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 VERSON**

APPENDIX OF EXHIBITS TO ABRAXIS'S OPENING BRIEF IN SUPPORT OF ITS MOTION FOR JUDGMENT AS A MATTER OF LAW OF NONINFRINGEMENT, INVALIDITY, INEQUITABLE CONDUCT, AND ITS MOTION FOR NEW TRIAL, ITS OPENING BRIEF IN SUPPORT OF ITS MOTION TO AMEND THE JUDGMENT AND ENTER FINDINGS THAT ELAN ENGAGED IN INEQUITABLE CONDUCT IN PROCURING THE '363 AND '025 PATENTS, AND THE PROPOSED FINDINGS OF FACT AND CONCLUSIONS OF LAW (VOLUME I OF II)

OF COUNSEL:
Michael A. Jacobs
MORRISON & FOERSTER, LLP
425 Market Street
San Francisco, CA 94105-2482
(415) 268-7000

Emily A. Evans
Eric S. Walters
Erik J. Olson
Paul F. Coyne
Diana B. Kruze
MORRISON & FOERSTER, LLP
755 Page Mill Road
Palo Alto, CA 94304-1018
(650) 813-5600

Anders T. Aannestad
MORRISON & FOERSTER LLP
12531 High Bluff Drive
San Diego, California 92130
(858) 720-5100

YOUNG CONAWAY STARGATT & TAYLOR, LLP
Josy W. Ingersoll (#1088)
Elena C. Norman (#4780)
Karen E. Keller (#4489)
Michele Sheretta Budicak (#4651)
Jeffrey T. Castellano (#4837)
The Brandywine Building
1000 West Street, 17th Floor
Wilmington, DE 19801
(302) 571-6600
jingersoll@ycst.com
enorman@ycst.com
kkeller@ycst.com
mbudicak@ycst.com
jcastellano@ycst.com

Attorneys for Defendant
ABRAXIS BIOSCIENCE, INC.

Dated: July 14, 2008

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JX005	Cover letter of IND 55974 and section VII. Chemistry, Manufacturing and Controls Information Excerpt (p 547)	9/30/1997	A 1-3
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JX021	Excerpts from Laboratory Notebook 24 (Neil Desai)	9/4/1996	A 140-144
JX039	Excerpts from File History of U.S. Patent No. 5,399,363 (Liversidge et al.) (Certified copy)	03/21/1995	A 145-169
JX040	Excerpts from File History of U.S. Patent No. 5,834,025 (de Garavilla et al) (Certified copy)	11/10/1998	A 170-182
JX047	Article - E Merisko-Liversidge, et al, "Formulation and Antitumor Activity Evaluation of Nanocrystalline Suspensions of Poorly Soluble Anticancer Drugs"	1/1/1996	A 183-189
JX053	Presentation - Nano-Taxol Update Presentation - Nano-Taxol/Objectives	00/00/1994	A 190-246
JX054	Memo from Gary Liversidge re Nanos	08/30/1995	A 247-254
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JX072	Article - G. Liversidge et al., "Particle Size Reduction for Improvement of Oral Bioavailability of Hydrophobic Drugs: I. Absolute Oral Bioavailability of Nanocrystalline Danazol in Beagle Dogs"	00/00/1995	A 473-479
JX081	U.S. Patent No. 5,399,363 ('363 patent) (Ribbon copy)	3/21/1995	A 480-492
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JX087	Sterling Memo from Cardiovascular Pharmacology to Distribution re Final Study Report for SWPRD Study Report No. 832	07/02/1992	A 529-547
DX015	United States Patent No. 5,834,025 - de Garavilla et al.	11/10/1998	A 548-557
DX041	Memorandum from E. Gresham from R. Selvaraj re XRPD of ABI-007 Samples Micron Inc. Analytical Services Report #27872 Micron Inc. Analytical Services Report #28217 Micron Inc. Analytical Services Report #28757 Micron Inc. Analytical Services Report #29007 Micron Inc. Analytical Services Report #29101 Micron Inc. Analytical Services Report #33063 Micron Inc. Analytical Services Report #33370 Abraxis Research Report PD06-NP/N-004 titled, "Comparative Study on Characterization Results of Abraxane Commercial and Stability Lots" Technical Report # PD03-NP/P-020 titled, "Paclitaxel and Human Albumin Assays in Supernatant Liquid and Pellet of ABI-007 Reconstituted Suspension" Laboratory Notebook 23938 (Maureen Connell)	03/21/2002 03/28/2002 06/21/2002 10/21/2002 12/13/2002 01/14/2003 02/27/2006 04/21/2006 00/00/2002	A 558-803
DX076	Laboratory Notebook 00123 (Vuong Trieu)	07/02/2007	A 804-820
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DX286	Email from Elaine Liversidge to Eugene Cooper et al. re Piposulfan Plan	11/12/1996	A 1231
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	Excerpts of Trial Transcripts	06/02-11/2008	A 2059-2206

