 illustrates this example. The curve in the lower plot indicates sensitization, with the coagulation value of sodium chloride lowered by as much as 60%. Zeta potential measurements can distinguish between sensitization by bridging and by charge neutralization. The charge reversal caused by adsorption of Polymer B shown in the upper plot pinpoints charge neutralization as the cause of sensitization. If Polymer B had a ζ potential–polymer concentration plot similar to Polymer A, sensitization would be ascribed to bridging.

Even water-soluble polymers which are too thoroughly hydrophilic to be adsorbed by hydrophobic sol particles can stabilize those sols. Their thickening action slows down Brownian motion and sedimentation, giving the particles less opportunity to come into contact and hence retarding flocculation.

Electrokinetic Phenomena—When a dc electric field is applied to a dispersion, the particles move towards the electrode of charge opposite to that of their surface. The counterions located inside their hydration shell are dragged along while the counterions in the diffuse double layer outside the plane of slip, in the free or mobile solvent, move towards the other electrode. This phenomenon is called *electrophoresis*. If the charged surface is immobile, as is the case with a packed bed of particles or a tube filled with water, application of an electric field causes the counterions in the free water to move towards the opposite electrode, dragging solvent with them. This flow of liquid is called *electroosmosis*, and the pressure produced by it, *electroosmotic pressure*. Conversely, if the liquid is made to flow past charged surfaces by applying hydrostatic pressure, the displacement of the counterions in the free water produces a potential difference between the two ends of the tube or bed called *streaming potential*.

The three phenomena depend on the relative motion of a charged surface and of the diffuse double layer outside the plane of slip surrounding that surface. The major part of the diffuse double layer is within the free solvent and can, therefore, move along the surface.[5-8] All three electrokinetic phenomena measure the identical ζ potential, which is the potential at the plane of slip.

The particles of pharmaceutical suspensions and emulsions are visible in the microscope or ultramicroscope, as are bacteria, erythrocytes and other isolated cells, latex particles, and many contaminant particles in pharmaceutical solutions. Their ζ potential is conveniently measured by *microelectrophoresis*. A potential difference E applied between two electrodes dipping into the dispersion and separated by a distance d produces the potential gradient or field strength E/d, expressed in v/cm. From the average velocity v of the particles, measured with the eyepiece micrometer of a microscope and a stopwatch, the ζ potential is calculated by the Smoluchowski equation

$$\zeta = \left(\frac{4\pi\eta}{D}\right)\left(\frac{v}{E/d}\right) = \left(\frac{4\pi\eta}{D}\right)u$$

The electrophoretic mobility $u = v/(E/d)$ is the velocity in a potential gradient of 1 v/cm. Particle size and shape do not affect the ζ potential according to the above equation. However, if the particle radius is comparable to δ or smaller (in which case the particles cannot be detected in a microscope), the factor 4 is replaced by 6. The viscosity η and the dielectric constant D refer to the aqueous medium in the double layer and cannot be measured directly.[21] Using the values for water at 25°, expressing the velocity in microns/sec and the electrophoretic mobility in (microns/sec)/(volts/cm), and converting into the appropriate units reduces the Smoluchowski equation to $\zeta = 12.9\eta u$, with ζ given in millivolts (mV). If the particle surface has appreciable conductance, the ζ potential calculated by this equation may be low.[6,21] Dispersions of hydrophobic particles with ζ potentials below 20–30 mV are frequently unstable and tend to coagulate. On the other hand, values as high as ±180 mV have been reported for the ζ potential.[1,5]

The chief experimental precautions in microelectrophoresis measurements are:

1. Electroosmosis causes liquid to flow along the walls of the cell containing the dispersion. This in turn produces a return flow in the center of the cell. The microscope must be focused on the stationary boundary between the two liquid layers flowing in opposite directions in order to measure the true velocity of the particles.
2. Only in very dilute dispersions is it possible to follow the motion of single particles in the microscope field and to measure their velocity. Since the ζ potential depends largely on the nature, ionic strength, and pH of the suspending medium, dispersions should be diluted not with water but with solutions of composition identical to their continuous phase, eg, with their own serum separated by ultrafiltration or centrifugation. The Zeta-Meter is a commercial microelectrophoresis apparatus of easy, fast and reproducible operation.

When the particles cannot be observed individually with a microscope or ultramicroscope, other electrophoresis methods are employed.[5,8,22,23] In *moving boundary electrophoresis*, the movement of the boundary formed between a sol or solution and the pure dispersion medium in an electric field is studied. If the disperse phase is colorless, the boundary is located by the refractive index gradient (Tiselius apparatus, used frequently with protein solutions). If several species of particles or solutes with different mobilities are present, each will form a boundary moving with a characteristic velocity. Unlike microelectrophoresis, this method permits the identification of different colloidal components in a mixture, the measurement of the electrophoretic mobility of each, and an estimation of the relative amounts present.

Zone electrophoresis theoretically permits the complete separation of all electrophoretically different components, requires much smaller samples than moving boundary electrophoresis, and can be performed in simpler and less expensive equipment. The method avoids convection by supporting the solution in an inert and porous solid like filter paper, cellulose acetate membrane, agar, starch or polyacrylamide gels cut into strips, or disks or columns of polyacrylamide gel.

A strip of filter paper or gel is saturated with a conducting buffer solution and a few microliters of the solution being analyzed is deposited as a spot or narrow band. A potential difference is applied between the ends of the strip which are in contact with the electrode compartments. The spot or band spreads and unfolds as each component migrates towards one or the other electrode at a rate determined primarily by its electrophoretic mobility. Evaporation of water due to the heating effect of the electric current may be mini-

mized by immersing the strip in a cooling liquid or sandwiching it between impervious solid sheets. After a sufficient time has elapsed to afford good separation, the strip is removed and dried. The position of the spots or bands corresponding to the individual components is detected by color reactions or radioactive counting.

Zone electrophoresis is applied mainly in analysis and for small-scale preparative separations. It does not permit mobility measurements. Because several samples can be analyzed simultaneously (in parallel strips or gel columns), because only minute amounts of sample are needed, and because the equipment is simple and easy to operate, zone electrophoresis is widely used to study the proteins in blood serum, erythrocytes, lymph and cerebrospinal fluid, saliva, gastric and pancreatic juices, and bile.

Immunodiffusion combined with electrophoresis is called *immunoelectrophoresis*.[22,24] The proteins in a fluid, including the antigens, are first separated by gel electrophoresis. A longitudinal trench is then cut along one or both sides of the gel strip near the edge in the direction of the electrophoresis axis. The trench is filled with the antibody solution. On standing, antibody and antigen proteins diffuse in all directions, including towards each other. Precipitation occurs along an elliptical arc (precipitin band) wherever an antigen meets its specific antibody. The precipitin bands are either visible directly or may be developed by staining. Since diseases frequently produce abnormal electrophoretic patterns in body fluids, zone electrophoresis and immunoelectrophoresis are convenient and powerful diagnostic techniques.

Isoelectric focusing[23,25] is a novel method using electrophoresis to separate proteins according to their isoelectric points. At pH values equal to their isoelectric points, proteins do not migrate in an electric field because their net charge is zero. In a liquid column on which a pH gradient is imposed, different species arrange themselves so that the protein with the highest isoelectric point will be located nearest to the cathode, which is immersed in the solution of a strong base. The protein with the lowest isoelectric point will be located nearest to the anode, which is immersed in the solution of a strong acid. The other proteins settle into intermediate positions, where the pH values are intermediate and equal to their isoelectric points.

Hydrophilic Dispersions

Most liquid disperse systems of pharmaceutical interest are aqueous. Therefore, most lyophilic colloidal systems discussed below consist of hydrophilic solids dissolved or dispersed in water. Most of the products mentioned below are official in the USP or NF, where more detailed descriptions may be found, also elsewhere in this text.

Hydrophilic colloids can be divided into particulate and soluble materials. The latter are water-soluble linear or branched polymers dissolved molecularly in water. Their aqueous solutions are classified as colloidal dispersions because the individual molecules are in the colloidal particle size range, exceeding 50 or 100 Å. Particulate or corpuscular hydrophilic colloidal dispersions are formed by solids which swell and are peptized in water but whose primary particles do not dissolve or break down into individual molecules or ions. One subdivision of particulate hydrophilic colloids is comprised of dispersions of cross-linked polymers whose linear, uncross-linked analogues are water-soluble.

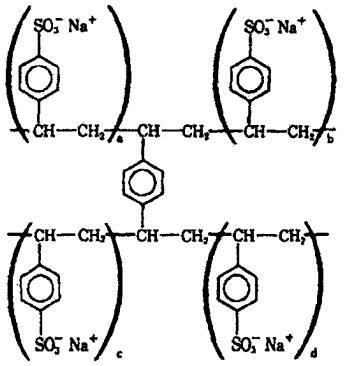
Particulate Hydrophilic Dispersions

The disperse phase of these sols consists of solids which in water swell and break up spontaneously into particles of colloidal dimensions. The disperse particles have high specific surface areas and are, therefore, extensively hydrated. They have characteristic shapes. If the attraction between individual particles is strong, the dispersions have yield values at relatively low solids content.

Bentonite is an aluminum silicate crystallizing in a layer structure (see above), with individual lamellas 9.4 Å thick. Their top and bottom surfaces are sheets of oxygen ions from silica plus an occasional sodium ion neutralizing a silicate ion-exchange site. The clay particles consist of stacks of these lamellas. Water penetrates inside the stacks between lamellas to hydrate the oxygen ions, causing extensive swelling. Bentonite particles in bentonite magma consist of single lamellas and packets of a few lamellas with intercalated water. The specific surface area amounts to several hundred square meters per gram. *Kaolin* also has a layer structure, but does not swell in water because water does not intercalate between individual lattice layers. Kaolin plates dispersed in water are, therefore, much thicker than those of bentonite, ca 0.04–0.2 μm. In kaolin, hydrated alumina lattice planes alternate with silica planes. Thus, one of the two external surfaces of a kaolin plate consists of a sheet of oxygen ions from silica, the other is a sheet of hydroxide ions from hydrated alumina. Both surfaces are well hydrated. Magnesium aluminum silicate (*Veegum*) is a clay similar to bentonite but contains magnesium; it is white whereas bentonite is gray.

Additional hydrophilic particles producing colloidal dispersions in water are listed below. *Colloidal silicon dioxide* consists of roughly spherical particles covered with siloxane and silanol groups (pages 278–279). *Titanium dioxide* is a white pigment with excellent covering power due to its high refractive index. *Microcrystalline cellulose* (page 277) is hydrophilic because of the hydroxyl and ether groups in the surface of the cellulose crystals. Gelatinous precipitates of hydrophilic compounds such as *aluminum hydroxide gel*, *aluminum phosphate gel*, and *magnesium hydroxide* consist of coarse flocs produced by agglomeration of the colloidal particles formed in the initial stage of the precipitation. They possess large internal surface areas, which is one of the reasons why the first two are used as substrates for adsorbed vaccines and toxoids.

Cross-linked Polymers—The polymers discussed below are polyelectrolytes, ie, they contain ionic groups and would be soluble in water in the absence of cross-linking. For instance, *sodium polystyrene sulfonate* is a copolymer of about 92% styrene and 8% divinylbenzene, which is sulfonated and neutralized to produce the cation-exchange resin



Chains a–b and c–d are water-soluble linear polymer chains. They are cross-linked or bound together via a phenylene group as shown. There are many such cross-links tieing every chain to two or more other chains, so that every atom in a grain of ion-exchange resin is bound to every other atom by primary,

covalent bonds. The grains swell in water until the cross-links are strained but do not dissolve, because this would involve the rupture of primary valence bonds. Swelling renders the ion-exchange sites in the interior of a grain accessible to the gastrointestinal fluids. Partial exchange of Na^+ by K^+ followed by excretion of the used resin in the feces reduces hyperkalemia resulting from acute renal failure. Partial replacement of Na^+ by H^+ could reduce acidosis.

Cholestyramine resin is an anion-exchange resin containing the same backbone of cross-linked polystyrene, but substituted with $—CH_2—N^+(CH_3)_3Cl^-$ instead of sodium sulfonate. Part of the chloride anions is exchanged or replaced by bile salt anions, which are thus eliminated in the feces bound to the resin grains rather than reabsorbed. *Colestipol hydrochloride* is another orally administered anion-exchange resin used to increase the fecal excretion of bile salts. It is an extensively cross-linked, insoluble but permeable copolymer made from diethylenetriamine, tetraethylenepentamine, and epichlorohydrin. Strong cation- and anion-exchange resins are used as sustained-release vehicles for basic and acid drugs, respectively (see Chapter 92).

Polycarbophil is a copolymer of acrylic acid cross-linked with a small amount of divinyl glycol. The weakly acidic carboxyl groups are not ionized in the strongly acid environment of the stomach but only in the more nearly neutral intestines. Therefore, swelling by osmotic influx of water occurs mostly in the intestines, where imbibition of water decreases the fluidity of stools associated with diarrhea. Among natural polymers, tragacanth consists of ½ of a water-soluble fraction, tragacanthin, and ⅔ of a gel fraction called bassorin which swells in water but does not dissolve. Starch consists of ⅙ of a fraction, soluble in hot water, called amylose. The remainder, amylopectin, merely absorbs water and swells. It owes its insolubility to extensive branching rather than cross-linking.

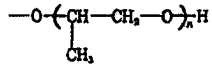
Soluble Polymers as Lyophilic Colloids

Most hydrophilic colloidal systems used in dosage forms are molecular solutions of water soluble, high molecular weight polymers. The polymers are either linear or slightly branched but not cross-linked.

Classifications—According to their origin, water-soluble polymers are divided into three classes. *Natural polymers* include polysaccharides (acacia, agar, heparin sodium, pectin, sodium alginate, tragacanth, xanthan gum) and polypeptides (casein, gelatin, protamine sulfate). Of these, agar and gelatin are only soluble in hot water.

Cellulose derivatives are produced by chemical modification of cellulose obtained from wood pulp or cotton to produce soluble polymers. *Cellulose* is an insoluble, linear polymer of glucose repeat units in the ring or pyranose form joined by β-1,4 glucosidic linkages. Each glucose repeat unit (except for the two terminal ones) contains a primary hydroxyl group on the No 6 carbon and two secondary hydroxyls on No 2 and 3 carbons. The primary hydroxyl is more reactive. Chemical modification of cellulose consists in reactions or substitutions of the hydroxyl groups. The extent of such reactions is expressed as *degree of substitution* (DS), namely, the number of substituted hydroxyl groups per glucose residue. The highest value is DS = 3.0. Fractional values are the rule because the DS is averaged over a multitude of glucose residues. A DS value of 0.6 indicates that some glucose repeat units are unsubstituted while others have one or even two substituents.

