IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ELAN PHARMA INTERNATIONAL LIMITED, Plaintiff, v. ABRAXIS BIOSCIENCE, INC., Defendant.	)	Case No. 06-438-GMS **REDACTED PUBLIC VERSION**

DECLARATION OF LISA A. CHIARINI IN SUPPORT OF PLAINTIFF ELAN PHARMA INTERNATIONAL LIMITED'S ANSWERING BRIEF TO DEFENDANT ABRAXIS BIOSCIENCE, INC.'S MOTION TO COMPEL RESPONSES TO ABRAXIS'S FOURTH SET OF INTERROGATORIES

I, Lisa A. Chiarini, hereby declare under penalty of perjury pursuant to 28 U.S.C. Section 1746 as follows:

1. I am a member in good standing of the New York State Bar, am admitted to this Court *pro hac vice* in this matter, and am an associate with the law firm of Baker Botts, L.L.P., counsel of record for Elan Pharma International Limited in the above captioned action. I submit this Declaration based upon personal knowledge. If called upon as a witness, I could, and would, competently testify to the truth of each statement herein.

2. Attached hereto as Exhibit 1 is a true and correct copy of a chart of Abraxis's Interrogatories prepared by Elan counsel in order to illustrate the number of Interrogatories that have been propounded by Abraxis to Elan.

3. Attached hereto as Exhibit 2 is a true and correct copy of a selected portion of the 1989 edition of the McGraw-Hill Dictionary of Scientific and Technical Terms.

4. Attached hereto as Exhibit 3 is a true and correct copy of U.S. Patent No. 5,145,684.

5. Attached hereto as Exhibit 4 is a true and correct copy of selected portions of the deposition transcript of Lawrence de Garavilla taken in the above captioned action on July 26, 2007.

6. Attached hereto as Exhibit 5 is a true and correct copy of selected portions of the deposition transcript of Elaine Liversidge taken in the above captioned action on July 30, 2007.

7. Attached hereto as Exhibit 6 is a true and correct copy of selected portions of the deposition transcript of Nancy Peltier taken in the above captioned action on July 12, 2007.

8. Attached hereto as Exhibit 7 is a true and correct copy of selected portions of the deposition transcript of Pramod Sarpotdar taken in the above captioned action on June 29, 2007.

9. Attached hereto as Exhibit 8 is a true and correct copy of selected information from Elan's website.

I declare and affirm under penalty of perjury under the laws of the United States of America that the foregoing is true and correct.

FURTHER DECLARANT SAYETH NOT.

Executed this 30th day of August, 2007 in New York, New York.

Lisa A. Chiarini

EXHIBIT 1

Elan Pharma International Ltd. v. Abraxis Bioscience, Inc.

D.Del. 06-438-GMS

Abraxis Interrogatory No.	**Elan's Contention of Number of Separate Interrogatories**
D.I. 16, Abraxis's First Set Of Interrogatories served 11/3/2006	
1	At least 1
D.I. 24, Abraxis's Second Set Of Interrogatories served 11/15/2006	
2	At least 2
3	At least 2
4	At least 2
5	At least 1
6	At least 1
7	At least 3
8	At least 6
9	At least 1
D.I. 117, Abraxis's Third Set Of Interrogatories served 5/24/2007	
10	At least 2
11	At least 2
12	At least 1
13	At least 3
D.I. 161, Abraxis's Fourth Set Of Interrogatories served 6/29/2007	
14	At least 1
15	At least 1
16	At least 3
17	At least 1