CERTIFICATE OF SERVICE

I, Karen E. Keller, Esquire, hereby certify that on July 21, 2008, I caused to be electronically filed a true and correct copy of the foregoing document with the Clerk of the Court using CM/ECF, which will send notification that such filing is available for viewing and downloading to the following counsel of record:

Steven J. Balick, Esquire
ASHBY & GEDDES
500 Delaware Avenue, 8th Floor
Wilmington, DE 19801

I further certify that on July 21, 2008**,** I caused a true and correct copy of the foregoing document to be served by e-mail on the above-listed counsel of record and on the following non-registered participants in the manner indicated:

BY E-MAIL

Stephen Scheve, Esquire
BAKER BOTTS L.L.P.
One Shell Plaza
910 Louisiana Street
Houston, TX 77002-4995
steve.scheve@bakerbotts.com

Paul F. Fehlner, Esquire
BAKER BOTTS L.L.P.
30 Rockefeller Plaza
New York, NY 10112-4498
paul.fehlner@bakerbotts.com

YOUNG CONAWAY STARGATT
& TAYLOR, LLP

/s/ *Karen E. Keller*
Josy W. Ingersoll (No. 1088)
jingersoll@ycst.com
Elena C. Norman (No. 4780)
enorman@ycst.com
Karen E. Keller (No. 4489)
kkeller@ycst.com
Michele Sherretta Budicak (No. 4651)
mbudicak@ycst.com
Jeffrey T. Castellano (No. 4837)
jcastellano@ycst.com
The Brandywine Building
1000 West Street, 17th Floor
Wilmington, Delaware 19899
(302) 571-6600

Attorneys for Defendant
ABRAXIS BIOSCIENCE, INC.

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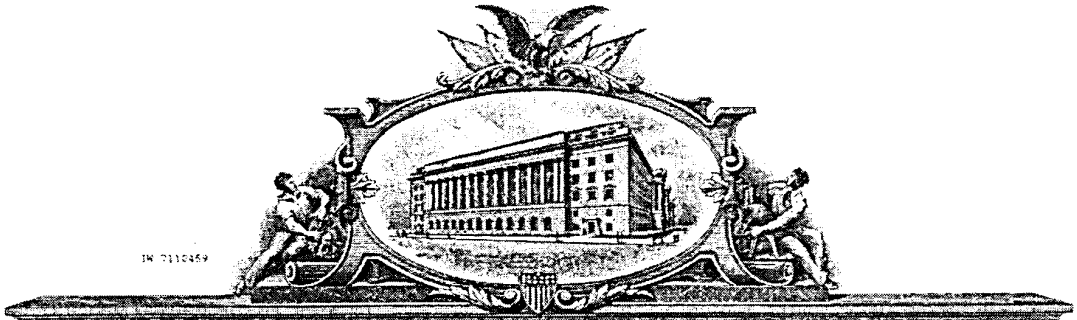

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

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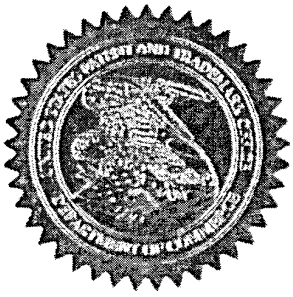
United States Patent and Trademark Office

February 07, 2008

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *07/908,125*
FILING DATE: *July 01, 1992*
PATENT NUMBER: *5,399,363*
ISSUE DATE: *March 21, 1995*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office



N. WILLIAMS
Certifying Officer

PART 0 OF (2 PART(S)

5399363

07/908125 | PATENT DATE | PATENT NUMBER

NUMBER	FILING DATE	CLASS	SUBCLASS		
908,125	07/01/92	514			

G. LIVERSIDGE, WEST CHESTER, PA; PLAINE LIVERSIDGE, WEST CHESTER
RAMOD P. SARPOTDAR, MALVERN, PA.

CONTINUING DATA********
VERIFIED THIS APPLN IS A CIP OF 07/647,105 01/25/91 PAT 5,145,684

Certificate

APR 25 2006

of Correction

07908125

FOREIGN/PCT APPLICATIONS
VERIFIED

FOREIGN FILING LICENSE GRANTED 08/11/92

Foreign priority claimed 35 USC 119 conditions met	☐ yes ☒ no ☐ yes ☒ no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
Verified and Acknowledged	Examiner's Initials		PA	0	27	1	$930.00	[illegible]