Soluble cellulose derivatives are listed below. The DS values correspond to the pharmaceutical grades. The groups shown are the replacements for the hydrogen atoms of the cellulosic hydroxyls. Official derivatives are *methylcellulose* (DS = 1.65–1.93), $—O—CH_3$ and *sodium carboxymethylcellulose* (DS = 0.60–1.00), $—O—CH_2—COO^-Na^+$. *Hydroxyethyl cellulose* (DS ≅ 1.0), $—O\text{-}(CH_2CH_2—O)_n\text{-}H$ and *hydroxypropyl cellulose* (DS ≅ 2.5) are manufactured by the addition of ethylene oxide and propylene oxide, respectively, to alkali-treated cellulose. The value of n is about 2.0 for the former and not much greater than 1.0 for the latter. *Hydroxypropyl methylcellulose* is prepared by reacting alkali-treated cellulose first with methyl chloride to introduce methoxy groups (DS = 1.1–1.8) and then with propylene oxide to introduce propylene glycol ether groups (DS = 0.1–0.3). In general, the introduction of hydroxypropyl groups into cellulose reduces the water solubility somewhat while promoting the solubility in polar organic solvents like short-chain alcohols, glycols and some ethers.



The molecular weight of native cellulose is so high that soluble derivatives of approximately the same degree of polymerization would dissolve too slowly, and their solutions would be excessively viscous even at concentrations of 1% and less. Controlled degradation is used to break the cellulose chains into shorter segments, reducing the viscosity of the solutions of the corresponding soluble derivatives. Commercial grades of a given cellulose derivative such as sodium carboxymethylcellulose come in various molecular weights or viscosity grades as well as with various degrees of substitution, offering the pharmacist a wide selection.

Official cellulose derivatives which are insoluble in water but soluble in some organic solvents include *ethylcellulose* (DS = 2.2–2.7), $—O—C_2H_5$; *cellulose acetate phthalate* (DS = 1.70 for acetyl and 0.77 for phthalyl); and *pyroxylin* or cellulose nitrate (DS ≅ 2), $—O—NO_2$. *Collodion*, a 4.0% w/v solution of pyroxylin in a mixture of 75% (v/v) ether and 25% (v/v) ethyl alcohol, constitutes a lyophilic colloidal system.

The third class, water soluble *synthetic polymers*, consists mostly of vinyl derivatives including *polyvinyl alcohol*, *povidone* or polyvinylpyrrolidone, and *carbomer* (*Carbopol*), a copolymer of acrylic acid. High molecular weight polyethylene glycols are also called *polyethylene oxides*.

A second classification of hydrophilic polymers is based on their charge. *Nonionic* or uncharged polymers include methylcellulose, hydroxyethyl and hydroxypropyl cellulose, ethylcellulose, pyroxylin, polyethylene oxide, polyvinyl alcohol and povidone. *Anionic* or negatively charged *polyelectrolytes* include the following carboxylated polymers: acacia, alginic acid, pectin, tragacanth, xanthan gum and carbomer at pH values leading to ionization of the carboxyl groups; sodium alginate and sodium carboxymethylcellulose; also polypeptides at pH values above their isoelectric points, eg, sodium caseinate. A stronger acid group is sulfuric acid, which exists as a monoester in agar and heparin and as a monoamide in heparin. *Cationic* or positively charged *polyelectrolytes* are rare. Examples are polypeptides at pH values below their isoelectric points. Protamines are strongly basic due to a high arginine content, with isoelectric points around pH 12, eg protamine sulfate.

Gel Formation—As described in Chapter 22 and illustrated in Fig 22-7-*A*, the flexible chains of dissolved polymers interpenetrate and are entangled because of the constant Brownian motion of their segments. The chains writhe and forever change their conformations. Each chain is encased in a sheath of solvent molecules that solvate its functional groups. In the case of aqueous solutions, water molecules are hydrogen-bonded to the hydroxyl groups of polyvinyl alcohol, hydroxyl groups and ether links of polysaccharides, ether links of polyethylene oxide or polyethylene glycol, amide groups of polypeptides and povidone, and carboxylate groups of anionic polyelectrolytes. The envelope of water of hydration

prevents chains segments in close proximity from touching and attracting one another by interchain hydrogen bonds and van der Waals forces as they do in the solid state. The slippage of solvated chains past one another when the solution flows is lubricated by the free solvent between their solvation sheaths.

Factors that lower the hydration of dissolved macromolecules reduce or thin out the sheath of hydration separating adjacent chains. When the hydration is low, contiguous chains tend to attract one another by secondary valence forces including hydrogen bonds and van der Waals forces. Hydrophobic bonding makes an important contribution to interchain attraction between polypeptide chains even in solution. Van der Waals forces and hydrogen bonds thus establish weak and reversible cross-links between chains at their points of contact or entanglement, bringing about phase separation or precipitation.

Most water-soluble polymers have higher solubilities in hot than in cold water and tend to precipitate on cooling, as the sheaths of hydration surrounding adjacent chains become too sparse to prevent interchain attraction. Dilute solutions separate into a solvent phase practically free of polymer and a viscous liquid phase containing practically all of the polymer but still a large excess of solvent. This process is called *simple coacervation* and the polymer-rich liquid phase a *coacervate.*[26] If the polymer solution is concentrated enough and/or the temperature low enough, cooling causes the formation of a continuous network of precipitating chains attached to one another through weak cross-links consisting of interchain hydrogen bonds and van der Waals forces at the points of mutual contact. Segments of regularly sequenced polymer chains even associate laterally into crystalline bundles or *crystallites.* Irregular chain structures as found in random copolymers, randomly substituted cellulose ethers and esters, and highly branched polymers like acacia prevent crystallization during precipitation from solution. Chain entanglements provide the sole temporary cross-links in those cases. The network of associated polymer chains immobilizes the solvent and causes the solution to set to a gel. Gelatinous precipitates or highly swollen flocs may separate when cooling more dilute polymer solutions.

Besides the chemical nature of polymer and solvent, the three most important factors causing phase separation, precipitation and gelation of polymer solutions are temperature, concentration and molecular weight. Lower temperatures, higher concentrations and higher molecular weights promote gelation and produce stronger gels.

For a typical *gelatin*, 10% solutions acquire yield values and begin to gel at about 25°, 20% solutions at about 30°, and 30% solutions at about 32°. The *gelation* is reversible: the gels liquefy when heated above these temperatures. Gelation is rarely observed above 34° regardless of concentration, so that gelatin solutions do not gel at 37°. Conversely, gelatin will dissolve readily in water at body temperature. The gelation temperature or gel point of gelatin is highest at the isoelectric point, where the attachment between adjacent chains by coulombic attraction or ionic bonds between carboxylate ions and alkylammonium, guanidinium or imidazolium groups is most extensive. Since the carboxyl groups are not ionized at gastric pH, interchain ionic bonds are practically nonexistent, and interchain attraction is limited to hydrogen bonds and van der Waals forces. The gelation temperature or the melting point of gelatin gels depends more strongly on temperature and concentration than on pH.[27] The combination of an acid pH considerably below the isoelectric point and a temperature of 37° completely prevents the gelation of gelatin solutions. Conversely, these two conditions promote rapid dissolution of gelatin capsules in the stomach. Agar and pectic acid solutions set to gels at only a few percent of solids.

Unlike most water-soluble polymers, methylcellulose, hydroxypropyl cellulose and polyethylene oxide are more soluble in cold than in hot water. Their solutions therefore tend to gel on heating (*thermal gelation*).

When dissolving powdered polymers in water, temporary gel formation often slows the process down considerably. As water diffuses into loose clumps of powder, their exterior frequently turns to a cohesive gel of solvated particles encasing dry powder. Such blobs of gel dissolve very slowly because of their high viscosity and the low diffusion coefficient of the macromolecules. Especially for large-scale dissolution, it is helpful to disperse the polymer powder in water before it can agglomerate into lumps of gel. In order to permit dispersion to precede hydration and to prevent temporary gel formation, the polymer powders are dispersed in water at temperatures where the solubility of the polymer is lowest. Most polymer powders, such as sodium carboxymethylcellulose, are dispersed with high shear in *cold* water before the particles can hydrate and swell to sticky gel grains agglomerating into lumps. Once the powder is well dispersed, the solution is heated with moderate shear to about 60° for fastest dissolution. Because methylcellulose hydrates most slowly in hot water, the powder is dispersed with high shear in ⅛ to ⅓ of the required amount of water heated to 80–90°. Once the powder is finely dispersed, the rest of the water is added cold or even as ice, and moderate stirring causes prompt dissolution. For maximum clarity, fullest hydration and highest viscosity, the solution should be cooled to 0°–10° for about an hour.

The following are two alternative methods for preventing the formation of gelatinous lumps upon addition of water. The powder is prewetted with a water-miscible organic solvent such as ethyl alcohol or propylene glycol that does not swell the polymer, in the proportion of from three to five parts solvent to each part of polymer. If other nonpolymeric powdered adjuvants are to be incorporated into the solution, these are dry-blended with the polymer powder. The latter should comprise ¼ or less of the blend for best results.

A pharmaceutical application of *gelation* in a nonaqueous medium is the manufacture of *Plastibase* or *Jelene*, which consists of 5% of a low-molecular-weight polyethylene and 95% of mineral oil. The polymer is soluble in mineral oil above 90°, which is close to its melting point. When the solution is cooled below 90°, the polymer precipitates and causes gelation. The mineral oil is immobilized in the network of entangled, and adhering, insoluble polyethylene chains which probably even associate into small crystalline regions. Unlike petrolatum, this gel can be heated to about 60° without substantial loss in consistency.

Large increases in the concentration of polymer solutions may lead to precipitation and gelation. One way of effectively increasing the concentration of aqueous polymer solutions is to add inorganic salts. The salts will bind part of the water of the polymer solution in order to become hydrated. Competition for water of hydration dehydrates the polymer molecules and precipitates them, causing gelation. This phenomenon is called *salting out*. Because of its high solubility in water, ammonium sulfate is often used by biochemists to precipitate and separate proteins from dilute solution. To the pharmacist, salting out usually represents an undesirable problem. It is reversible, however, and subsequent addition of water redissolves the precipitated polymers and liquefies their gels. Salting out may cause the polymer to separate as a concentrated and viscous liquid solution or simple coacervate rather than as a solid gel.

The effectiveness of electrolytes to salt out, precipitate or gel hydrophilic colloidal systems depends on how extensively the electrolytes are hydrated. The *Hofmeister* or *lyotropic series* arranges ions in the order of increasing hydration and increasing effectiveness in salting out hydrophilic colloids. The series, for monovalent cations, is

$$Cs^+ < Rb^+ < NH_4^+ < K^+ < Na^+ < Li^+$$

and for divalent cations,

$$Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+}$$

This series also arranges the cations in the order of decreasing coagulating power or increasing coagulation values for negative hydrophobic sols (see Table III) and of increasing ease of their displacement from cation exchange resins: K^+ displaces Na^+ and Li^+. For anions, the lyotropic series in the order of decreasing coagulating power and decreasing effectiveness in salting out is

$$F^- > \text{citrate}^{3-} > HPO_4^{2-} > \text{tartrate}^{2-} > SO_4^{2-} > \text{acetate}^- > Cl^- > NO_3^- > ClO_3^- > Br^- > ClO_4^- > I^- > CNS^-$$

Iodides and thiocyanates and to a lesser extent bromides and nitrates actually tend to increase the solubility of polymers in water, salting them in.[1,3,5–7,9] These large polarizable anions destructure water, reducing the extent of hydrogen bonding among water molecules and thereby making more of the hydrogen-bonding capacity of water available to the solute. Most salts except nitrates, bromides, perchlorates, iodides and thiocyanates raise the temperature of precipitation or gelation of most hydrophilic colloidal solutions or their gel melting points. Exceptions among hydrophilic colloids are methylcellulose, hydroxypropyl cellulose and polyethylene oxide whose gelation temperatures or gel points and gel melting points are lowered by salting out.

Hydrophobic aqueous dispersions are coagulated by electrolytes at 0.0001–0.1 *M* concentrations (see Table III). Moreover, the coagulation is irreversible, ie, removal of the coagulating salt does not allow the coagulum to be redispersed, because the hydrophobic sols are intrinsically unstable. By contrast, most hydrophilic sols require electrolyte concentrations of 1 *M* or higher for precipitation. Their precipitation or gelation can be reversed, and the polymer redissolved by removing the salt through dialysis or by adding more water. Hydrophilic colloids disperse or dissolve spontaneously in water, and their sols are intrinsically stable.

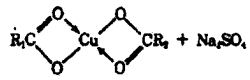
Most of the hydrophilic and water-soluble polymers mentioned above are only slightly soluble or insoluble in alcohol. Addition of alcohol to their aqueous solutions may cause precipitation or gelation because (i) alcohol is a nonsolvent or precipitant, lowering the dielectric constant of the medium, and (ii) alcohol tends to dehydrate the hydrophilic solute. Alcohol lowers the concentrations at which electrolytes salt out hydrophilic colloids. Phase separation through the addition of alcohol to an aqueous polymer solution may cause coacervation, ie, the separation of a concentrated viscous liquid phase, rather than precipitation or formation of a gel. Sucrose also competes for water of hydration with hydrophilic colloids, and may cause phase separation. However, most hydrophilic sols tolerate substantially higher concentrations of sucrose than of electrolytes or alcohol. Lower viscosity grades of a given polymer are usually more resistant to electrolytes, alcohol and sucrose than grades of higher viscosity and higher molecular weights.

Whenever hydrophilic colloidal dispersions undergo irreversible precipitation or gelation, chemical reactions are involved. Neither dilution with water nor heating nor attempts to remove the gelling or precipitating agent by washing or dialysis will liquefy those gels or redissolve the gelatinous precipitates formed at lower polymer concentrations. Carboxyl groups are not ionized in strongly acid media. If a polymer owes its solubility to the ionization of these weakly acid groups, reducing the pH of its solution below 3 may lead to precipitation or gelation. This is observed with such carboxylated polymers as many gums, sodium carboxymethylcellulose and carbomer. Hydrogen carboxymethylcellulose swells and disperses but does not dissolve in water. Neutralization to higher pH values returns the carboxyl groups to their ionized state and reverses the gelation or precipitation.

Only the sodium, potassium, ammonium and triethanolammonium salts of carboxylated polymers are well soluble in water. In the case of carboxymethylcellulose, salts with heavy metal cations (silver, copper, mercury, lead) and trivalent cations (aluminum, chromic, ferric) are practically insoluble. Salts with divalent cations, especially of the alkaline earth metals, have borderline solubilities. Generally, higher degrees of substitution tend to increase the tolerance of the carboxymethylcellulose to salts.