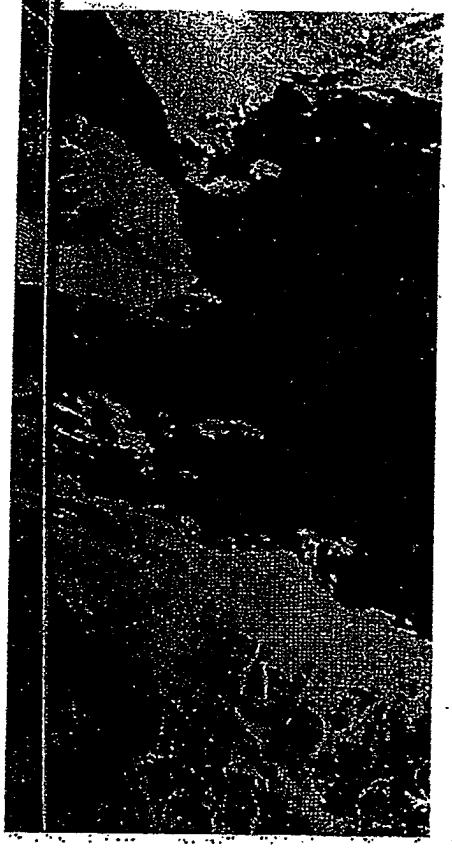
Abraxis Interrogatory No.	Elan's Contention of Number of Separate Interrogatories
18	At least 1
19	At least 1
20	At least 1
21	At least 1
22	At least 1
23	At least 1
24	At least 1
25	At least 1
26	At least 1
27	At least 1
28	At least 1
29	At least 1
30	At least 1
31	At least 1
32	At least 1
33	At least 1
34	At least 1
	TOTAL 50

EXHIBIT 2

Q
123
.M34
1989

McGraw-Hill DICTIONARY OF SCIENTIFIC AND TECHNICAL TERMS

Fourth Edition



Sybil P. Parker
EDITOR IN CHIEF

McGRAW-HILL BOOK COMPANY

New York
St. Louis
San Francisco

Auckland, Bogotá, Caracas, Colorado Springs, Hamburg, Lisbon, London, Madrid, Mexico, Milan, Montreal, New Delhi, Oklahoma City, Panama, Paris, San Juan, São Paulo, Singapore, Sydney, Tokyo, Toronto

BAKER BOTTS LLP
FEB 24 2004
HOUSTON LIBRARY

BAKER BOTTS LLP

On the cover: Pattern produced from white light by a computer-generated diffraction plate containing 529 square apertures arranged in a 23 × 23 array. (R. B. Hoover, Marshall Space Flight Center)

On the title pages: Aerial photograph of the Sinai Peninsula made by Gemini spacecraft. (NASA)

Included in this Dictionary are definitions which have been published previously in the following works: F. B. Jordain, *Condensed Computer Encyclopedia*, Copyright © 1969 by McGraw-Hill, Inc. All rights reserved. J. Markus, *Electronics and Nucleonics Dictionary*, 4th ed., Copyright © 1960, 1966, 1978 by McGraw-Hill, Inc. All rights reserved. J. Quick, *Artists' and Illustrators' Encyclopedia*, Copyright © 1969 by McGraw-Hill, Inc. All rights reserved. *Blakiston's Gould Medical Dictionary*, 3d ed., Copyright © 1956, 1972 by McGraw-Hill, Inc. All rights reserved. T. Baumeister and L. S. Marks, eds., *Standard Handbook for Mechanical Engineers*, 7th ed., Copyright © 1958, 1967 by McGraw-Hill, Inc. All rights reserved.

In addition, material has been drawn from the following references: R. E. Huschke, *Glossary of Meteorology*, American Meteorological Society, 1959; *U.S. Air Force Glossary of Standardized Terms*, AF Manual 11-1, vol. 1, 1972; *Communications-Electronics Terminology*, AF Manual 11-1, vol. 3, 1970; W. H. Allen, ed., *Dictionary of Technical Terms for Aerospace Use*, 1st ed., National Aeronautics and Space Administration, 1965; J. M. Gilliland, *Solar-Terrestrial Physics: A Glossary of Terms and Abbreviations*, Royal Aircraft Establishment Technical Report 67158, 1967; *Glossary of Air Traffic Control Terms*, Federal Aviation Agency; *A Glossary of Range Terminology, White Sands Missile Range, New Mexico*, National Bureau of Standards, AD 467-424; *A DOD Glossary of Mapping, Charting and Geodetic Terms*, 1st ed., Department of Defense, 1967; P. W. Thrush, comp. and ed., *A Dictionary of Mining, Mineral, and Related Terms*, Bureau of Mines, 1968; *Nuclear Terms: A Glossary*, 2d ed., Atomic Energy Commission; F. Casey, ed., *Compilation of Terms in Information Sciences Technology*, Federal Council for Science and Technology, 1970; *Glossary of Stinfo Terminology*, Office of Aerospace Research, U.S. Air Force, 1963; *Naval Dictionary of Electronic, Technical, and Imperative Terms*, Bureau of Naval Personnel, 1962; *ADP Glossary*, Department of the Navy, NAVSO P-3097.