ADDRESS
PATENT DEPARTMENT
STERLING WINTHROP, INC.
~~31 COLUMBIA TURNPIKE~~
~~RENSSELAER, NY 12144~~
9 Great Valley Parkway
Malvern, Pennsylvania 19355

SURFACE MODIFIED ANTICANCER NANOPARTICLES

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (rev. 10-78)

PARTS OF APPLICATION
FILED SEPARATELY

NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE 9-29-94	CLAIMS ALLOWED
9-19-94	William E. Benston, Assistant Examiner; D. Gassaway, Docket Clerk	Total Claims 17; Print Claim 1
ISSUE FEE: Amount Due 1511.00; Date Paid 12-19-94	Thurman K. Page, SUPERVISORY PATENT EXAMINER, ART UNIT 152, Primary Examiner	DRAWING: Sheets Drwg. 4; Figs. Drwg. 4; Print Fig. 4
Label Area	ISSUE CLASSIFICATION: Class 424; Subclass 490	ISSUE BATCH NUMBER E61

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Form PTO-436
Rev. 9/80

0002

15X William Benton JR.

MAIL ROOM OCT 29 1992 PAT. & TRADEMARK OFF.



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#2 R. Gassaway 12-17-92

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GROUP 120 RECEIVED

In re Application of
Elaine Liversidge et al

Serial No. : 07/908,125 : Group Art Unit: ~~[illegible]~~

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)
William Davis 30744
(Reg. No.)
William Davis 26 Oct 1992
(Date of Signature)

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

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

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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

William J. Davis
Registration No. 30,744
Attorney for Applicants
(215) 889-8802

Address:
Patent Department
Sterling Winthrop Inc.
81 Columbia Turnpike
Rensselaer, NY 12144

WJD:mmm

#6/A
10/5/93



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Riversidge 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	:	

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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:

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

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

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

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31. 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 $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$.

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32. 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.

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33. 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

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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
Attorney for Applicants
Reg. No. 30,744

Sterling Winthrop Inc.
9 Great Valley Parkway
Malvern, PA 19355
Tele: (215) 889-8802

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)

#17

MAIL ROOM SEP 13 1993 PAT. & TRADEMARK OFF.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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 30744
(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

BENSTON, JR., W — EXAMINER

D 6

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 2-6, 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)

0347

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.

WEB W.E. Benston:jaw
December 19, 1993

THURMAN K. PAGE
SUPERVISORY PRIMARY EXAMINER
GROUP 1502

Gp 1502 #12 B

EK DN 62,937 PATENT

CERTIFICATE UNDER 37 CFR 1.8(a)

MAIL ROOM JUN 27 1994 PAT. & TRADEMARK OFF.

RECEIVED JUL 18 1994 GROUP 150

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)

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:

B

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
Attorney for Applicants
Reg. No. 30,744

Date: June 22, 1994

Sterling Winthrop Inc.
Patent Department
9 Great Valley Parkway
P.O. Box 3026
Malvern, PA 19355
Telephone: (610) 889-8802
Facsimile: (610) 889-6364

IN 7110459

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

February 07, 2008

THIS IS TO CERTIFY THAT ANNEXED IS A TRUE COPY FROM THE RECORDS OF THIS OFFICE OF THE FILE WRAPPER AND CONTENTS OF:

APPLICATION NUMBER: *08/696,754*
FILING DATE: *August 14, 1996*
PATENT NUMBER: *5,834,025*
ISSUE DATE: *November 10, 1998*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

M. K. CARTER
Certifying Officer

0001

ISSUE CLASSIFICATION: Class 424 Subclass 501

[redacted]

P 5834025

5834025

UTILITY SERIAL NUMBER	PATENT DATE	PATENT NUMBER
08/696754	NOV 10 1998	

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
08/696,754	08/14/96	424	501	~~1502~~ 1615	AZPURU

APPLICANTS: LAWRENCE DE GARAVILLA, DOWNINGTOWN, PA; ELAINE M. LIVERSIDGE, WEST CHESTER, PA; GARY G. LIVERSIDGE, WEST CHESTER, PA.