Precipitation or gelation occur due to metathesis when inorganic salts of heavy or trivalent cations are mixed with alkali metal salts of carboxylated polymers in solution. For instance, if a soluble copper salt is added to a solution of sodium carboxymethylcellulose, the double decomposition can be written schematically as

$$R_1COO^-Na^+ + R_2COO^-Na^+ + CuSO_4 \longrightarrow$$



$+ Na_2SO_4$

R_1 and R_2 represent two carboxymethylcellulose chains which are cross-linked by a chelated copper ion. Dissociation of the cupric carboxylate complex is negligible.

Association Colloids

Solution Properties and Micelle Formation of Surfactants

Surface activity characterizes compounds which, while soluble in a given liquid, tend to accumulate in the interfaces between this liquid and air, another immiscible liquid, or a solid. Because of their high concentration in these interfaces, surface-active compounds lower the corresponding interfacial tensions or free energies. This discussion deals mainly with aqueous media, where some soluble high polymers such as methylcellulose and vinyl methyl ether–maleic anhydride copolymer and many small molecules such as soaps and quaternary ammonium salts are surface-active. Because the small molecules in dilute solutions tend to associate into aggregates (micelles) of equivalent diameters in the 30–100 Å range, ie, of colloidal dimensions, they are called *association colloids*. Other names are *surfactants* or *surface-active agents*.[6,28–30]

Surfactants are amphiphiles because they contain a hydrocarbon (hydrophobic and lipophilic) portion and one or more ionic or otherwise strongly hydrophilic groups in the same molecule (see Chapters 19 and 21). This dual nature causes them to be preferentially adsorbed at air-water, oil-water, and solid-water interfaces, forming oriented monolayers in which the hydrophilic groups are in the aqueous phase and the hydrocarbon chains are pointed towards the air, in contact with the solid surface, or immersed in the oil phase.

As increasing amounts of a solid surfactant are dissolved in a beaker full of water and its concentration in solution increases, its monolayers adsorbed at the air-water and glass-water interfaces become more and more crowded until they are so tightly packed that further occupancy requires excessive compression of the surfactant molecules already in the two monolayers (saturation adsorption, see Chapter 19). Further increments in the amount of dissolved surfactant beyond that concentration cause amounts equivalent to the new molecules to aggregate into micelles. This process begins at a characteristic concentration called the *critical micelle concentration*

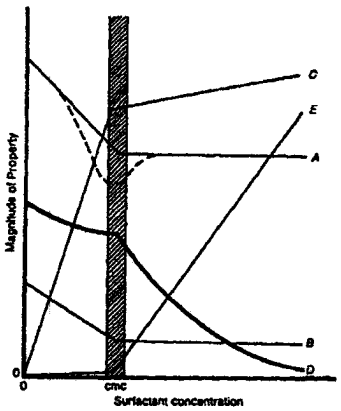


Fig 20-14. Effect of surfactant concentration and micelle formation on various properties of the aqueous solution of an ionic surfactant. *A:* Surface tension; *B:* interfacial tension; *C:* osmotic pressure; *D:* equivalent conductivity; *E:* solubility of compound with very low solubility in pure water. (Courtesy, Schott H, Martin AN, in Dittert LW, ed: *American Pharmacy*, 7th ed, Lippincott, Philadelphia, Chap 8, 1974).

(cmc). From then on, the concentration of monomeric or nonassociated surfactant molecules hardly increases, rising only slightly above the cmc, but the concentration of micellar or associated molecules increases in direct proportion to the increase in overall surfactant concentration. In dilute solutions, the micelles are approximately of the same size; increments in surfactant concentration merely increase their number.

The magnitudes of several properties of surfactant solutions are plotted against concentration in Fig 20-14. All curves undergo profound changes in slope over a narrow range of concentration which defines the cmc. The reason is that the bulk of the surfactant molecules present at concentrations in excess of the cmc are aggregated into micelles. Since the number of solute particles increases much more slowly with concentration above the cmc than below it, so do the colligative properties like osmotic pressure. The surface and interfacial tensions of surfactant solutions decrease rapidly until the cmc is reached, but remain nearly constant at higher concentrations. Surface-active impurities sometimes produce a minimum in the surface tension–concentration curve in the vicinity of the cmc, shown as a dotted line in curve *A* of Fig 20-14. The equivalent conductivity of ionic surfactant solutions, obtained by dividing the specific electric conductivity by the concentration expressed as equivalents/cm^3, decreases at a faster rate above the cmc than below. This indicates that micelles transport electricity more slowly than nonassociated surfactant ions.[28,30]

The partial molal volume of the surfactant and the relative viscosity and turbidity of its solutions increase faster with increasing concentration above the cmc than below it. Aggregates, because of their larger size, engender more resistance to flow than nonassociated surfactant molecules. The higher turbidity results from the increased amount of light scattered by the comparatively large micelles. However, to the naked eye, solutions above and below the cmc appear equally clear. The rate of increase of the refractive index of surfactant solutions with concentration is higher below than above the cmc.

The shape of micelles formed in dilute surfactant solutions is approximately spherical (see Fig 20-15*A*). The polar headgroups of the surfactant molecules are arranged in an outer spherical shell whereas their hydrocarbon chains are oriented towards the center, forming a spherical core for the micelle. The hydrocarbon chains are randomly coiled and entangled; the micellar interior has a nonpolar, liquid-like character resembling a liquid normal paraffin such as dodecane. In the micelles of polyoxyethylated nonionic surfactants, the polyoxyethylene moieties are oriented outwards and permeated by water while the hydrocarbon moieties form an "oil droplet" core as in ionic micelles (see Fig 20-15*B*).[28-33]

This arrangement is energetically favorable all around. The hydrophilic headgroups, located externally, are in contact with water and remain extensively hydrated. The hydrocarbon moieties are removed from the aqueous medium and partly shielded from contact with water by the polar headgroups. They no longer interfere with hydrogen bonding among water molecules. This interference is the reason why surfactant molecules are pushed out of aqueous media towards interfaces. The hydrocarbon tails of the surfactant molecules, located in the interior of the micelle, interact with one another by weak London–van der Waals forces (hydrophobic bonding).

Micelles are not static aggregates but dissociate, regroup, and reassociate rapidly. The half-life of micelles of ionic surfactants in the absence of additives is a small fraction of one second. Nonionic micelles dissociate much less rapidly.

Representative values of the critical micelle concentration and of the aggregation number, ie, the number of surfactant molecules/micelle, are listed in Table IV.[28,31-34] Cmc values depend primarily on whether the surfactant is ionic or nonionic. The lateral electrostatic repulsion of the charged headgroups located in the periphery of ionic micelles is an obstacle to micelle formation, deferring it to higher surfactant concentrations. Whether the surfactant is anionic (negatively charged headgroups) or cationic (positively charged headgroups) is of secondary importance in determining the cmc. The addition of simple salts reduces these repulsive forces and, therefore, lowers the cmc of ionic surfactants.

Within a homologous series, the cmc decreases regularly with increasing length of the hydrocarbon chain and, therefore, with increasing surface activity of the surfactant (Traube's rule). As is seen in Table IV, each additional methylene group approximately halves the cmc. For polyoxyethylated nonionic surfactants, the cmc increases with increasing hydrophile-lipophile balance (defined in Chapter 21) and with decreasing temperature.

The size of a micelle or its aggregation number is governed largely by geometric factors. The radius of the hydrocarbon core cannot exceed the length of the extended hydrocarbon chain of the surfactant molecule. Therefore, increasing the chain length or ascending a homologous series increases the aggregation number of spherical micelles. For surfactants whose hydrocarbon portion is a single normal alkyl chain, the maximum aggregation numbers consistent with spherical shape are approximately 27, 39, 54, 72, and 92 for C_8, C_{10}, C_{12}, C_{14}, and C_{16}, respectively. The micelles of sodium lauryl sulfate in the saline solution and of the nonionic surfactants of Table IV are too large to be spherical. The nonionic surfactants form larger micelles at the higher temperature because of lower hydration, ie, the system is moving in the direction of the cloud point. Micellar diameters range from 25 Å for sodium octane sulfonate to 60 Å for the equivalent spherical diameter of the 10 ethylene oxide adduct of dodecanol at 25°. The micelles are thus at the lower end of the particle size range for colloids.

On increasing the surfactant concentration beyond a few percent, on adding electrolytes in the case of ionic surfactants, and on raising the temperature in the case of nonionic sur-

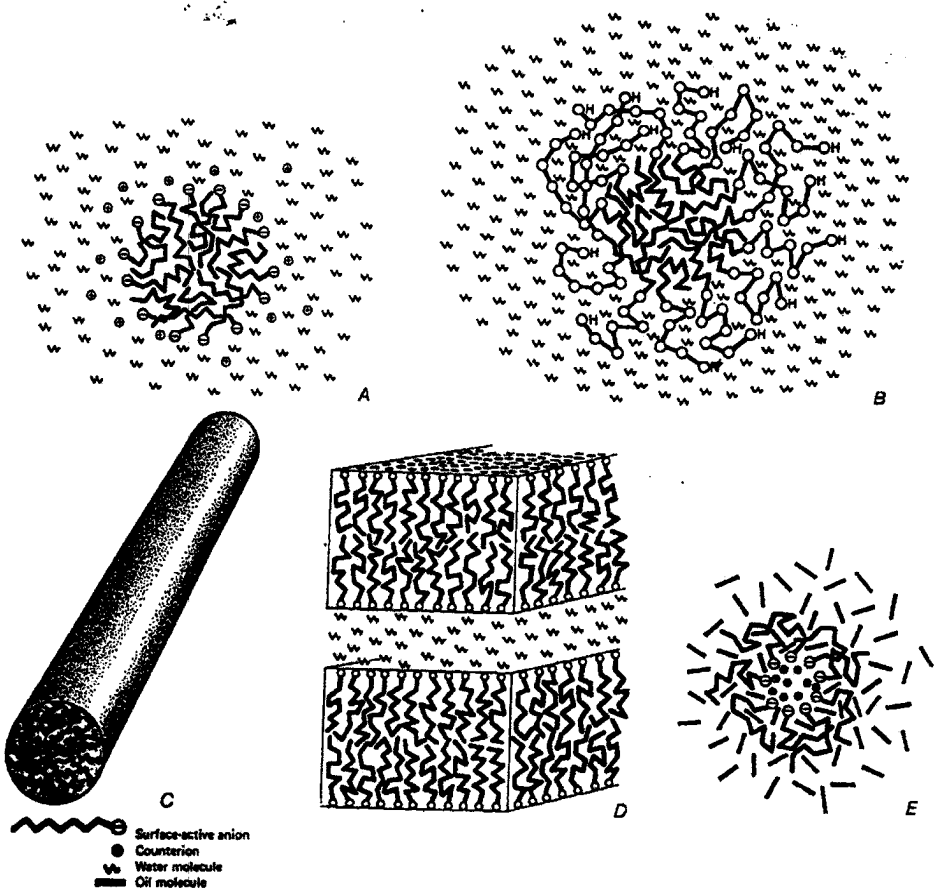


Fig 20-15. Different types of micelles. *A:* Spherical micelle of an anionic surfactant; *B:* spherical micelle of a nonionic surfactant; *C:* cylindrical micelle of an ionic surfactant; *D:* lamellar micelle of an ionic surfactant; *E:* reverse micelle of an anionic surfactant in oil. (Courtesy: Schott H, Martin AN, in Dittert LW, ed: *American Pharmacy*, 7th ed, Lippincott, Philadelphia, Chap 6, 1974.)

factants, the micelles increase in size. Being too large to remain spherical, they change to ellipsoidal, to cylindrical, and finally to lamellar shapes. In cylindrical micelles, the polar headgroups form the periphery and the hydrocarbon tails fill the interior of cylinders (Fig 20-15C). In lamellar micelles, the surfactant molecules are arranged in parallel bimolecular sheets with a tail-to-tail orientation, ie, the hydrocarbon tails form the inner layer. Water is stratified between sheets, hydrating the external polar headgroups (Fig 20-15D). In both types of micelles, the hydrocarbon tails are randomly coiled and in a liquid-like state.[15,30]

In concentrated aqueous solutions containing 20% or more surfactant, cylindrical micelles often line up parallel and arrange themselves into hexagonal arrays. Likewise, lamellar micelles are often parallel and equidistant from each other, with the intervening water layers of equal thickness. These ordered solutions are birefringent and quite viscous. They are in the liquid crystalline or mesomorphic state, ie, they are liquids which have some of the properties of crystalline solids. Since the membrane and the protoplasm of cells may be in the liquid crystalline state, such structures are of biological importance.