McGRAW-HILL DICTIONARY OF SCIENTIFIC AND TECHNICAL TERMS, Fourth Edition

Copyright © 1989, 1984, 1978, 1976, 1974 by McGraw-Hill, Inc. All rights reserved. Printed in the United States of America. Except as permitted under the United States Copyright Act of 1976, no part of this publication may be reproduced or distributed in any form or by any means, or stored in a data base or retrieval system, without the prior written permission of the publisher.

2 3 4 5 6 7 8 9 0 DOW/DOW 8 9 5 4 3 2 1 0 9

ISBN 0-07-045270-9

Library of Congress Cataloging-in-Publication Data

McGraw-Hill dictionary of scientific and technical terms.

1. Science—Dictionaries. 2. Technology—Dictionaries.
I. Parker, Sybil P.
Q123.M34 1989 503'/21 88-13490
ISBN 0-07-045270-9

For more information about other McGraw-Hill materials, call 1-800-2-MCGRAW in the United States. In other countries, call your nearest McGraw-Hill office.

dicular or oblique to the axis of another fold. Also known as subsequent fold; superimposed fold; transverse fold. { 'krós ,fōld }

crossfoot [COMPUT SCI] To add numbers in several different ways in a computer, for checking purposes. { 'krós,fůt }

cross furring ceiling [BUILD] A ceiling in which furring members are attached perpendicular to the main runners or other structural members. { 'krós ,fər·iŋ ,sēl·iŋ }

cross gateway *See* cross heading. { 'krós 'gāt,wā }

cross hair [ENG] An inscribed line or a strand of hair, wire, silk, or the like used in an optical sight, transit, or similar instrument for accurate sighting. { 'krós ,her }

crosshatch generator [ELECTR] A signal generator that generates a crosshatch pattern for adjusting color television receiver circuits. { 'krós,hach ,jen·ə,rād·ər }

crosshaul [MECH ENG] A device for loading objects onto vehicles, consisting of a chain that is hooked on opposite sides of a vehicle, looped under the object, and connected to a power source and that rolls the object onto the vehicle. { 'krós,hól }

crosshead [MECH ENG] A block sliding between guides and containing a wrist pin for the conversion of reciprocating to rotary motion, as in an engine or compressor. [MET] A device generally employed in wire coating which is attached to the discharge end of the extruder cylinder; designed to facilitate extruding material at an angle. [MIN ENG] A runner or guide positioned just above a sinking bucket to restrict excessive swinging. { 'krós,hed }

cross heading [MIN ENG] Mine passage driven for ventilation from the airway to the gangway, or from one breast through the pillar to the adjoining working. Also known as cross gateway; cross hole; headway. { 'krós ,hed·iŋ }

cross hole *See* cross heading. { 'krós ,hōl }

crossing angle [NAV] The angle at which two lines of position intersect. Also known as angle of cut. { 'krós·iŋ ,aŋ·gəl }

crossing over [GEN] The exchange of genetic material between paired homologous chromosomes during meiosis. Also known as crossover. { ¦krós·iŋ 'ō·vər }

crossing plates [CIV ENG] Plates placed between a crossing and the ties to support the crossing and protect the ties. { 'krós·iŋ ,plāts }

crossing symmetry [PARTIC PHYS] The amplitude for a process that involves creation of a particle with four-momentum P_μ is equal to the amplitude for a process which is the same except it involves destruction of the antiparticle with four-momentum $-P_\mu$. { 'krós·iŋ ,sim·ə·trē }