CONTINUING DATA********
VERIFIED PROVISIONAL APPLICATION NO. 60/004,488 09/29/95

CA

FOREIGN/PCT APPLICATIONS********
VERIFIED

FOREIGN FILING LICENSE GRANTED 09/04/96

Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged (Examiner's Initials)	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐ yes ☒ no / ☐ yes ☐ no		PA	0	20	5	$986.00	729930

ADDRESS: ~~REDMAN & BALOGH 1400 CRYSTAL VALLEY DRIVE AMBLER PA 19002-4042~~
Morgan, Lewis & Bockius 1800 M Street, N.W. Washington, D.C. 20036

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

U.S. DEPT. OF COMM./PAT. & TM — PTO-436L (Rev. 12-94)

PARTS OF APPLICATION FILED SEPARATELY

Applications Examiner

NOTICE OF ALLOWANCE MAILED	Assistant Examiner	CLAIMS ALLOWED: Total Claims	Print Claim
04-02-98		20	

ISSUE FEE: Amount Due	Date Paid	DRAWING: Sheets Drwg.	Figs. Drwg.	Print Fig.
1320.00	7/1/98			

CARLOS AZPURU
PRIMARY EXAMINER
GROUP 1500
Primary Examiner

ISSUE BATCH NUMBER C 53

Label Area

PREPARED FOR ISSUE

WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.

Form PTO-436A (Rev. 8/92)

BW

(FACE)

0002

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/696,754	08/14/96	DE GARAVILLA L	72993A

15M1/0930

MORGAN, LEWIS & BOCKIUS
1800 M STREET, N. W.
WASHINGTON DC 20036

EXAMINER	
AZPURU,C	
ART UNIT	PAPER NUMBER
1502	7

DATE MAILED: 09/30/97

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

PTO-90C (Rev. 2/95)
*U.S. GPO: 1997-422-198/60031

1- File Copy

Office Action Summary	Application No. 08/696,754	Applicant(s) De Garavilla et al.
	Examiner Carlos Azpuru	Group Art Unit 1502

☐ Responsive to communication(s) filed on _____.

☐ This action is **FINAL.**

☐ Since this application is 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.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-20* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ *is/are allowed.*

☒ Claim(s) *1-20* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

U. S. Patent and Trademark Office
PTO-326 (Rev. 9-95) Office Action Summary Part of Paper No. 7

DETAILED ACTION

Receipt is acknowledged of the preliminary amendment filed 08/14/97.

Claim Rejections - 35 USC § 103

1. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

2. Claims 1-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wong et al.

Wong et al disclose a nanoparticulate formulation (see Abstract) directed towards poorly soluble therapeutic agents (having a solubility less than about 10 mg/ml as cited at col. 1, lines 18-20).Particle size is less than 400 nm (see col. 6, lines 7-9). Therapeutic agents if found at a concentration of between 0.1-60% (col. 5, lines 63-65). The surface modifier is found at a concentration of 0.1 to 90% (col. 6, lines 1-2). Pharmaceutical carriers and dosage forms are disclosed at cols 11-12. Intravenous administration is disclosed at col. 4, line 40. Wong et al differ only in their lack of disclosure of a precise infusion rate.

However, the specification is clear at col 12, lines 64-68; and col. 13, lines 1-15, that such a dosage level varies according to therapeutic agent, severity of disease or condition being treated, duration of treatment, etc. Further, it would have been well within the skill of the ordinary

practitioner to select the proper therapeutic infusion rate of the therapeutic agents given that they are well, known, as being presented in well known nanoparticles as disclosed by Wong et al, and have an expectation of similar therapeutic efficacy. Therefore, it is deemed that it would have been well within the skill of the ordinary practitioner to claim the instant method utilizing the nanoparticles as disclosed by Wong et al, and further to select a therapeutic infusion (dosage) rate appropriate to the agent, disease and individual being selected. The instant method would have been obvious given the disclosure of Wong et al.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carlos Azpuru whose telephone number is (703) 703-0237. The examiner can normally be reached on Tuesday-Friday from 6:30 am to 6:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Thurman Page, can be reached on (703) 308-2927. The fax phone number for this Group is (703) 305-5408 and 308-5433.

Communications via Internet e-mail regarding this application, other than those under 35 U.S.C. 132 or which otherwise require a signature, may be used by the applicant and should be addressed to [**thurman.page@uspto.gov**].