Oil-soluble surfactants such as heavy-metal soaps, sodium dioctylsulfosuccinate, and sorbitan monoesters form aggregates when dissolved in hydrocarbons, chlorinated hydrocarbons, and other nonaqueous liquids of low polarity. These micelles are inverted or turned inside out (see Fig 20-15E): the hydrocarbon tails are oriented outwards into the oil phase while the polar headgroups are in the center of the micelle, where water can be solubilized. Because the bulky headgroups are in the center, the aggregation numbers of such reverse micelles are small, usually between 3 and 20.[35]

Drugs as Association Colloids

Association into micelles is of importance in the case of surface-active drugs. The following are examples of drugs that behave as cationic or anionic surfactants; they are sur-

Table IV—Critical Micelle Concentrations and Micellar Aggregation Numbers of Various Surfactants in Water at Room Temperature

Structure	Name	CMC, mM/L	Surfactant molecules/micelle
n-$C_{11}H_{23}COOK$	Potassium laurate	24	50
n-$C_8H_{17}SO_3Na$	Sodium octant sulfonate	150	28
n-$C_{10}H_{21}SO_3Na$	Sodium decane sulfonate	40	40
n-$C_{12}H_{25}SO_3Na$	Sodium dodecane sulfonate	9	54
n-$C_{12}H_{25}OSO_3Na$	Sodium lauryl sulfate	8	62
n-$C_{12}H_{25}OSO_3Na$	Sodium lauryl sulfate[a]	1	96
	Sodium di-2-ethylhexyl sulfosuccinate	5	48
n-$C_{16}H_{21}N(CH_3)_3Br$	Decyltrimethylammonium bromide	63	36
n-$C_{12}H_{25}N(CH_3)_3Br$	Dodecyltrimethylammonium bromide	14	50
n-$C_{14}H_{29}N(CH_3)_3Br$	Tetradecyltrimethylammonium bromide	3	75
n-$C_{14}H_{29}N(CH_3)_3Cl$	Tetradecyltrimethylammonium chloride	3	64
n-$C_{12}H_{25}NH_3Cl$	Dodecylammonium chloride	13	55
n-$C_{12}H_{25}O(CH_2CH_2O)_8H$	Octaoxyethylene glycol monododecyl ether	0.13	132
n-$C_{12}H_{25}O(CH_2CH_2O)_8H$[b]		0.10	301
n-$C_{12}H_{25}(CH_2CH_2O)_{12}H$	Dodecaoxyethylene glycol monododecyl ether	0.14	78
n-$C_{12}H_{25}O(CH_2CH_2O)_{12}H$[b]		0.091	116
t-C_8H_{17}-C_6H_4-$O(CH_2CH_2O)_{9.7}H$	Decaoxyethylene glycol mono-p,t-octylphenyl ether (octoxynol 9)	0.27	100

[a] Interpolated for physiologic saline, 0.154 M NaCl.
[b] At 55°C instead of 20°C.

face-active and associate into micelles: Antibacterials (hydrochlorides of acridines, benzalkonium chloride, cetylpyridinium chloride), tranquilizers (hydrochlorides of reserpine and phenothiazine derivatives), local anesthetics (procaine hydrochloride, tetracaine hydrochloride, dibucaine hydrochloride, lidocaine hydrochloride), nonnarcotic analgesics (propoxyphene hydrochloride), narcotic analgesics (morphine sulfate, meperidine hydrochloride), some prostaglandin salts, antimuscarinic drugs (propantheline bromide, methantheline bromide, methixene hydrochloride), cholinergic agents (pilocarpine hydrochloride and other alkaloidal salts), antihistamines (pyrilamine maleate, tripelennamine hydrochloride, chlorcyclizine hydrochloride, diphenhydramine hydrochloride), anthelmintics (lucanthone hydrochloride), and antibiotics (sodium fusidate, some penicillins, and cephalosporins).[36] In the presence of common surfactants, surface-active drugs may form mixed micelles, ie, aggregates containing molecules of both compounds. The properties (composition, cmc, aggregation number) of mixed micelles are frequently a weighted average of the corresponding properties of the individual surfactants.[28,30]

According to the *Ferguson principle*, the toxicity to microorganisms or the therapeutic efficacy of a drug depends on its chemical potential or thermodynamic activity rather than on its concentration. If the external phase to which the drug was administered, eg, blood or gastrointestinal fluid, is in equilibrium with the internal phase where the receptor site is located (eg, tissue or bacteria), the chemical potential of the drug in both phases is the same (see Chapter 15). For drugs capable of associating into micelles, the therapeutically active species are the nonassociated molecules. Micelles are mere reservoirs for monomeric drug molecules into which they dissociate on dilution. Thus, according to the Ferguson principle, no increase in efficacy can be expected by raising the blood level of such drugs or their concentration in the gastrointestinal fluid above their cmc, because the chemical potential of the nonassociated molecules remains nearly constant.

Micellar Solubilization[30,37–47]

The pharmacists Engler and Dieckhoff discovered, in the 19th century, that water-insoluble materials like tars and cresols could be dissolved in concentrated aqueous solutions of soaps to form clear solutions as opposed to milky emulsions. The mechanism was elucidated 30 years later by Hartley and McBain.[37]

The interior of surfactant micelles formed in aqueous media consists of hydrocarbon tails in liquid-like disorder. The micelles resemble minuscule pools of liquid hydrocarbon like dodecane surrounded by shells of polar headgroups, as sketched in Figs 20-15A–D. Compounds which are poorly soluble in water but well soluble in hydrocarbon solvents can be dissolved inside micelles, ie, they are brought into solution in an overall aqueous medium. In fact, if an aqueous surfactant solution is in contact with a bulk solid or liquid water-insoluble, oil-soluble organic compound, the surfactant molecules penetrate into the organic mass, detach organic molecules and form micelles around them. The organic compound is thus gradually dissolved in the aqueous medium.

Being hydrophobic and oleophilic, the solubilized molecules are located primarily in the hydrocarbon core of the micelle (see Fig 20-16A). Even water-insoluble drugs usually contain polar functional groups such as hydroxyl, carbonyl, ether, amino, amide, and cyano. Upon solubilization, these hydrophilic groups locate on the periphery of the micelle among the polar headgroups of the surfactant in order to become hydrated (see Fig 20-16B). For instance, when cholesterol or dodecanol is solubilized by sodium lauryl sulfate, their hydroxyl groups penetrate between sulfate ions and are even bound to them by hydrogen bonds, while their hydrocarbon portions are immersed among the dodecyl tails of the surfactant which make up the core of the micelle.

Micelles of polyoxyethylated nonionic surfactants consist of an outer shell of hydrated polyethylene glycol moieties and a core of hydrocarbon moieties (see Fig 20-16B). Compounds like phenol, cresol, benzoic acid, salicylic acid, and esters of *p*-hydroxybenzoic and *p*-aminobenzoic acids have some solubility in water and in oils but considerable solubility in liquids of intermediate polarity like ethanol, propylene glycol or aqueous solutions of polyethylene glycols. When solubilized by nonionic micelles, they are located in the hydrated outer polyethylene glycol shell as shown in Fig 20-16C. Since these compounds have hydroxyl or amino groups, they frequently form complexes with the ether oxygens of the surfactant by hydrogen bonding.[28,41,42,44,46]

Solubilization is generally nonspecific: any drug which

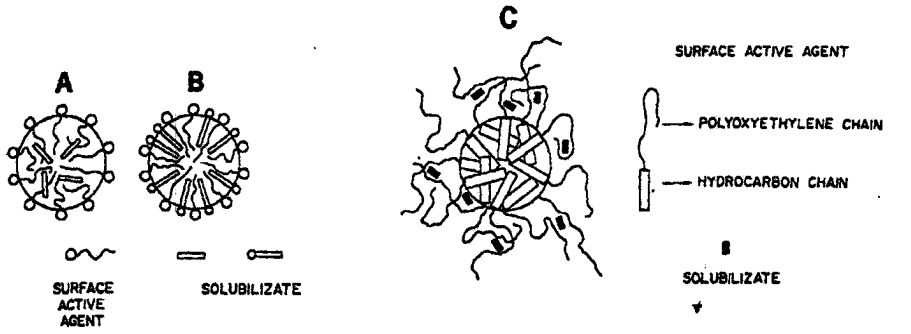


Fig 20-16. The locations of solubilizates in spherical micelles. A: Ionic surfactant (solubilized molecule has no hydrophilic groups); B: Ionic surfactant (solubilized molecule has a hydrophilic group); C: nonionic surfactant (polar solubilizate). (Courtesy, Shinoda K, *et al: Colloidal Surfactants*, Academic, New York, Chap 2, 1963.)

appreciably soluble in oils can be solubilized. Each has a solubilization limit, comparable to a limit of solubility, which depends on temperature and on the nature and concentration of the surfactant. Hartley distinguishes two categories of solubilizates. The first consists of comparatively large, asymmetrical and rigid molecules forming crystalline solids, such as steroids and dyes. These do not blend in with the normal paraffin tails which make up the micellar core; because of dissimilarity in structure, they remain distinct as solute molecules. They are sparingly solubilized by surfactant solutions, a few molecules/micelle at saturation (see Table V). The number of carbon atoms in the micellar hydrocarbon core required to solubilize a molecule of steroid or dye at saturation is of the same order of magnitude as the number of carbon atoms of bulk liquid dodecane or hexadecane per molecule of steroid or dye in their saturated solutions in these liquids.

Since solubilization depends on the presence of micelles, it does not take place below the cmc. It can, therefore, be used to determine the cmc, particularly when the solubilizate is a dye or another compound easy to assay. Plotting the maximum amount of a water-insoluble dye solubilized by aqueous surfactant, or the absorbance of its saturated solutions, versus the surfactant concentration produces a straight line which intersects the surfactant concentration axis at the cmc.

Table V—Micellar Solubilization Capacities of Different Surfactants for Estrone[a]

Surfactant	Concentration range, molarity	Temp, °C	Moles surfactant/mole solubilized estrone
Sodium laurate	0.025–0.023	40	91
Sodium oleate	0.002–0.35	40	53
Sodium lauryl sulfate	0.004–0.15	40	71
Sodium cholate	0.09–0.23	20	238
Sodium deoxycholate	0.007–0.36	20	476
Diamyl sodium sulfosuccinate	0.08–0.4	40	833
Dioctyl sodium sulfosuccinate	0.002–0.05	40	196
Tetradecyltrimethylammonium bromide	0.005–0.08	20	45
Hexadecylpyridinium chloride	0.001–0.1	20	32
Polysorbate 20	0.002–0.15	20	161
Polysorbate 60	0.0008–0.11	20	83

[a] Courtesy, Sjöblom L, in Shinoda K, ed: *Solvent Properties of Surfactant Solutions*, Dekker, New York, Chap 5, 1967.

Above the cmc, the amount of solubilized dye is directly proportional to the number of micelles and, therefore, proportional to the overall surfactant concentration. Below the cmc, no solubilization takes place. This is represented by Curve E of Fig 20-14.

The second category of compounds to be solubilized are often liquid at room temperature and consist of relatively small, symmetrical, and/or flexible molecules such as many constituents of essential oils. These molecules mix and blend in freely with the hydrocarbon portions of the surfactants in the core of the micelles, so as to become indistinguishable from them. Such compounds are extensively solubilized and in the process usually swell the micelles: they augment the volume of the hydrocarbon core and increase the number of surfactant molecules per micelle. Their solubilization frequently lowers the cmc.

Pharmaceutical Applications of Micellar Solubilization—Saponated cresol solution, which consists of 49% *v/v* of cresol solubilized in concentrated aqueous sodium or potassium soap solution, is the oldest official preparation making use of solubilization. Hexachlorophene liquid soap contains 0.24% *w/w* of hexachlorophene solubilized in an 11% solution of a potassium soap. Both are clear liquids. With the advent of nonionic surfactants, it became possible to formulate solubilized preparations for internal use. Polyoxyethylated surfactants with hydrophile-lipophile balance numbers (defined in Chapter 19) between 13 and 18 are the most widely used solubilizing agents. Among them are polysorbate 80, polysorbate 20, and to a lesser extent polyoxyl 40 stearate and octoxynol 9.

Many water-insoluble drugs are formulated as clear, aqueous solutions by processes of micellar solubilization.[41,42,44,46,47] For example, adrenal cortical hormones, including cortisol, prednisolone and their acetates, cortisone acetate, and fluorometholone are solubilized by polysorbate 80 in ophthalmic solutions. The fat-soluble vitamins A alcohol and palmitate, D, E and its acetate, and K are solubilized in aqueous media by polyoxyethylated surfactants. In this state, they are often more stable than when molecularly dissolved in vegetable oils. Antibiotics of low water-solubility like chloramphenicol, tyrothricin, griseofulvin, and amphotericin B are readily solubilized by aqueous solutions of nonionic surfactants, as are sulfonamides of low water-solubility like sulfapyridine, sulfisoxazole, and sulfaethidole. Other poorly soluble drugs which have been solubilized by nonionic surfactants include nonnarcotic analgesics and antipyretics (aspirin, acetanilid, and phenacetin), barbiturates (phenobarbital, secobarbital and others whose soluble sodium salts

are unstable and sometimes irritant), anticoagulants (dicumarol, ethyl biscoumacetate), and alkaloidal and glycosidal drugs (cinchona and tropane alkaloids, cardiac glycosides) whose extraction from plant material is facilitated by polysorbates.

Iodine and nitrofurazone are solubilized by octoxynol 9, the former possibly through complexation with the ether oxygens.[41] Polysorbate 80 solubilizes coal tar and Peruvian balsam. Tinctures (eg, benzoin, and lemon) and elixirs (eg, phenobarbital, and terpin hydrate and codeine) can be diluted with water after polysorbate 20 or 80 has been added to solubilize the drugs, resinous components and essential oils which would otherwise come out of solution. Essential oils consist largely of terpenes and their oxygenated derivatives, which fall into Hartley's second category of solubilizates. One gram of micellar polysorbate can solubilize between 0.10 and 0.25 g of essential oil, depending on the nature and content of its polar constituents.[44]

Ichthammol is a dark and very viscous liquid derived from a shale oil, which in turn is produced by destructive distillation of bituminous schists. Sulfonation of the distillate and neutralization with ammonia converts a portion into ammonium alkyl- and arylsulfonates, which are anionic surfactants. When mixed with water, they dissolve, form micelles, and solubilize the water-insoluble portion. Bile salts, the sodium salts of cholic acid and its derivatives, are anionic surfactants. Their solubilization of monoglycerides is an important step in the digestion of triglycerides.

Oil-soluble, water-insoluble drugs are often more stable when solubilized by surfactant micelles in water than when molecularly dissolved in oils. On oral and parenteral administration, they are frequently better absorbed from the aqueous vehicle. The surfactant solutions may be diluted below the cmc by body fluids, causing the solubilized drugs to precipitate. However, the precipitated drugs have very small particle sizes because their dispersions are stabilized by the surfactants. This results in fast and complete absorption. Solubilization of lipophilic drugs by micellar bile salts may play an important role in their intestinal absorption.[48]

Drugs which are sufficiently soluble in water to be effective in the absence of surfactants may have their availability and activity lowered by micellar solubilization. For instance, solubilization of parabens and other preservatives which are somewhat soluble in water reduces their antimicrobial effectiveness: the preservatives are partitioned between the micelles and water. The solubilized fraction is inactive against microorganisms; the fraction molecularly dissolved in water, which is active, has its concentration lowered by solubilization. Such observations have also been made for the antibacterial chloroxylenol in the presence of polyoxyethylated nonionic surfactants. While the total amount dissolving in aqueous solutions was increased manyfold by micellar solubilization, the bactericidal activity of such saturated solutions was no greater than that of a saturated solution of chloroxylenol in pure water. Chloroxylenol solubilized by micelles was inactive.[41,46]

The fact that phenols are complexed by polyoxyethylated surfactants through specific binding via hydrogen bonds may have contributed to their inactivation.[44,46] Such binding is stronger than nonspecific micellar solubilization. On the other hand, antibacterials like thymol, resorcinol, *n*-hexylresorcinol and trichlorophenol had their solubility in aqueous media as well as their antibacterial activity enhanced by micellar soaps and sodium lauryl sulfate. Anionic surfactants have some antibacterial activity of their own. Overall, several of the studies based on anionic and nonionic surfactants indicated an increase in the activity of a variety of antimicrobial drugs of high and low water solubility, antibiotics, and preservatives at low surfactant concentration and a reduction in activity at high surfactant concentration.[46]

Microemulsions[49,50]

Microemulsions are liquid dispersions of water and oil that are made homogeneous, transparent, and stable by the addition of relatively large amounts of a surfactant and a cosurfactant. *Oil* is defined as a liquid of low polarity and low miscibility with water, eg, toluene, cyclohexane, mineral or vegetable oils.