cross joint [GEOL] A fracture in igneous rock perpendicular to the lineation caused by flow magma. Also known as transverse joint. { 'krós ,jóint }

cross-lamination *See* cross-bedding. { ¦krós lam·ə'nā·shən }

cross-level [ENG] To level at an angle perpendicular to the principal line of sight. { 'krós ,lev·əl }

crossline screen [GRAPHICS] In halftone photography, a grid that has opaque lines intersecting at right angles, forming transparent squares (screen apertures). Also known as a glass screen. { 'krós,līn ,skrēn }

cross-linking [ORG CHEM] The setting up of chemical links between the molecular chains of polymers. { 'krós ,liŋk·iŋ }

cross-magnetizing effect [ELECTROMAG] The distortion in the flux-density distribution in the air gap of an electric rotating machine caused by armature reaction. { ¦krós 'mag·nə,tīz·iŋ i'fekt }

crossmarks [GRAPHICS] Register marks used for the exact positioning of images in step-and-repeat, double, or multicolor printing; also used for superimposing overlays onto a base or onto each other. { 'krós,märks }

cross matching [IMMUNOL] Determination of blood compatibility for transfusion by mixing donor cells with recipient serum, and recipient cells with donor serum, and examining for an agglutination reaction. { 'krós ,mach·iŋ }

cross modulation [COMMUN] A type of interference in which the carrier of a desired signal becomes modulated by the program of an undesired signal on a different carrier frequency; the program of the undesired station is then heard in the background of the desired program. { ¦krós ,mäj·ə'lā·shən }

cross multiplication [MATH] Multiplication of the numerator of each of two fractions by the denominator of the other, as when eliminating fractions from an equation. { ¦krós ,məl·tə·plə'kā·shən }

cross-neutralization [ELECTR] Method of neutralization used in push-pull amplifiers, whereby a portion of the plate-cathode alternating-current voltage of each vacuum tube is applied to the grid-cathode circuit of the other vacuum tube through a neutralizing capacitor. { ¦krós ,nü·trə·lə'zā·shən }

cross office switching time [COMMUN] Time required to connect any input through the switching center to any selected output. { 'krós ,òf·əs 'swich·iŋ ,tīm }

Crossopterygii [PALEON] A subclass of the class Osteichthyes comprising the extinct lobefins or choanate fishes and represented by one extant species; distinguished by two separate dorsal fins. { krā,sāp·tə'rij·ē,ī }

Crossosomataceae [BOT] A monogeneric family of xerophytic shrubs in the order Dilleniales characterized by perigynous flowers, seeds with thin endosperm, and small, entire leaves. { krā·sə,sō·mə¦tās·ē,ē }

crossover [CIV ENG] An S-shaped section of railroad track joining two parallel tracks. [ELEC] A point at which two conductors cross, with appropriate insulation between them to prevent contact. [ENG] The portion of a draw works' drum containing grooves for angle control so the wire rope can cross over to begin a new wrap. [GEN] *See* crossing over. { 'krós,ō·vər }

crossover distortion [ELECTR] Amplitude distortion in a class B transistor power amplifier which occurs at low values of current, when input impedance becomes appreciable compared with driver impedance. { 'krós,ō·vər dis'tór·shən }

crossover experiment [MED] An experiment or clinical investigation in which subjects are divided randomly into at least as many groups as there are kinds of treatment to be given, and then the groups are interchanged until every subject has received each treatment. { 'krós,ō·vər ik'sper·ə·mənt }

crossover flange [ENG] Intermediate pipe flange used to connect flanges of different working pressures. { 'krós,ō·vər ,flanj }

crossover frequency [ENG ACOUS] 1. The frequency at which a dividing network delivers equal power to the upper and lower frequency channels when both are terminated in specified loads. 2. *See* transition frequency. { 'krós,ō·vər ,frē·kwən·sē }