All Internet e-mail communications will be made of record in the application file. PTO employees do not engage in Internet communications where there exists a possibility that sensitive information could be identified or exchanged unless the record includes a properly signed express waiver of the confidentiality requirements of 35 U.S.C. 122. This is more clearly set forth in the Interim Internet Usage Policy published in the Official Gazette of the Patent and Trademark on February 25, 1997 at 1195 OG 89.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-2351.

CARLOS AZPURU
PRIMARY EXAMINER
GROUP 1500

OIPE JC23 FEB 09 1998 PATENT & TRADEMARK OFFICE

PATENT
Attorney Docket No. 47988-224
Parent Application No. 08/696,754

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
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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.

B

PATENT
Attorney Docket No. 47988-224
Parent Application No. 08/696,754

IN THE CLAIMS:

B1

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:

(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.

Ex note [Claim 2, line ~~1~~ 2, change "is of from" to –is from--.

B2

5. (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 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.

B3

9. (Amended) A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic [effect] 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

30

B3 amended

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.

31

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

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

McDermott, Will & Emery
227 West Monroe Street
Chicago, IL 60606-5096
Phone: (312) 984-7746
Fax: (312) 984-7700

Pharmaceutical Research, Vol. 13, No. 2, 1996 *Report*

Formulation and Antitumor Activity Evaluation of Nanocrystalline Suspensions of Poorly Soluble Anticancer Drugs

E. Merisko-Liversidge,[1,3] P. Sarpotdar,[1] J. Bruno,[1] S. Hajj,[1] L. Wei,[1] N. Peltier,[1] J. Rake,[1] J. M. Shaw,[1] S. Pugh,[2] L. Polin,[2] J. Jones,[2] T. Corbett,[2] E. Cooper,[1] and G. G. Liversidge[1]

Purpose. Determine if wet milling technology could be used to formulate water insoluble antitumor agents as stabilized nanocrystalline drug suspensions that retain biological effectiveness following intravenous injection.

Methods. The versatility of the approach is demonstrated by evaluation of four poorly water soluble chemotherapeutic agents that exhibit diverse chemistries and mechanisms of action. The compounds selected were: piposulfan (alkylating agent), etoposide (topoisomerase II inhibitor), camptothecin (topoisomerase I inhibitor) and paclitaxel (antimitotic agent). The agents were wet milled as a 2% w/v solids suspension containing 1% w/v surfactant stabilizer using a low energy ball mill. The size , physical stability and efficacy of the nanocrystalline suspensions were evaluated.

Results. The data show the feasibility of formulating poorly water soluble anticancer agents as physically stable aqueous nanocrystalline suspensions. The suspensions are physically stable and efficacious following intravenous injection.

Conclusions. Wet milling technology is a feasible approach for formulating poorly water soluble chemotherapeutic agents that may offer a number of advantages over a more classical approach.

KEY WORDS: anticancer agents; poorly water soluble agents; nanoparticles; etoposide; camptothecin; piposulfan and paclitaxel.

INTRODUCTION

Frequently in drug discovery poorly water soluble agents are identified as actives using *in vitro* assays but are discarded because they are unable to be formulated for further evaluation *in vivo*. Thus many promising agents are discarded due to poor water solubility and the lack of a generally applicable formulation approach to address this problem. Though this predicament is a likely scenario in any therapeutic/diagnostic discovery program it has been a recurring issue in cancer research. The development of a number of promising anticancer agents have been delayed or abandoned due to issues resulting from the poor water solubility of the drug (1).

Currently, the poorly water soluble chemotherapeutic agents used in the clinic and that are in various phases of development are formulated by conventional methods. Routinely, these agents are formulated using a co-solvent plus other excipients which act to solubilize the drug and provide for stability of the formulation during storage and on injection (2). Commonly, the use of co-solvents produce toxic side effects e.g., anaphylaxis, pain at the site of injection, emboli formation and paradoxically precipitation which results in poor intravenous bioavailability. For instance, etoposide (VP-16), a semisynthetic analog of podophyllotoxin, is a sparingly water soluble drug that is used to treat small-cell lung cancer and various other neoplasms (3,4). Etoposide being a poorly water soluble drug is formulated using benzyl alcohol, polyoxyethylated sorbitan ester (Tween 80) and polyethyleneglycol (PEG 300). Prior to injection the formulation is diluted then slowly infused. Even though etoposide has been used in the clinic for a number of years, formulation related toxicity issues still occur (5–7).