Microemulsions are intermediate in properties between micelles containing solubilized oils and emulsions. While emulsions are lyophobic and unstable, microemulsions are on the borderline between lyophobic and lyophilic colloids. True microemulsions are thermodynamically stable.[51] Therefore, they are formed spontaneously when oil, water, surfactants, and cosurfactants are mixed together. The unstable emulsions require input of considerable mechanical energy for their preparation, which may be supplied by colloid mills, homogenizers or ultrasonic generators.

Both emulsions and microemulsions may contain high volume fractions of the internal phase. For instance, some O/W systems contain 75% (v/v) of oil dispersed in 25% water, although lower internal phase volume fractions are more common.

At low surfactant concentrations, viz, low multiples of the cmc, micelles are spheres (Figs 20-15 A, B, and E) or ellipsoids. When an oil is solubilized by micelles in water, it blends into the micellar core formed by the hydrocarbon tails of the surfactant molecules (Fig 20-16A) and swells the micelles.

Spherical or ellipsoidal micelles are nearly monodisperse, and their mean diameters are in the range of 25 to 60 Å. Microemulsion droplets also have a narrow droplet size distribution with a mean diameter range of approximately 60 to 1,000 Å. Since the droplet diameters are less than ¼ of the wavelength of light (4,200 Å for violet and 6,600 Å for red light), microemulsions scatter little light and are, therefore, transparent or at least translucent.

Emulsions have very broad droplet size distributions. Only the smallest droplets, with diameters of about 1,000 to 2,000 Å, are below the resolving power of the light microscope. The upper size limit is 25 or 50 μm (250,000 or 500,000 Å). Because emulsion droplets are comparable in size, or larger than the wavelength of visible light, they scatter it more or less strongly depending on the difference in refractive index between oil and water. Thus, most emulsions are opaque.

The three disperse systems—micellar solutions, microemulsions, and emulsions—can be of the O/W (oil-in-water) or W/O type. Aqueous micellar surfactant solutions can solubilize oils and lipid-soluble drugs in the core formed by their hydrocarbon chains. Likewise, oil-soluble surfactants like sorbitan monooleate and docusate sodium form "reverse micelles" in oils (Fig 20-15E) capable of solubilizing water in the polar center. The solubilized oil in the former micelles and the solubilized water in the latter may in turn enhance the micellar solubilization of oil-soluble and water-soluble drugs, respectively.

Oil-soluble drugs have been incorporated into O/W emulsions by dissolving them in the oil phase before emulsification (see Chap 21 and Ref 52). By the same token, it may be possible to dissolve oil-soluble drugs in a vegetable oil and make an oral or parenteral O/W microemulsion. The advantage of such microemulsion systems over conventional emulsions is their smaller droplet size and superior shelf stability. Aqueous micellar solutions[53] and O/W microemulsions[54] have both been used as aqueous reaction media for oil-soluble compounds.

Emulsions and micellar solutions of oils solubilized in aqueous surfactant solutions consist of three components, oil, water and surfactant. Microemulsions generally require a 4th component, called *cosurfactant*. Commonly used cosurfactants are linear alcohols of medium chain length, which are

Table VI—Microemulsion Formulations

Compound	Function	Content in microemulsions, % O/W	W/O
Sodium lauryl sulfate	Surfactant	13	10
1-Pentanol	Cosurfactant	8	25
Xylene	Oil	8	50
Water		71	15

sparingly miscible with water. Since the cosurfactants as well as the surfactants are surface-active, they promote the generation of extensive interfaces through the spontaneous dispersion of oil in water, or vice-versa, resulting in the formation of microemulsions. The large interfacial area between oil and water permits the extensive formation of a mixed interfacial film consisting of surfactant and cosurfactant. This film is called the "interphase" because it is thicker than the surfactant monolayers formed at oil-water interfaces in emulsions. The interfacial tension at the oil-water interface in microemulsions approaches zero, which also contributes to their spontaneous formation. According to another viewpoint, microemulsions are regarded as micelles extensively swollen by large amounts of solubilized oil.

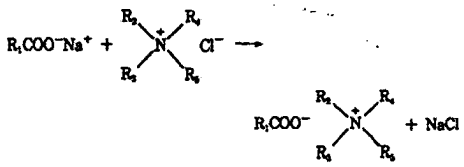
Typical formulations for an O/W and a W/O microemulsion are shown in Table VI. The ratio, g surfactant/g solubilized or emulsified oil or water is in the range of 2–20 for micellar solutions and 0.01–0.1 for emulsions. Microemulsions have intermediate values: The ratios for the formulations in Table VI are near unity. In industrial formulations, the ratios are closer to 0.1 to reduce costs. Microemulsions are used in such diverse applications as floor polish and agricultural pesticide formulations and in tertiary petroleum recovery. The use of O/W microemulsions as aqueous vehicles for oil-soluble drugs to be administered by the oral or parenteral route is worth investigating.

Chemical Incompatibilities Involving Hydrophilic Polymers and Surfactants

Ionic surfactants and polyelectrolytes sometimes exhibit chemical incompatibilities in aqueous solution among themselves and with ionizable drugs. Because of the dual hydrophilic and hydrophobic nature of ionic surfactants and because of the large number of ionic groups in a single molecule of a polyelectrolyte, precipitation effects involving these two classes of compounds frequently occur even at low concentrations and are extensive.

Micelles of nonionic surfactants and, to a lesser extent, water-soluble polymers with a multiplicity of hydroxyl or ether groups tend to bind drug molecules which contain phenolic or other hydroxyl groups or un-ionized carboxyl groups and possess some solubility in water. This binding, which occurs mainly through hydrogen bonding, may reduce the effectiveness or bioavailability of such drugs (see above). Otherwise, nonionic surfactants and polymers are generally compatible with most drugs and adjuvants in aqueous solutions.

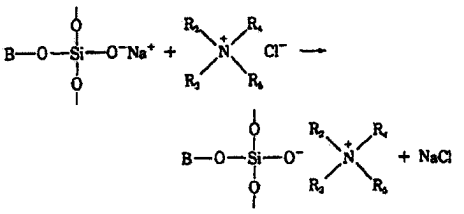
Basic drugs, cationic surfactants and cationic polyelectrolytes are generally incompatible with anionic surfactants and with acid drugs and anionic polyelectrolytes in their ionized forms. The following equation schematically represents a typical precipitation reaction involving a carboxylate, eg, sodium stearate or glycocholate, sodium salicylate or aminosalicylate, sodium alginate or carboxymethylcellulose. Alkyl sulfates and aryl sulfonates undergo similar reactions, as does tannic acid because of a high concentration of phenolic hydroxyl groups. If R_1 through R_5 are relatively hydrophobic organic moieties (although R_3 and/or R_4 and/or R_5 may be hydrogen atoms), two low-molecular-weight surface-active



compounds are involved in this precipitation reaction. It should be remembered that most, if not all, basic organic drugs behave as cationic surfactants at pH values low enough to produce substantial ionization. They lower the surface tension of water, aggregate into micelles, have a pronounced tendency to become adsorbed at interfaces of lipophilic organic solids or tissues and water, and precipitate from aqueous solutions in the presence of anionic surface-active compounds. The precipitates consist of carboxylates (or alkyl sulfates or alkylaryl sulfonates) of the mono-, di-, tri-, or tetraalkyl or tetralkylaryl ammonium cations. They form and are held together by the ionic primary valence bond and by hydrophobic bonding between R_1 and R_2 through R_5.

The same type of precipitation reaction takes place if R_1 represents a polymer molecule with many carboxylate (or sulfate) groups and/or if R_2 represents a polymer molecule with many basic nitrogen groups. There are four possibilities: (i) anionic surfactant + cationic surfactant; (ii) anionic polyelectrolyte + cationic surfactant; (iii) anionic surfactant + cationic polyelectrolyte; (iv) anionic polyelectrolyte + cationic polyelectrolyte. Charge neutralization in the first three cases generally produces gelatinous precipitates. In the last instance, where hydrophilic sols or polyelectrolytes of opposite charge are involved, either a gelatinous precipitate or droplets of a viscous liquid phase may separate. The droplets, which tend to coalesce, constitute a *complex coacervate*. Complex coacervates differ from simple coacervates, obtained by the phase separation of a single polymer, in that heating or dilution with water does not reverse the complex coacervation because it involves a chemical reaction.[1] Complex coacervates formed between two oppositely charged hydrophilic sols, such as solutions of gelatin at a pH below its isoelectric point and of acacia, are used in the NCR microencapsulation process described in Chapter 90. A few examples of complex coacervate formation have also been reported for combinations (i) and (ii).

Aqueous dispersions of sodium bentonite are also incompatible with basic drugs or cationic surface-active agents. Chemisorption involving the silicate cation-exchange sites of the clay takes place according to the schematic reaction:



where B represents part of a bentonite lamella. The cationic drug is bound to the surface of the lamella by the ionic bond and by van der Waals forces attracting its hydrophobic organic groups R_2 through R_5 to the clay surface. Cationic drugs chemisorbed on bentonite coagulate the dispersed clay and are excreted in the feces together with the clay rather than being absorbed in the gastrointestinal tract. Bentonite chemisorbs basic drugs extensively because of its high cat-

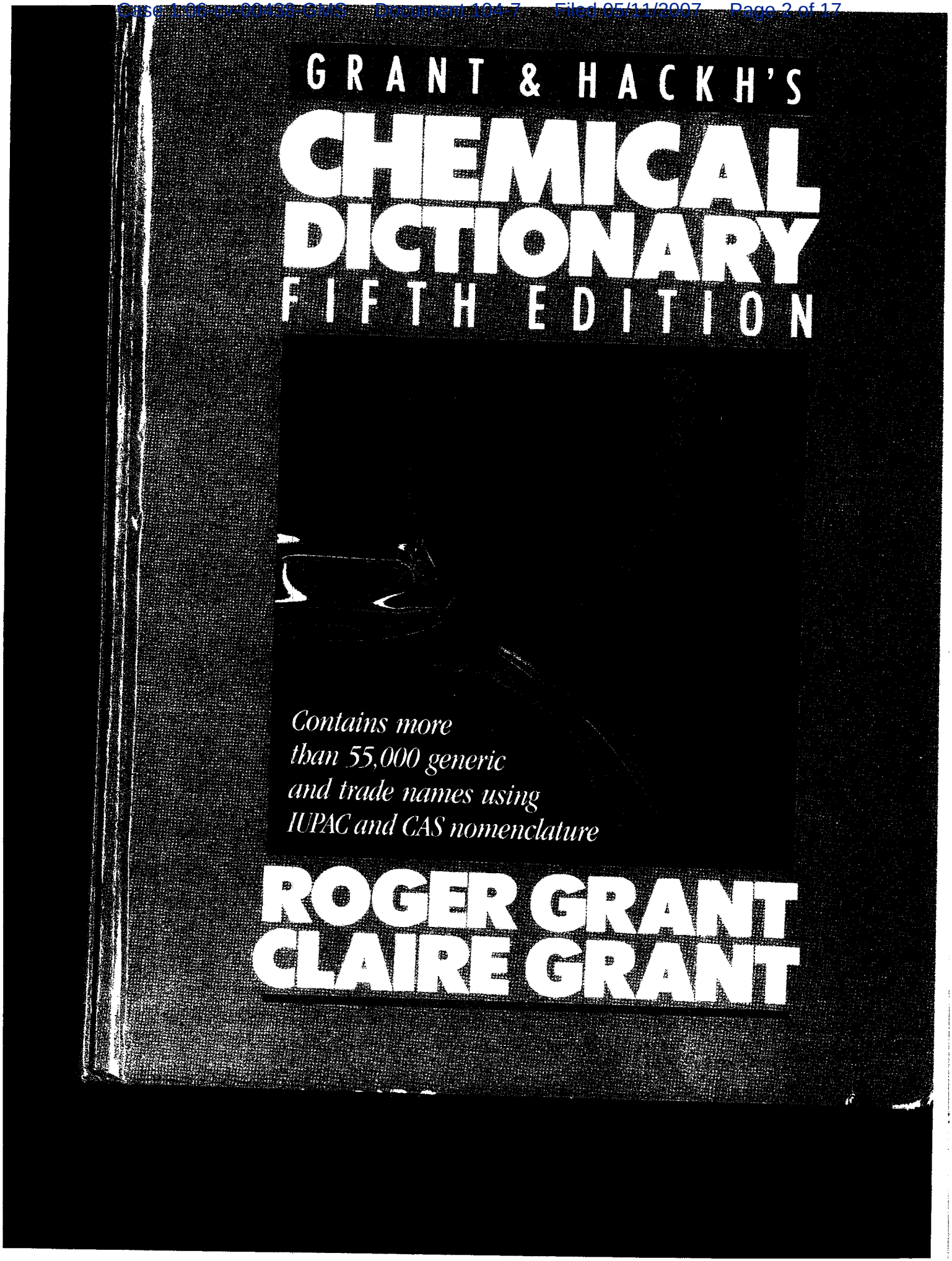
ion-exchange capacity, 0.7–1 milliequivalent/gram. Even kaolin, with the lower cation exchange capacity of 0.01–0.1 milliequivalents/gram, should not be taken simultaneously with a basic drug to avoid chemisorption of the latter.