crossover joint [PETRO ENG] A casing length with different threads at either end to permit a change from one thread to another in a casing string. { 'krós,ō·vər ,jóint }

crossover length [MATH] A length characteristic of a fractal network such that at scales which are small compared with this length the fractal nature of the structure is manifest in its dynamics, whereas at scales which are large compared with this length the dynamics resemble those of a crystalline structure. { 'krós,ō·vər ,leŋkth }

crossover network [ENG ACOUS] A selective network used to divide the audio-frequency output of an amplifier into two or more bands of frequencies. Also known as dividing network; loudspeaker dividing network. { 'krós,ō·vər ,net,wərk }

crossover spiral *See* lead-over groove. { 'krós,ō·vər ,spī·rəl }

crossover voltage [ELECTR] In a cathode-ray storage tube, the voltage of a secondary writing surface, with respect to cathode voltage, on which the secondary emission is unity. { 'krós,ō·vər ,vōl·tij }

cross-peen hammer [ENG] A hammer with a wedge-shaped surface at one end of the head. { 'krós ,pēn 'ham·ər }

cross-pointer indicator [NAV] A flight instrument having two needles which cross in the center when the aircraft is on course; the vertical needle indicates the position of the aircraft with respect to the localizer course, and the horizontal needle serves a similar purpose for the glide slope of an instrument landing system. { 'krós ,póint·ər ,in·də,kād·ər }

cross-polarization [ELECTROMAG] The component of the electric field vector normal to the desired polarization component. { ¦krós ,pō·lə·rə'zā·shən }

cross-pollination [BOT] Transfer of pollen from the anthers of one plant to the stigmata of another plant. { ¦krós ,pä·lə,nā·shən }

cross product [MATH] An anticommutative multiplication on the vectors of euclidean three-dimensional space. Also known as vector product. { 'krós ,prä·dəkt }

cross-reaction [IMMUNOL] Reaction between an antibody and a closely related, but not complementary, antigen. { 'krós rē'ak·shən }

cross-referencing program [COMPUT SCI] A computer pro-

EXHIBIT 3

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

[75] Inventors: Gary G. Liversidge, West Chester; Kenneth C. Cundy, Pottstown, both of Pa.; John F. Bishop, Rochester; David A. Czekai, Honeoye Falls, both of N.Y.

[73] Assignee: Sterling Drug Inc., New York, N.Y.

[21] Appl. No.: 647,105

[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

[56] References Cited

U.S. PATENT DOCUMENTS

2,671,750	3/1954	Macek	514/179
4,107,288	8/1978	Oppenheim	424/499
4,540,602	9/1985	Motoyama	424/495
4,826,689	5/1989	Violanto	424/489
4,851,421	7/1989	Iwasaki et al.	514/352

FOREIGN PATENT DOCUMENTS

411629	2/1991	European Pat. Off. .
2282330	11/1990	Japan .
2185397	7/1987	United Kingdom .
2200048	7/1988	United Kingdom .

OTHER PUBLICATIONS

Lachman et al., "the Theory and Practice of Industrial Pharmacy", Chapter 2, Milling (1986).

Remington's Pharmaceutical Sciences 17th Edition, Chapter 20, Schott, H., "Colloidal Dispersions".

Primary Examiner—Thurman K. Page

Assistant Examiner—William E. Benston, Jr.

Attorney, Agent, or Firm—Arthur H. Rosenstein; William J. Davis

[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 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.

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 4

REDACTED

EXHIBIT 5

REDACTED

EXHIBIT 6

REDACTED

EXHIBIT 7

REDACTED

EXHIBIT 8

Elan Research & Development



Discovery Research Projects

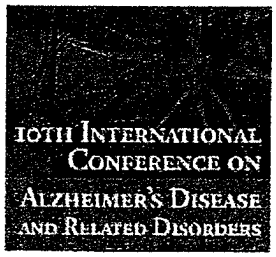
Elan's discovery research efforts are centered on bringing science and innovation to patients in its core therapeutic focus areas of neurology, autoimmune diseases and severe pain.