Extensive literature is available on other technologies concerned with delivery issues of hard to formulate chemotherapeutic agents. Although significant progress has been made in each of these areas including liposomes (8,9), emulsions (10,11) and polymeric carriers (12,13), there is a need for additional methodologies that provide a safe, effective and economical means for intravenous administration of poorly water soluble therapeutics and/or diagnostics.

In this study, a delivery system well suited for sparingly water soluble chemotherapeutic agents is described wherein the compounds are formulated as dispersible particles consisting essentially of a crystalline drug substance stabilized with a surface modifier. The methods of preparation and characterization of nanocrystalline dosage forms suitable for intravenous administration of poorly water soluble chemotherapeutic agents are presented together with preclinical efficacy data performed in the mammary 16C murine tumor model.

MATERIALS AND METHODS

Chemicals

With the exception of piposulfan which was custom synthesized at Kodak Research Laboratories, Rochester, NY., agents were obtained from the following vendors: etoposide (Sigma Chemical Co, St. Louis, MO), camptothecin (Aldrich, Milwaukee, WI) and paclitaxel (Biolyse Corporation, Port-Daniel, Que.). Pluronic and Tetronic surfactant stabilizers were obtained from BASF (Parsnippany, NJ.) and the polyoxyethylated sorbitan esters were purchased from ICI, Wilmington, DE.

Nanoparticle Formulations

Nanoparticle formulations were prepared under aseptic conditions as a drug suspension (2% wt/v) containing 1% (wt/v) surfactant stabilizer (14,15). An aqueous suspension containing drug and stabilizer was wet milled using preconditioned zirconium oxide media (Zircoa Inc., Solon, OH.) For screening, milling was performed in a 28 ml bottle using a media bead volume of 7.5 ml and 3.75 ml of the drug/surfactant slurry. The slurry was milled on a low energy mill (U.S. Stoneware, East Palestine, OH) 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. Milling efficiency was dependent on a number of factors including drug substance and choice of stabilizer. The particle size of the slurry was assayed daily during milling. Routinely, the drug/surfactant slurry was milled to a final size of less than 400 nm based on

[1] NanoSystems, Collegeville, Pennsylvania 19426.
[2] Division of Hematology and Oncology, Wayne State University, Detroit, Michigan 48202.
[3] To whom correspondence should be addressed.

ELANP 0036387

photon correlation spectroscopy (PCS) and generally this could be achieved in a 4 day period. Milled nanosuspensions were evaluated for chemical stability, physical stability, and physical stability in plasma. Formulations with acceptable physical stability in plasma were submitted for efficacy studies. Acceptable physical stability was defined as absence of agglomeration or negligible particle size growth in the presence of plasma when the nanosuspension was incubated with plasma for 60 min at 37°C.

The stabilizers used for each of the formulations were: 1) polyoxyethylene sorbitan monooleate (Tween 80)/sorbitan monooleate (Span 80) for piposulfan; 2) Pluronic F108 for camptothecin, and 3) Pluronic F127 for etoposide and paclitaxel (Table 1).

Particle Size Analysis

Particle size analysis was determined using photon correlation spectroscopy (PCS). Prior to sizing, samples were diluted with freshly filtered deionized water. Sizing measurements were routinely performed using the Coulter Model N4MD Submicron Particle Analyzer (Coulter, Miami, Fl.) or the Malvern Zetamaster (Malvern Instruments Ltd., Worcester, England) using unimodal analysis of intensity distributions for mean particle size determination. PCS results were confirmed using scanning electron microscopy (SEM). For SEM, samples were diluted and an aliquot of the diluted preparation was dried, sputter coated and visualized using the Topco SM510 (Topcon Technologies, Inc., Pleasanton, Ca.).

Plasma Stability Assays

Stability of nanoparticle suspensions in plasma was monitored by photon correlation spectroscopy (PCS) and light microscopy. Rat plasma was delipidated prior to use by centrifugation at 150,000 × g for 1hr. The supernatant was carefully decanted and filtered through a 0.1 micron filter. The plasma was then pretested in the Coulter N4MD submicron particle analyzer to ensure that the sample was particle-free. Nanosuspensions of piposulfan, camptothecin, etoposide and paclitaxel were diluted 1:2, 1:10, and 1:100 with lipid-free plasma. Samples were vortexed and incubated at 37°C. For PCS analysis, samples were diluted with water and assayed. Presence or absence of aggregation was also monitored using the Leica DMRB optical microscope with a 100 × phase-contrast objective.