Chemical incompatibilities involving anionic polyelectrolytes and polyvalent cations are discussed above, in the section on *Hydrophilic Dispersions, Gel Formation.*

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EXHIBIT M


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Case 1:06-cv-00438-GMS Document 104-7 Filed 05/11/2007 Page 3 of 17

supercarbonate Hydrogencarbonate*.
superchlorination Sterilizing water by chlorinating in excess, and then adding an antichlor.
superconductivity Supraconductivity. The property of some elements of becoming particularly good electrical conductors at temperatures below about 11 K; e.g.: Pb 7.2, Hg 4.2, Sn 3.7, In 3.4, Tl 2.4 K. **s. motor** A device utilizing the s. of copper to produce considerable reductions in the size and weight of conventional electric motors.
supercooled Over- or undercooled. Cooled below the freezing point of a liquid without the separation of solid matter.
superfluid Supafluid. A fluid having exceptionally good heat-transfer and penetrating properties, e.g., liquid $helium_{II}$.
Supergas Trademark for triptane.
superheated Overheated. Describing a liquid or gas heated above its boiling point in the liquid state; as superheated steam (water vapor at above 100°C).
supermalloy The alloy Ni 75, Mo 5, Fe + Mn 20%. It has a high magnetic permeability; used in transformer cores.
supernatant Describing the liquid above a sediment or precipitate.
supernickel The alloy Cu 70, Ni 30%; retains its strength at high temperatures.
supernormal A volumetric solution of concentration greater than normal.
superoxide Hyperoxide*.
superpalite A poison gas. Cf. *palite*.
superphosphate (1) An acid phosphate. (2) A fertilizer mixture of calcium phosphate and calcium sulfate obtained by the action of concentrated sulfuric acid on phosphate minerals (apatite, phosphorite). **ammoniated ~** See *ammoniated superphosphate*. **double ~** A fertilizer made by the action of phosphoric acid on rock phosphate (40–50% phosphorus pentaoxide).
superpolymer A polymer whose molecular weight exceeds 10,000.
superpotential Overvoltage.
supersaturated More than saturated. **s. solution** A solution containing an excess of dissolved substance over that normally required for saturation at a particular temperature, obtainable by slowly cooling a saturated solution.
supersolubility Supersaturation. **s. curve** The curve relating the concentration of a supersaturated solution with the temperature; analogous to and parallel with the *solubility curve*, q.v.
supersonic Describing speed or velocity greater than that of sound. Cf. *ultrasonic*.
supersteel A high-speed *steel*, q.v.
supertension Overvoltage.
suppository Pharmaceutical term for a medicated, conical body for insertion into the rectum.
suppurative An agent that produces pus.
supra- Prefix (Latin) indicating "above."
supraconductivity Superconductivity.
Supramid Trademark for a polyamide synthetic fiber.
suprarenal gland Adrenal gland. A gland above the kidney.
suprarenaline Adrenaline.
suprasterols Sterols produced by irradiation of lumisterol. **s. II** $C_{28}H_{44}O$ = 396.7. m.110.
Suprema Trademark for a viscose synthetic fiber.
Supron Trademark for a polyamide synthetic fiber.
suramin sodium ~ $C_{51}H_{34}O_{23}N_6S_6$ = 1291. Bayer 205. Germanin. Bitter, hygroscopic powder, insoluble in ether; a urea complex, used to treat African trypanosomiasis.
surcharge The sum of the errors involved in an assay.
surface The outer part of a body having length and breadth but not thickness. **s.-active agent** See *surfactant*. **s. charge density*** The electric charge per unit surface area. Cf. current *density*. **s. energy** The product obtained by multiplying surface tension by the two-thirds power of the molecular weight and specific volume. **s. tension*** The contractile surface force of a liquid which makes it tend to assume a spherical form, e.g., to form a meniscus. It also exists at the junction of 2 liquids. It is measured directly (1 mN/m = 1 dyne/cm) or indirectly by determining the capillarity. The s. t. (at constant temperature) γ is in a constant ratio to the 4th power of the orthobaric densities of liquid D and gas d; hence $\gamma = K(D-d)^4$. Cf. *parachor*. **s. tension apparatus** Tensiometer. A device for determining the s. t., based upon the flexibility of a wire.
surfactant A surface-active agent; i.e., one that modifies the nature of surfaces, this often involving reducing the surface tension of water. Widely used as wetting agents, detergents, emulsifiers, dispersing agents, penetrants, and antifoams. Four types: cationic (as, modified onium salts); anionic (alkylarylsulfonates); nonionic (polyethylene oxides), and ampholytic (dodecyl-β-alanine).
surfusion The unstable condition of a liquid cooled below its freezing point without solidifying. Cf. *supercooled*.
surpalite Diphosgene.
surrogate A substitute for another substance; as, margarine for butter.
surrosion The increase in weight of a substance due to corrosion.
susceptible Sensitive. Readily capable of responding to an action or force; as, magnetic susceptibility. Cf. *immune*.
susotoxin $C_{10}H_{26}N_2$ = 174.3. Sustoxin. A ptomaine from cultures of hog cholera bacillus.
suspension (1) Suspensoid. (2) A thin thread on which the mirror and magnet of a galvanometer hang. **s. method** The determination of the density of a solid by placing it in a solution of known density. See *density fluids*.
suspensoid Suspension, soliquoid. Finely divided colloidal particles floating in a liquid, too small to settle, but kept in motion by Brownian motion.
sussexite A native, hydrated magnesium manganese diborate.
sustoxin Susotoxin.
Sutherland S.-Einstein equation $D = RT/Lf$, where D is the diffusion constant, R the gas constant, T the thermodynamic temperature, L the Avogadro constant, and f the frictional force on each molecule having unit velocity. **S.'s formula** The viscosity of a gas (η) at thermodynamic temperature T is given from that at 0°C (η_0) by: $\eta = \eta_0(T/273.15)^{3/2} \times (k + 273.15)/(k + T)$, where k is the S. constant.
suture A stitch, or the operation of stitching, or the s. material, used in surgery. **absorbable ~** A sterile s. prepared from the intestine of healthy animals (USP). **nonabsorbable ~** A s. that resists enzyme digestion in living animal tissues, e.g., of stainless steel.
suxamethonium chloride BP name for succinylcholine chloride.
Sved A unit of sedimentation rate.
Svedberg S., The(odor) (1884–1971) Swedish physical chemist, Nobel prize winner (1926); noted for research on colloids. **S.'s equation** The amplitude A of Brownian movement of a particle is proportional to its vibration period t. **S. unit** Abbrev. S; used in sedimentation; equal to 10^{-13} seconds.
swage To fashion metal, particularly iron, by drawing it into a groove, mold, or die having a desired shape.

EXHIBIT N

9/B



PATENT
Attorney Docket No. 47988-224 Bef
Parent Application No. 08/696,754 3-5-98

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231

on February 4, 1998
Date of Deposit

Signature

2/4/98
Date of Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
De Garavilla et al.	
Application No.: 08/696,754	Group Art Unit: 1502
Filed: August 14, 1996	Examiner: C. Azpuru
For: REDUCTION OF INTRAVENOUSLY ADMINISTERED NANOPARTICULATE-FORMULATION-INDUCED ADVERSE PHYSIOLOGICAL REACTIONS	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

AMENDMENT

This Amendment is filed in response to the Office Action mailed on September 30, 1997. Filed here with is a Petition for a two month extension of time to extend the period for response to **February 28, 1998**.

Please amend this application as follows.

PATENT
Attorney Docket No. 47988-224
Parent Application No. 08/696,754

IN THE CLAIMS:

1. (Amended) A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic [effect] effects comprising[:] intravenously administering to said mammal an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, wherein said drug composition [comprising] comprises:

B1

(a) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml[,];

(b) [said drug substance having] a surface modifier adsorbed on the surface of the drug substance [thereof] in an amount of from about 0.1[-] to about 99.9% by weight and sufficient to maintain an effective average particle size of from about 50 nm to about 1000 nm[,]; and

(c) a pharmaceutically acceptable carrier therefor.

Claim 2, line 2, change "is of from" to –is from--.

5. (Amended) A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

B2

(a) intravenously administering to said mammal an antihistamine in the amount of from about 5 to about 10 mg/kg of body weight; and

(b) subsequently intravenously administering to said mammal an effective amount of a nanoparticulate drug composition comprising: (1) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml[,]; and (2) [said drug substance having] a surface modifier adsorbed on the surface of the drug substance [thereof] in an amount of from about 99.9 to about 0.1% by weight and sufficient to maintain an effective average particle size of less than about 1000 nm.

9. (Amended) A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic [effect] effects comprising:

B3

(a) intravenously administering to said mammal a desensitizing amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min; and

(b) intravenously administering an effective amount of said nanoparticulate composition comprising: (1) particles consisting essentially of from about 0.1 to about 99.9% by

B3 cancel

weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml[,]; (2) [said drug substance having] a surface modifier adsorbed on the surface of the drug substance [thereof] in an amount sufficient to maintain an effective average particle size of from about 100 to about 1000 nm[,]; and (3) a pharmaceutically acceptable carrier therefor.

B4

13. (Amended) A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic [effect] effects comprising[:] intravenously administering to said mammal an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, wherein said drug composition [comprising] comprises:

(a) particles having an effective average particle size of from about 50 to about 1000 nm and consisting essentially of from about 0.1 to about 99.9% by weight of an organic drug substance entrapped in from about 99.9 to about 0.1% by weight of liposome or a colloidal polymeric material; and

(b) a pharmaceutically acceptable carrier therefor.

B5

17. (Amended) A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal an antihistamine in the amount of from about 5 to about 10 mg/kg of body weight; and

(b) subsequently intravenously administering to said mammal an effective amount of a nanoparticulate drug composition comprising: (1) particles having an effective average particle size of from about 50 to bout 1000 nm and consisting essentially of from about 0.1 to about 99.9% by weight of an organic drug substance entrapped in from about 99.9 to about 0.1% by weight of liposome or a colloidal polymeric material; and (2) a pharmaceutically acceptable carrier therefor.

REMARKS

Applicants respectfully request reconsideration and reexamination of this application.

I. STATUS OF THE CLAIMS

Applicants have amended claims 1, 2, 5, 9, 13, and 17 to correct minor grammatical errors and to more clearly and particularly point out the invention. In addition, claims 1 and 5 have been amended to recite a surface modifier present in an amount of "from about" 0.1 to "about" 99.9% by weight. Support for this amendment can be found in the specification at, for example, page 17, lines 1-4. Finally, claims 5 and 9 have been amended to recite an effective average particle size for the drug substance of less than "about" 1000 nm and from "about" 100 to "about" 1000 nm, respectively. Support for this amendment can be found in the specification at, for example, page 22, line 34, through page 23, line 4. Because Applicants' amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

II. SUMMARY OF THE INVENTION

The present invention is directed to intravenous methods of administering a nanoparticulate composition in which the elicitation of adverse hemodynamic effects are eliminated. Applicants observed that intravascular administration of nanoparticulate suspensions can cause significant cardiovascular dysfunction, such as reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility. *See* page 1, line 35, through page 2, line 8, of the specification. Applicants unexpectedly discovered that such adverse hemodynamic effects associated with intravenous administration of nanoparticulate formulations can be eliminated, or substantially reduced, by maintaining the intravenous infusion rate at less than 10 *mg/ml*, or by pretreating the patient with an antihistamine. *See e.g.*, page 10, lines 3-5 and 22-24; and page 10, line 28, through page 11, line 3, of the specification. This is not taught or suggested in the cited prior art.

III. REJECTION OF THE CLAIMS UNDER 35 U.S.C. § 103

Claims 1-20 were rejected under 35 U.S.C. § 103(a) as being allegedly unpatentable over Wong et al., U.S. Patent No. 5,565,188. *See* page 2 of the Office Action. Applicants respectfully traverse this ground for rejection.

Wong et al. is directed to a nanoparticulate composition comprising a therapeutic or diagnostic agent and having a specified triblock copolymer as a surface modifier adsorbed on the

surface of the therapeutic or diagnostic agent. The triblock copolymer contains one or more polyoxyethylene blocks and one or more polyoxy(higher alkylene) blocks, wherein at least some of the blocks are linked together by an oxymethylene linking group. *See* col. 1, line 51, through col. 2, line 4, of Wong et al. This does not teach or suggest Applicants' claimed invention.

In support of the rejection, the Examiner stated that:

> Wong et al disclose a nanoparticulate formulation . . . directed towards poorly soluble therapeutic agents . . . Wong et al differ only in their lack of disclosure of a precise infusion rate.
>
> However, the specification is clear . . . that such a dosage level varies according to therapeutic agent, severity of disease or conditions being treated, duration of treatment, etc.

See page 2 of the Office Action. Applicants respectfully disagree.

Intravenous administration of compositions according to Wong et al. resulted in deleterious hemodynamic effects, as observed by Applicants and other investigators. *See* page 2, lines 3-8 of the specification. Wong et al. provide no guidance as to how to eliminate such deleterious hemodynamic effects.

After identifying the problem to be solved (*i.e.*, adverse hemodynamic reactions associated with intravenous administration of nanoparticulate compositions), Applicants developed experiments designed to distinguish and separate effects arising from components of nanoparticulate formulations and effects of intravenous injections. The experiments comprised using inert polystyrene nanoparticles, surfactant-coated polystyrene nanoparticles, and surfactant alone in *in vivo* tests. *See* page 3, lines 2-6, of the specification. Applicants discovered that hemodynamic effect is associated with the surfactant-particle combination. *See* page 10, lines 14-21, of the specification. Only after evaluating the results of the *in vivo* tests were Applicants able to develop intravenous injection parameters which eliminate or dramatically reduce hemodynamic side effects. Moreover, the set of parameters developed by Applicants are critical to controlling *in vivo* hemodynamic effects. *See* page 10, lines 3-5, of the specification. Such parameters

are neither suggested nor taught by Wong et al.

As to the use of a pretreatment with an antihistamine, Wong et al. contain no suggestion whatsoever of the use of such a method to eliminate or reduce hemodynamic side effects associated with intravenous administration of nanoparticulate formulations.

As to the use of a infusion rate of less than 10 mg/ml, Wong et al. do not suggest such a parameter to eliminate or reduce hemodynamic side effects associated with intravenous administration of nanoparticulate formulations. Moreover, the use of an infusion rate of less than 10 mg/ml is not merely an optimization of an administration variable, as the use of this specific infusion rate results in dramatic, unexpected results, *i.e.*, the reduction or elimination of hemodynamic side effects. *See In re Woodruff*, 16 U.S.P.Q.2d. 1934, 1936 (Fed. Cir. 1990); *In re Antonie*, 195 U.S.P.Q. 6, 8-9 (CCPA 1977) (The court held that discovery of an optimum value of a variable in a known process is normally obvious, but exceptions to this rule include where the results of optimizing a variable were unexpectedly good.).

At issue in *Antonie* was the patentability of a water treatment device in which wastewater passed through a tank having continuously rotating contactors. The primary prior art reference showed the device's basic structure, but was silent regarding quantitative design parameters. The applicants in *Antonie* claimed the device with a specified ratio of tank volume to contactor area, asserting that this volume-contactor ratio maximized treatment capacity. The Federal Circuit reversed the PTO's rejection of the claimed invention based on obviousness, holding that the prior art reference did not disclose or suggest the functional relation of treatment capacity to tank volume-to-contactor area. *Antonie* at 8.

Similarly, Wong et al. do not teach the relationship between an intravenous infusion rate or a pretreatment of an antihistamine and hemodynamic side effects resulting from intravenous administration of nanoparticulate formulations. Thus, there is no motivation to one of ordinary skill in the art at the time the claimed invention was made to

modify the disclosure of Wong et al. by either using a pretreatment of an antihistamine or controlling the infusion rate of an intravenous formulation to obtain Applicants' claimed invention. Because Wong et al. do not teach or suggest the claimed invention, withdrawal of this ground for rejection by the Examiner is courteously requested.

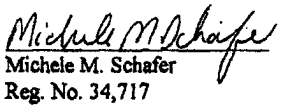
IV. CONCLUSION

Applicants respectfully request reconsideration of the present application in view of the above amendments and remarks. This application is now in condition for allowance and early notice to that effect is respectfully solicited.