In neurology, Elan is focused on building upon its breakthrough research and extensive experience in the area of neuropathology-related disorders such as Alzheimer's disease, where the company's efforts include programs focused on small molecule inhibitors of beta secretase and gamma secretase, enzymes whose actions are thought to affect the accumulation of the amyloid plaques found in the brains of patients with Alzheimer's disease. Elan is also studying other neurodegenerative diseases, such as Parkinson's disease.

In autoimmune diseases, Elan's primary emphasis is studying cell trafficking to discover ways to provide disease-modifying therapies for autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and inflammatory bowel disease; and in severe pain, research efforts focus on inflammatory and neuropathic pain.

Products in Development

In neurology, Elan, in collaboration with Wyeth, initiated a Phase II clinical trial for an experimental humanized monoclonal antibody with a targeted indication of immunotherapeutic treatment of mild to moderate Alzheimer's disease (AD). The humanized monoclonal antibody is designed and engineered to clear the neurotoxic beta-amyloid peptide that accumulates in the brains of patients with AD.



In autoimmune diseases, Elan's primary emphasis is studying and developing ways to provide disease-modifying therapies for a wide range of diseases.

View Elan's pipeline
View Elan's marketed products

TYSABRI is a registered trademark of Elan Pharmaceuticals, Inc. in the United States and of Elan Pharma International Ltd. in various countries outside the United States.

PRIALT is a registered trademark of Elan Pharmaceuticals, Inc.

Copyright © 2001- 2004 Elan Corporation, plc, Dublin, Ireland. All rights reserved. | Privacy Policy | Terms of use

elan

Pipeline and Products

Elan Corporation, plc is a neuroscience based biotechnology company that is focused on discovering, developing, manufacturing and marketing advanced therapies in neurodegeneration, autoimmune diseases and severe pain.

Medical Inquiries:
Medical Information Services can be reached at: Toll-free Phone: **1-888-638-7605**

An Elan Medical Information Services representative is available to answer medical information inquiries from 9:00 am – 7:00 pm EST Monday through Friday.

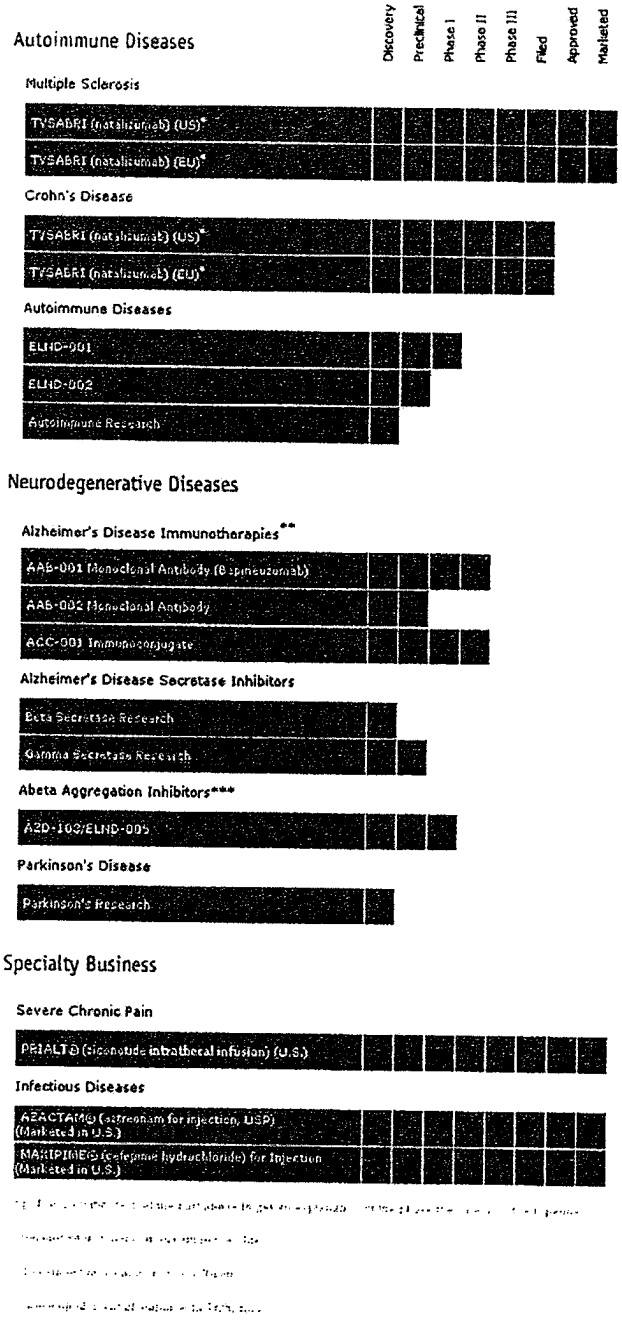
TYSABRI is a registered trademark of Elan Pharmaceuticals, Inc. in the United States and of Elan Pharma International Ltd. in various countries outside the United States. Prialt is a registered trademark of Elan Pharmaceuticals, Inc.