Animal Studies

Studies were performed in accordance with Wayne State University Medical School's animal use and care administrative policies. As previously described (16), tumor fragments (~30 mg) were seeded subcutaneously by trocar into 7–10 week-old female C3H mice (National Institute of Health, Bethesda, Md.). Chemotherapy of either a nanoparticle suspension or a control formulation was initiated within 6 days of tumor implantation. For chemotherapy, animals were injected via the tail vein using a multiple injection regimen. Dose and dosing schedule were selected based on the toxicity of the compound with the intention of administering the drug at its maximum tolerated dose (MTD).

To assess antitumor effectiveness, median tumor burden of treated animals was compared to median tumor burden of untreated controls. Results are expressed as a percentage determined by comparing the average weight of tumors in treated animals (expressed as "T") to the average weight of tumors in untreated controls (expressed as "C"). This value is expressed as percent T/C. A T/C = 0% is indicative of a highly active agent while a T/C >42% is considered inactive (16).

RESULTS

Particle Size Reduction

Figure 1 shows the particle size reduction profile achieved during ball milling. Generally, within the first 24 h a significant reduction in particle size is observed. Depending on the drug core and stabilizer utilized, additional milling rendered finer particle dispersions. For this study, nanosuspensions were milled for 4 days and particle size analysis was performed every 12 hrs. After four days of milling nanosuspensions of piposulfan and etoposide with a mean diameter ~200 nm were harvested. For camptothecin and paclitaxel, milling was continued for an additional 3 to 4 days to further reduce particle size. As shown in Table 1, routinely nanosuspensions were prepared with a mean particle size that averaged 200–250 nm for all four agents studied.

Characterization of Nanoparticle Drug Suspensions

In Figure 2, the particle size distribution for nano-piposulfan, nano-etoposide, nano-camptothecin and nano-paclitaxel is shown. Intensity distributions were obtained using photon correlation spectroscopy (PCS). Different drug cores with an optimal stabilizer produce a relatively uniform dispersion. The gross uniformity or homogeneity of the dispersions was verified using scanning electron microscopy (SEM). In Figure 3, the scanning electron micrographs show representative images of the nanoparticle drug suspensions. The milled nanosuspensions for each agent were homogeneous. However, the morphology of the

Table 1. Average Particle Size: Nano-Suspensions[a]

Compound (solubility)[b]	Formulation	Average Size in nm. (number of batches)
Piposulfan (125μg/ml)	2% Piposulfan, 0.33% Tween 80, 0.67% Span 80	210.2 ± 38.9 (8)
Camptothecin (50μg/ml)	2% Camptothecin, 1% F108	202.3 ± 30.5 (6)
Etoposide (200μg/ml)	2% Etoposide, 1% F127	256.2 ± 53.0 (12)
Paclitaxel (<10 μg/ml)	2% Paclitaxel, 1% F127	279.2 ± 29.60 (7)

[a] Chemotherapeutic agents were roller milled as a 2% wt/v suspension using surfactant satbilizers at a core to surfactant ratio of 2:1. Samples were sized using photon correlation spectroscopy (PCS). The average size was determined using the said number of batches.
[b] Aqueous solubility @ 25°C.

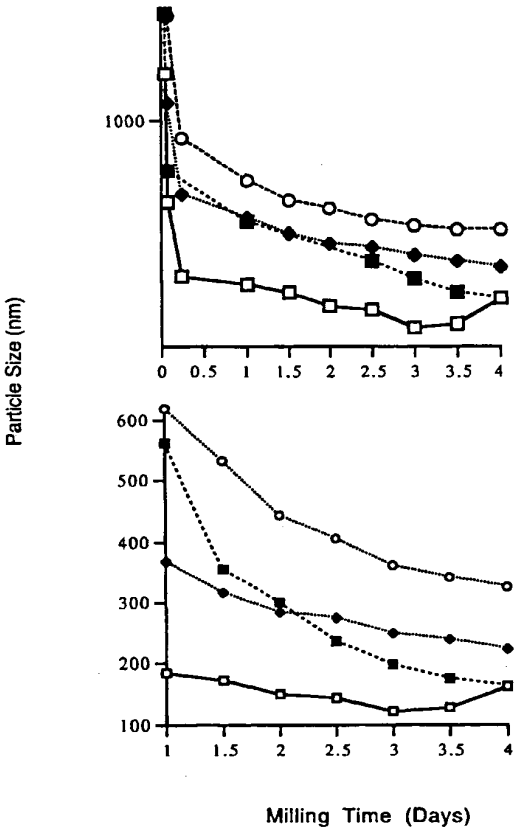

Fig. 1. Effectiveness of particle size reduction using wet milling technology to formulate water insoluble chemotherapeutic agents. Compounds (piposulfan—□; camptothecin—◆; etoposide—■; and paclitaxel—○) were roller milled using the surfactant stabilizers described in Table 1. During milling, particle size was monitored every 12 hrs. For particle size analysis samples were diluted with deionized water, vortexed and sized using photon correlation spectroscopy (PCS). Data show weight average diameters of particles in suspension.