Should the Examiner have any questions or comments regarding the pending application or this Amendment, the Examiner is urged to call the undersigned at 312-984-7746.

If there are any fees due in connection with the filing of this Amendment, please charge the fees to our Deposit Account No. 13-0206. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
McDermott, Will & Emery



Michele M. Schafer
Reg. No. 34,717

Dated: February 4, 1998

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EXHIBIT O

Acute Hemodynamic Effects and Blood Pool Kinetics of Polystyrene Microspheres following Intravenous Administration

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Abstract □ The acute hemodynamic effect of intravenous administration of polystyrene microspheres was investigated and correlated with their distribution pattern and kinetics. Microspheres of three diameters (3.4, 7.4, and 11.6 μm) were administered. The 7.4- and 11.6-μm diameter microspheres were filtered by the pulmonary capillary network following intravenous administration, the majority during the first pass. There was no significant hemodynamic effect following administration of the 7.4- and 11.6-μm diameter microspheres in doses as high as 3.0×10^8 and 6.1×10^8, respectively (total cross-sectional area of 1.3×10^{11} and 6.4×10^{10} μm^2, respectively). Intravenous administration of 3.4-μm diameter microspheres produced significant dose-dependent systemic hypotension and depression of myocardial performance at dosages as low as 1.0×10^{10} (cross-sectional area of 9.1×10^{10} μm^2). These differences in acute hemodynamic effect from the 7.4- and 11.6-μm diameter microspheres may be due to the differences in distribution kinetics and fate of the 3.4-μm diameter microspheres, which readily pass through the lungs to the systemic circulation, with late disposition primarily in the liver and spleen. Although elimination of the smaller spheres from the blood during the first 6–8 min was rapid, *i.e.*, $t_{1/2} = 1.62$ and 1.72 min from the venous and arterial blood circulation, respectively, levels of 10^3 spheres/g of blood were present in the circulation for >1 hr. These findings must be considered in the planning of intravenous administration of microspheres as a drug delivery system to target organs.

Keyphrases □ Microspheres, polystyrene—acute hemodynamic effects and blood pool kinetics following intravenous administration to dogs, drug delivery system to target organs □ Drug delivery system—polystyrene microspheres, acute hemodynamic effects and blood pool kinetics following intravenous administration, dogs □ Kinetics—polystyrene microspheres, distribution and clearance in dogs following intravenous administration, drug delivery system □ Radionuclide imaging agents, potential—^{141}Ce-labeled polystyrene microspheres, acute hemodynamic effects and blood pool kinetics evaluated in dogs following intravenous administration

Systemic toxicity of pharmaceutical agents, particularly those used in the chemotherapy of malignancy, has prompted a search for alternative drug delivery methods. Ideally, these methods should be safe, comfortable, and convenient to the patient. To date, attempts at local therapy with surgical implants or chronic catheter placement have achieved only limited success. Intravascular administration of radioactive microspheres has been utilized safely in animals and humans for many years to assess blood flow patterns (1). Detailed investigation regarding their distribution patterns (2, 3), hemodynamic effects (4–6), and induced pathological changes (7, 8) has begun only recently, stimulated by evidence that pharmaceutical agents may be incorporated into subvisible particulates for intravenous administration, with subsequent local release after the particle lodges in the microcirculation (9–11).

While some deaths were recorded in previous studies on microsphere size, distribution time, and distribution pattern in beagle dogs (2, 4, 8), there was little evidence of tissue damage that could be attributed to the microspheres in specific organs. Due to the lack of significant clinical changes, the present study was undertaken to assess the influence of particle size on the hemodynamic changes associated with the intravenous administration of relatively large aggregate-weight bolus injections of microspheres.

EXPERIMENTAL

Administration of Microspheres—Polystyrene divinylbenzene microspheres labeled with cerium 141 were obtained as a suspension in physiological saline from a commercial supplier[1]. The specific activity was ~50 mCi/g; ~500 μCi was administered to each dog. The number of radioactive spheres was calculated from:

$$\text{number of spheres/mg} = \frac{1.55 \times 10^9}{D^3} \qquad \text{(Eq. 1)}$$

where D is the mean sphere diameter in microns. The nonradioactive spheres[2] were obtained as a 10% suspension in saline. The actual size and number of the microspheres were determined microscopically[3] and with an electronic particle counter[4], respectively. The appropriate dose of microspheres was drawn into a syringe, and an accurate radioactivity count was determined by a radioisotope calibrator[5]. The microspheres then were injected intravenously using a three-way stopcock. After thorough flushing of the syringe and stopcock with saline, the syringe was analyzed to exclude significant retention of the microspheres. Immediately prior to dosage administration, the vial of microspheres was placed in an ultrasonic bath for 15 min to ensure dispersion of the spheres. A surfactant, polysorbate 80, was not used in the preparation of the microspheres due to its adverse hemodynamic effect (12).

Preparation of Animals—Female beagle dogs, 13.0–15.0 kg, were randomly assigned to receive 3.4, 7.4, or 11.6-μm diameter microspheres. Following endotracheal intubation, anesthesia was maintained with a mixture of 1–2.5% halothane (depending on the blood pressure response and desired level of anesthesia) and oxygen delivered by a mechanical respirator. A left thoracotomy was performed, and a micromanometer-tipped catheter[6] was inserted into the left ventricle to measure systolic pressure. The rate of change of the left ventricular pressure (dp/dt) was derived electronically. A pair of ultrasonic piezoelectron crystals was implanted in the left ventricular subendocardial wall to measure regional myocardial wall motion and, by inference, left ventricular volume.

Micromanometer-tipped catheters[7] were inserted into the pulmonary artery and descending aorta to measure pulmonary artery pressure. A thermistor-tipped catheter[8] also was placed in the pulmonary artery to enable serial thermodilution cardiac output determinations. Catheters were positioned in the right atrium and descending aorta to allow ease in blood sampling from the venous and arterial circulation, respectively. The surface ECG was monitored continuously. The micromanometer-tipped catheters were referenced to zero and calibrated with a mercury manometer before insertion. Correction for micromanometer transducer drift was performed by superimposing electronically derived mean

[1] Nuclear Products Division, 3M Co., St. Paul, Minn.
[2] Dow Diagnostics, Indianapolis, Ind.
[3] Zetopan universal research microscope, Reichert, Austria.
[4] Coulter Counter model TAII, Coulter Electronics, Hialeah, Fla.
[5] Model CRC-6A, E. R. Squibb & Sons, Princeton, N.J.
[6] Konigsberg Co., Pasadena, Calif.
[7] Millar Co., Houston, Tex.
[8] 4F pediatric thermodilution catheter 702216, Edwards Laboratory, Santa Ana, Calif.

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Figure 1—Distribution of ^{141}Ce-labeled microspheres as determined by external nuclear imaging 3.5 hr following intravenous administration. The larger microspheres (7.4 μm in Dog 6 and 11.6 μm in Dog 4) were localized in the lungs, whereas the smaller (3.4 μm in Dog 1) microspheres distributed to the liver and spleen.

pressure against that of a fluid-filled catheter system connected to a pressure transducer[9] positioned at midchest level.

Hemodynamic Protocol—Following the surgical instrumentation, each animal was monitored for 30 min to ensure hemodynamic stability. Continuous direct measurements of the surface ECG, pulmonary artery pressure, systemic artery pressure, left ventricular pressure, rate of change of left ventricular pressure (dp/dt), and left ventricular segmental wall motion were recorded on magnetic tape[10]. Data were printed at predetermined intervals[11]. Serial duplicate cardiac output determinations were recorded at 20-min intervals[12]. Maximum values of $+dp/dt$ and $-dp/dt$ were determined from electronic differentiation of the left ventricular pressure waveform.

The systolic shortening fraction, which is a measure of the left ventricular regional wall motion, was calculated with ultrasonic segment-length measurement *via* the piezoelectron crystals implanted in the left ventricular subendocardium using the following expression: (end diastolic length − end systolic length)/end diastolic length. All animals were stable throughout the 30-min control period. A control animal was monitored for 3 hr following a placebo injection of physiological saline; this animal remained stable.

Following the 30-min control period, a measured number of ^{141}Ce-labeled microspheres of a given size (Table I) was administered as a bolus injection into the right atrium. Two dogs received the 3.4-μm diameter size, two received the 7.4-μm diameter size, and two received the 11.6-μm diameter size microspheres. Hemodynamic measurements and venous and arterial blood samples were taken every 2 min for 40 min, every 5 min for 20 min, and then every 10 min for the next 2 hr.

At the conclusion of the 3-hr monitoring period, a second bolus injection of nonradioactive microspheres of the same diameter (Table I) was administered slowly (~8 ml/min) to assess possible hemodynamic effects of larger doses. Then 3.5 hr after administration of the radioactive microspheres, each animal was sacrificed and sent for nuclear scanning. Two additional dogs were instrumented similarly for additional dosing with nonradioactive 3.4-μm diameter microspheres to complete the dose-response curve.

Scanning of Dogs—With a digital computer[13] attached to a scintillation camera[14], distribution of the radioactive microspheres was assessed by whole body scanning.

Blood Level of Spheres—Blood samples removed during the 3-hr study were analyzed for radioactivity using a well-type γ-counter[15]. Application of the appropriate calibration equation yielded results in microspheres per gram of blood.

RESULTS

Nuclear Scanning—Consistent with previous results, the radioactivity was confined principally to the lungs (>90%) in animals administered ^{141}Ce-labeled 7.4- and 11.6-μm diameter microspheres. The animals given 3.4-μm diameter microspheres had little activity (~12%) remaining in the lungs 3.5 hr following administration, the majority (~83%) of the radioactive particles having accumulated in the liver and spleen (Fig. 1). Table II summarizes the results of external analysis of these organs and demonstrates the clear tendency of the microspheres to distribute in a size-dependent organ-specific pattern.

Table I—Dosing Information

Dog	Microsphere Size, μm	Number	CSA[a], μm²	Weight, mg
Control	—	—	—	—
Control-NR[b]	3.4	1.41×10^{11}	1.28×10^{12}	3525
1	3.4	6.25×10^{8}	5.68×10^{9}	15.6
2	3.4	8.62×10^{8}	7.83×10^{9}	21.6
2NR	3.4	9.45×10^{9}	8.58×10^{10}	236
3	11.6	1.09×10^{7}	1.15×10^{9}	10.9
3NR	11.6	2.02×10^{8}	2.14×10^{10}	202
4	11.6	2.13×10^{7}	2.25×10^{9}	21.3
4NR	11.6	6.06×10^{8}	6.40×10^{10}	606
5	7.4	4.26×10^{7}	1.83×10^{9}	11.2
5NR	7.4	1.50×10^{9}	6.45×10^{10}	394
6	7.4	5.52×10^{7}	2.37×10^{9}	14.5
6NR	7.4	3.00×10^{9}	1.29×10^{11}	789
7NR	3.4	1.00×10^{9}	9.08×10^{9}	25.0
7LNR[c]	3.4	1.00×10^{10}	9.08×10^{10}	250
8NR	3.4	9.55×10^{10}	8.67×10^{11}	2388

[a] CSA = cross-sectional area. [b] NR = dose of nonradioactive spheres. [c] LNR = large dose of nonradioactive spheres.

Blood Pool Kinetics—Serial measurements of circulating microsphere levels from venous and arterial sites drawn following intravenous administration of ^{141}Ce-labeled microspheres are shown graphically by blood level profiles in Fig. 2. The elimination rate of the initial stage following intravenous administration is summarized in Table III. The elimination rate constant was determined by the least-squares method using first-order kinetics. These data, along with the ratios of venous and arterial blood levels presented in Table IV, demonstrate that few of the 7.4- and 11.6-μm spheres reached the arterial circulation; 98% of the 7.4- and 11.6-μm spheres were eliminated from the circulating blood pool in the first 2 min. For the 3.4-μm spheres, 10–12 min was required before 98% was eliminated. Within 10 min, the concentrations of the 11.6, 7.4, and 3.4-μm spheres in venous circulation were <10, ~10, and ~10^3–10^4 spheres/g of blood, respectively. Therefore, a ratio of 0.81 for venous to arterial circulation for the 3.4-μm spheres indicates that the smaller spheres passed readily from the venous to arterial circulation, with high arterial levels present virtually immediately after administration. The

Table II—Microsphere Distribution in Specific Organs 3.5 hr after Intravenous Administration Determined by External Imaging

Size, μm	Lungs, %	Liver, %	Spleen, %	Total in the Three Organs, %
3.4	12	73	10	95
7.4	92	(~2%)[a]		94
11.6	93	—[b]	—[b]	93

[a] Combined level of activity in liver and spleen was ~2%. [b] Negligible level in liver and spleen.

[9] Bentley Trantec mode 800, Irvine, Calif.
[10] Honeywell Co., Denver, Colo.
[11] Brush recorder 200, Gould Co., Cleveland, Ohio.
[12] Model 9520 Cardiac Output Computer, Edwards Laboratories, Santa Ana, Calif.
[13] PDP-11/15, Digital Equipment Corp., Maynard, Mass.
[14] Pho/gamma LFOV, Searle Radiographics, Des Plaines, Ill.
[15] Spectroscaler, Picker Nuclear Corp., Northford, Conn.

Table III—Elimination of Microspheres from Blood at Initial Stage following Intravenous Administration [a]

Sphere Size, μm	Elimination Rate, min^{-1}		$t_{1/2}$, min	
	Venous Blood	Arterial Blood	Venous Blood	Arterial Blood
3.4	−0.426 ± 0.078	−0.403 ± 0.053	1.62	1.72
7.4	−0.810 ± 0.168	−0.776 ± 0.140[b]	0.86	0.89
11.6	−1.33 ± 0.320	—	0.52	—

[a] Average of two dogs in each size. [b] Essentially all 11.6-μm spheres and >95% of the 7.4-μm spheres were removed from the systemic circulation during the first pass. Therefore, this elimination rate is for a relatively small number of spheres that reached the arterial circulation.

blood level profiles show that the venous and arterial levels of 3.4-μm spheres remained essentially the same over ~3 hr, suggesting that these spheres recirculate rather freely. Even after 2 hr, the level in the circulation was 10^2–10^3/g of blood.

Hemodynamic Data—The 7.4- and 11.6-μm diameter microspheres were not associated with significant hemodynamic changes in the dosage ranges administered. The 3.4-μm diameter microspheres, administered at a similar total cross-sectional area dosage, induced significant dose-dependent deterioration in some of the hemodynamic parameters monitored (Fig. 3). These changes are illustrated more clearly in Fig. 4, which depicts a slow continuous recording of the first 7 min following intravenous administration of 1.41×10^{11} 3.4-μm diameter microspheres. A profound drop in the systemic blood pressure, with associated deterioration in $-dp/dt$, $+dp/dt$, and systolic segment shortening, occurred within several minutes of microsphere administration. A later secondary elevation in pulmonary artery pressure also occurred, probably due to a neurohumoral reflex response to the severe systemic hypotension.