Azactam and Maxipime are registered trademarks of Bristol-Myers Squibb Company and licensed exclusively in the U.S. to Elan Pharmaceuticals, Inc.

© 2003 Elan Pharmaceuticals, Inc. ELN 022 0104.

Last Updated July 24, 2007

Pipeline and Products



U.S Products

All product information provided is intended for U.S. healthcare professionals only. If you are not a healthcare professional and would like more information about any of these products, please contact your doctor. These products may not be available in countries outside the United States or may be available under a different trademark; in different strengths; or for different indications. Countries outside the United States may have different regulatory requirements that would call for different information to be available; product information for countries other than the United States is not available at this time.

For an overview of Elan's research and development program, please click here.
For more information about Elan's pipeline and products, please click here.

U.S. Products

TYSABRI (natalizumab)

Tysabri® (natalizumab)

» Tysabri Information Center
» Pipeline and Products

prialt ZICONOTIDE INTRATHECAL INFUSION

PRIALT® (ziconotide **intrathecal infusion**)

» Fact Sheet » Prescribing Information
» Pipeline and Products

Azactam aztreonam IV/IM lg/2g

Azactam®(aztreonam for injection, USP)

» Fact Sheet » Prescribing Information
» Pipeline and Products

MAXIPIME

Maxipime®(cefepime hydrochloride) for Injection

» Fact Sheet » Prescribing Information
» Pipeline and Products

Elan has a Patient Assistance Program to provide TYSABRI and PRIALT to patients who might not otherwise be able to afford them. For more information on Elan's Medical Needs Program, you may contact the following: TYSABRI at 1-888-489-7227 or PRIALT at 1-888-774-2581

For Elan product-related inquiries, please contact **Medical Information Services:**
Toll-Free Phone: 1-888-638-7605

An Elan Medical Information Services representative is available to answer m
information inquiries from 8 a.m. - 7 p.m. Eastern Time, Monday through Fric

For Elan **U.S. Customer Service**-related inquiries, including order placement
and product availability, for U.S. distributed products other than **PRIALT (zic
intrathecal infusion**), please call:
Toll-Free Phone: 1-800-859-8586
An Elan Customer Service representative is available to answer inquiries from
Eastern Time, Monday through Friday.

Regarding questions concerning **PRIALT (ziconotide intrathecal infusion)**
Toll-Free Phone: 1-888-PRIALT-1 (1-888-774-2581)
A representative is available to answer medical information inquiries from 8 a
Eastern Time, Monday through Friday.

TYSABRI is a registered trademark of Elan Pharmaceuticals, Inc. in the United States and of El
International Ltd. in various countries outside the United States.

PRIALT is a registered trademark of Elan Pharmaceuticals, Inc.

AZACTAM and MAXIPIME are registered trademarks of Bristol-Myers Squibb Company and lic
the U.S.to Elan Pharmaceuticals, Inc.

Copyright © 2001- 2004 Elan Corporation, plc. Dublin,
Ireland All rights reserved. | Privacy Policy | Terms of use