These acute changes are most consistent with peripheral vascular collapse, not unlike those seen in anaphylactic shock. That direct myocardial depression occurs, but is not the predominant cause of shock, is evidenced by the lack of elevation in left ventricular end diastolic pressure and the reduction in end diastolic segment length. Only after a period of profound hypotension was there evidence of depressed myocardial function, *i.e.*, decreased $+dp/dt$ and decreased systolic segment shortening. Sustained ventricular tachycardia developed late following administration of 3.4-μm diameter microspheres in one animal. Cardiac output also was well maintained initially, dropping only after sustained hypotension, with its deleterious effect on the myocardium, had been present. Progressive increases in dosage of these 3.4-μm diameter microspheres resulted in progressive worsening of the severity (Fig. 5) and duration of the systemic hypotension.

DISCUSSION

Intravascular injection of particulate matter has been performed to image organs and assess blood flow characteristics. Early investigators utilized distribution kinetics of different size microspheres to assess the physical dimensions and biological characteristics of the microcirculation of various organs (1). As early as 1961, it was reported that the majority of microspheres less than 4.0 μm in diameter injected into the femoral vein passed through the pulmonary capillary network, whereas only a relatively small portion of similarly injected microspheres of >8.0 μm diameter passed into the systemic circulation (13). Previous work in our laboratories amplifies these points and, along with the present results, demonstrates that localization of the 7.4- and 11.6-μm diameter microspheres in the lungs, presumably by mechanical filtration, occurs in the first 2 min. This rapid localization suggests passive filtration and is consistent with ultrastructure studies of the pulmonary capillary network (14, 15) and the results of other investigators working with similarly sized microspheres (13, 16). The localization in the lungs appears to be complete for the 11.6-μm spheres, but there is evidence of gradual clearance and relocation in the liver and spleen of the 7.6-μm spheres.

Because of the recirculation characteristics of the microspheres of <4.0-μm diameter, their use was generally abandoned since they were of little value in organ imaging or assessing blood flow patterns. Of considerable interest is the recent documentation (2, 8) that, following intravenous administration, the 3.4-μm diameter microspheres pass through the pulmonary circulation and distribute in a pattern consistent with reticuloendothelial system uptake. This localization of the 3.4-μm diameter microspheres in the liver and spleen requires considerably more time than localization of the larger (≥7.4-μm diameter) microspheres in the lungs, suggesting a biologically active process, *e.g.*, phagocytosis (17) rather than passive filtration. The prolonged time course required for the 3.4-μm diameter microsphere clearance from the circulating blood pool correlates well with the duration of their acute hemodynamic effects (*vide supra*) and suggests some association with blood cells, a gradual release from the lungs, or a combination of the two.

Allen *et al.* (18) suggested that pulmonary hypertension is the most sensitive sign of hemodynamic deterioration following intravenous injection of microspheres larger than 13.5-μm in diameter. These authors also demonstrated a dose–response curve that correlated the increase in pulmonary artery pressure with increasing dosage and increasing sizes of administered microspheres (5). As expected from their dose–response curve, no significant pulmonary artery pressure rise was observed following intravenous administration of 7.4- and 11.6-μm diameter microspheres. While these particles are smaller than those that Allen utilized, they are nearly completely filtered by the pulmonary capillary network during the first pass through the pulmonary circulation.

The number of 3.4-μm diameter microspheres required to produce serious hemodynamic compromise is much smaller than would be predicted using the formula suggested by Allen *et al.* (5). This result appears due to the demonstrated ability of these small microspheres to pass through the pulmonary vascular network. Indeed, the period of hemodynamic compromise coincides with the time during which the 3.4-μm

Table IV—Ratio of Microsphere Levels in Venous and Arterial Blood (VBC/AVC) Samples following Intravenous Administration

Minutes	VBC/AVC		
	3.4 μm	7.4 μm	11.6 μm
2	0.81	6.4	85
4	0.69	3.1[a]	18[a]
10	0.81[b]	—[c]	—[c]
20	1.0	—	—
30	1.1	—	—

[a] Approximate venous concentration is <50 spheres/g of blood. [b] Venous concentration is 10^3–10^4 spheres/g of blood. [c] Venous concentration is <10 spheres/g of blood.

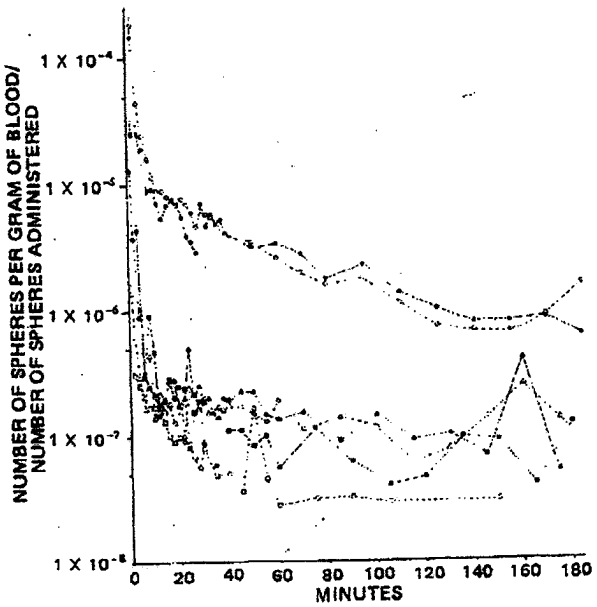


Figure 2—*Level of* ^{141}Ce*-labeled microspheres in venous and arterial samples following intravenous administration in beagle dogs. Key:* ●, *3.4 μm, venous;* ○, *3.4 μm, arterial;* ▲, *7.4 μm, venous;* △, *7.4 μm, arterial;* ■, *11.6 μm, venous; and* □, *11.6 μm, arterial.*

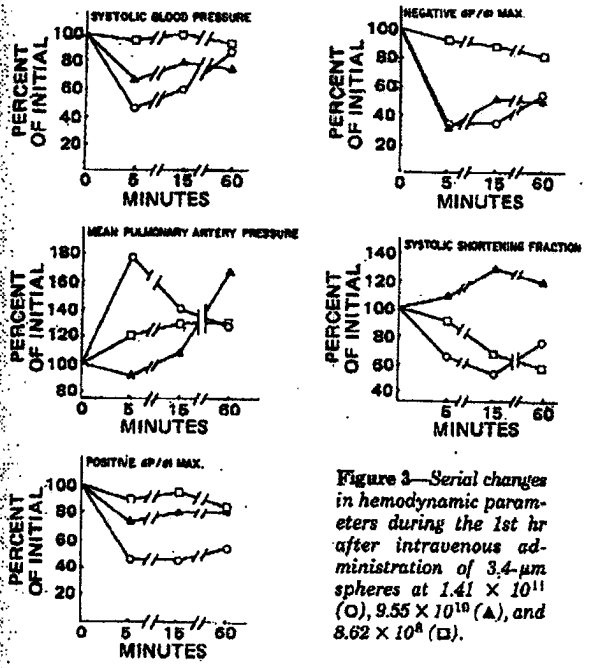


Figure 3—*Serial changes in hemodynamic parameters during the 1st hr after intravenous administration of 3.4-μm spheres at 1.41 × 10^{11} (○), 9.55 × 10^{10} (▲), and 8.62 × 10^{8} (□).*

diameter microspheres are numerous in the circulating blood pool (Figs. 2–4).

At present, the cause of the observed acute hemodynamic deterioration is unknown. Because of its similarity to anaphylaxis, a possible explanation would be the liberation of vasoactive amines as the microspheres pass through the pulmonary and peripheral vascular networks (19, 20). Alternatively, the hypotension could reflect a neurogenic reflex caused by high levels of the microspheres entering the central nervous system (21, 22). The former appears more likely based on the sequence of hemodynamic changes and the platelet clumping that occurred only around the 3.4-μm diameter microspheres upon histological examination (8, 23). The possibility that toxic monomers leaching from the smaller microspheres could promote vasomotor instability and initiate anaphylaxis was considered and discarded when injection of 20 ml of supernate from the sphere suspension into a dog caused no change in blood pressure over 0.5 hr. Furthermore, *in vitro* analyses of the supernate included UV spectra devoid of any peaks characteristic of monomer and a clean TLC analysis of a chloroform extract. The amount of residue left after drying the chloroform extract was too small to quantitate or to obtain an IR spectrum.

Incorporation of pharmacotherapeutic agents into microspheres for intravascular administration appears feasible from a technological standpoint and may have the advantages of achieving locally high drug concentrations in the target organ. Larger size (7.4- and 11.6-μm diameter) microspheres lodge in the pulmonary capillary network whereas 3.4-μm diameter microspheres appear to be taken up by the reticuloendothelial system. These differences in distribution fate following intravenous administration may be of significance in planning future chemotherapy of disease of the lungs and reticuloendothelial system.

CONCLUSION

This study demonstrates that intravenous administration of particulate matter may be associated with significant acute hemodynamic deterioration in the experimental animal and reveals that very small microspheres (3.4-μm diameter) have a different distribution fate and time course than larger microspheres (≥7.4-μm diameter). Since the difference in distribution is associated with a more severe, acute hemodynamic effect than would be expected based on size and number alone, the limitation of particulate material for target organ drug administration may be the ability to incorporate a therapeutic dose in a finite number of particles

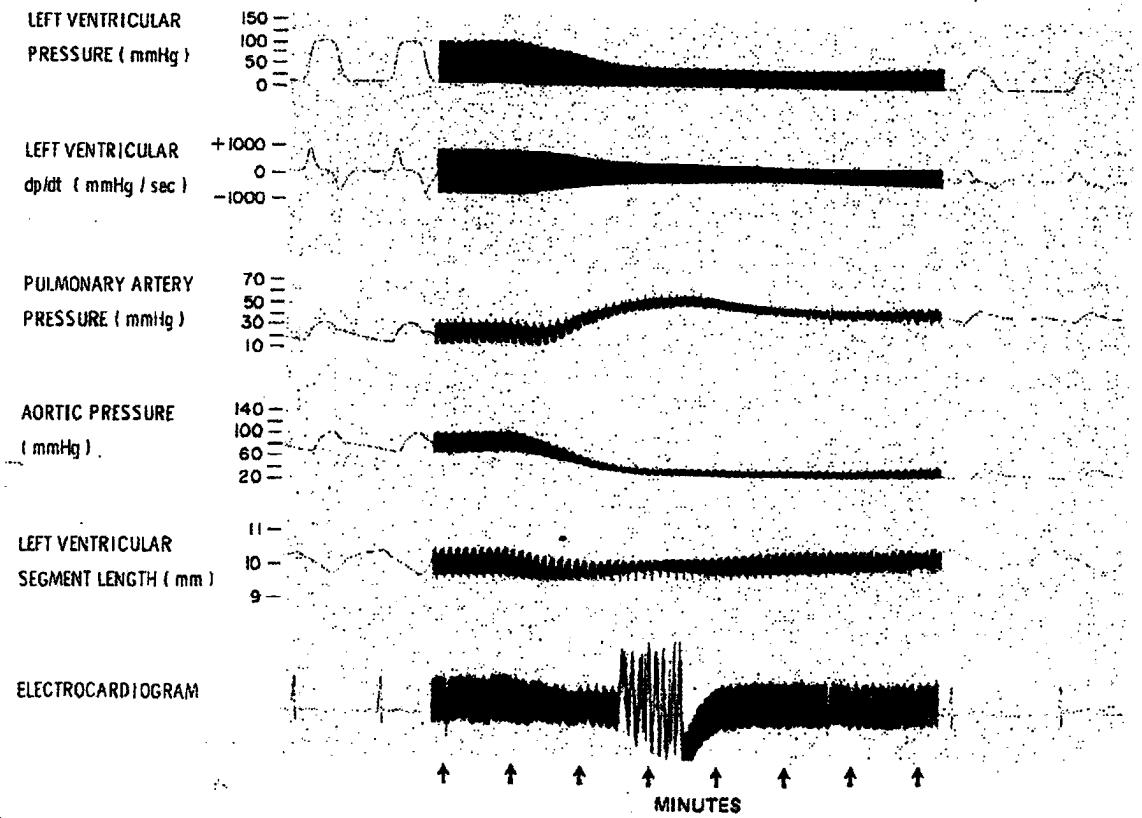


Figure 4—*Continuous display of the hemodynamic changes in the first 7 min following intravenous administration of 1.41 × 10^{11} microspheres of 3.4-μm size (area = 1.28 × 10^{12} $μm^2$ and weight = 3525 mg). A short lead and exit strip at chart speed of 25 mm/sec are provided to allow appreciation of individual waveforms.*

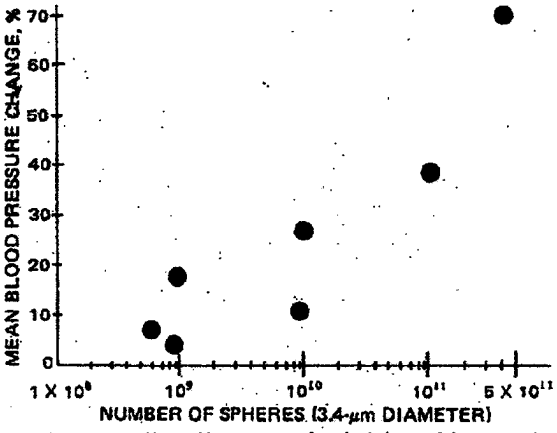


Figure 5—*Acute effect of intravenously administered 3.4-μm spheres on mean blood pressure as a function of concentration (r = 0.82).*

determined by their hemodynamic effect. Nevertheless, bolus intravenous injections of ~1 g of microspheres were administered to beagle dogs (13–15 kg) without serious consequences.

Therefore, the potential exists to incorporate sufficient amounts of drug into the microsphere during manufacture to provide therapeutic dosages. The influences of particle composition, surface charge, dissolution rate, and antigenicity will need to be determined. Furthermore, the acute hemodynamic effect evidenced following administration of the 3.4-μm diameter microspheres was consistently brief and reversible in the smaller dosage range. This finding suggests that a different dosage pattern, i.e., smaller, more frequent administrations rather than a large bolus injection, or slower administration could avoid the risks of hemodynamic effects, thereby delivering a selected chemotherapeutic agent in relatively high local tissue concentrations to the target organ with safety.

Finally, both histological and hemodynamic studies of the long-term effects of repeated intravascular injections of microspheres must be performed to exclude the possibility of irreversible tissue damage with chronic administration.

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