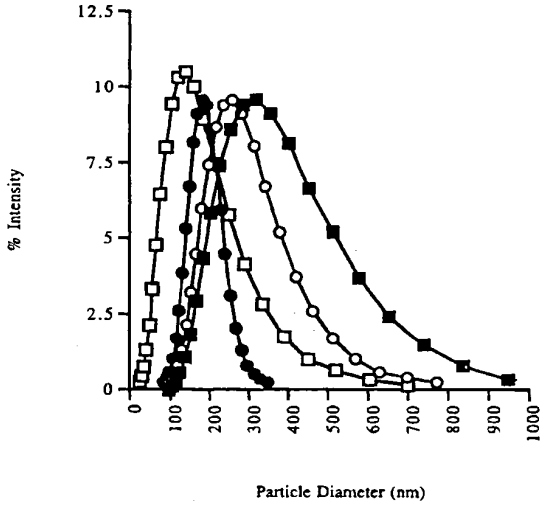

Fig. 2. Size distribution profiles of nanoparticle suspensions were obtained using the Malvern Submicron Particle Sizer. Samples were diluted with deionized water, vortexed for ~20 sec and sized. Size distribution profiles and cumulant z averages are shown for: ● nanopiposulfan (z Ave = 299.9); ○ nanocamptothecin (z Ave = 227.8; □ nanoetoposide (z Ave = 155.4); and ■ nanopaclitaxel (z Ave = 291.3).

various nanosuspensions differed from the cuboidal appearance of nanocamptothecin (Fig. 3B) to the rather elongated rod-like structures of nano-paclitaxel (Fig. 3D). Also, as monitored by both PCS and SEM, heterogeneity with respects to particle size and shape appears to be primarily governed by interactive properties of both the drug core and the surfactant stabilizer used during milling. When optimally stabilized, the suspensions did not aggregate and remained physically stable for at least four weeks post preparation (Fig. 4).

Since the intended use of these nanosuspensions is for intravenous administration, physical stability in the presence of plasma was monitored using particle size analysis and optical microscopy. Nanosuspensions were diluted with plasma and incubated at 37°C . As shown in Figure 5, for camptothecin and paclitaxel no significant change could be detected in the mean particle size of the preparations throughout a 60 min incubation period. For piposulfan and etoposide, particle growth was observed but there was no evidence of particle aggregation and/or agglomeration based on both PCS sizing and optical microscopy. Physical stability of the nanocrystalline suspensions was also not affected by time of incubation and dilution. Samples diluted 1:100 with plasma and incubated at 37°C for 24 hrs remained physically stable.

Efficacy of Nanoparticle Drug Suspensions

To evaluate efficacy, the nanocrystalline drug suspensions were tested in mice previously injected with mammary adenocarcinoma (16/C). The nanosuspensions were compared with the conventional formulation of the same agent or an agent of similar mechanism of action. Results of these studies are illustrated in Table 2. For all nanoparticle suspensions evaluated, tumor regression expressed as the percentage of tumor weight in treated animals to tumor weight in untreated controls was significant. In addition, the suspensions were suitable for intravenous bolus injection as suggested without increased incidence of acute toxicity in comparison to controls. For certain drugs, such as piposulfan, only the nanoparticle formulation was tolerated as an i.v. injectable. Control formulations were extremely toxic unless administered subcutaneously.

DISCUSSION

A technology is described for formulating water insoluble anticancer agents as nanoparticle drug suspensions that are stable. The method is versatile and suitable for many agents whose solubility is less than 200 ug/ml. The potential value of this approach was demonstrated using two well-known drugs, etoposide and paclitaxel, which are in the clinic but have reportedly been associated with formulation-related safety issues (6–8,17–19). The other agents investigated, piposulfan and camptothecin, are older drug candidates that could not be successfully formulated as an injectable. In the case of piposulfan

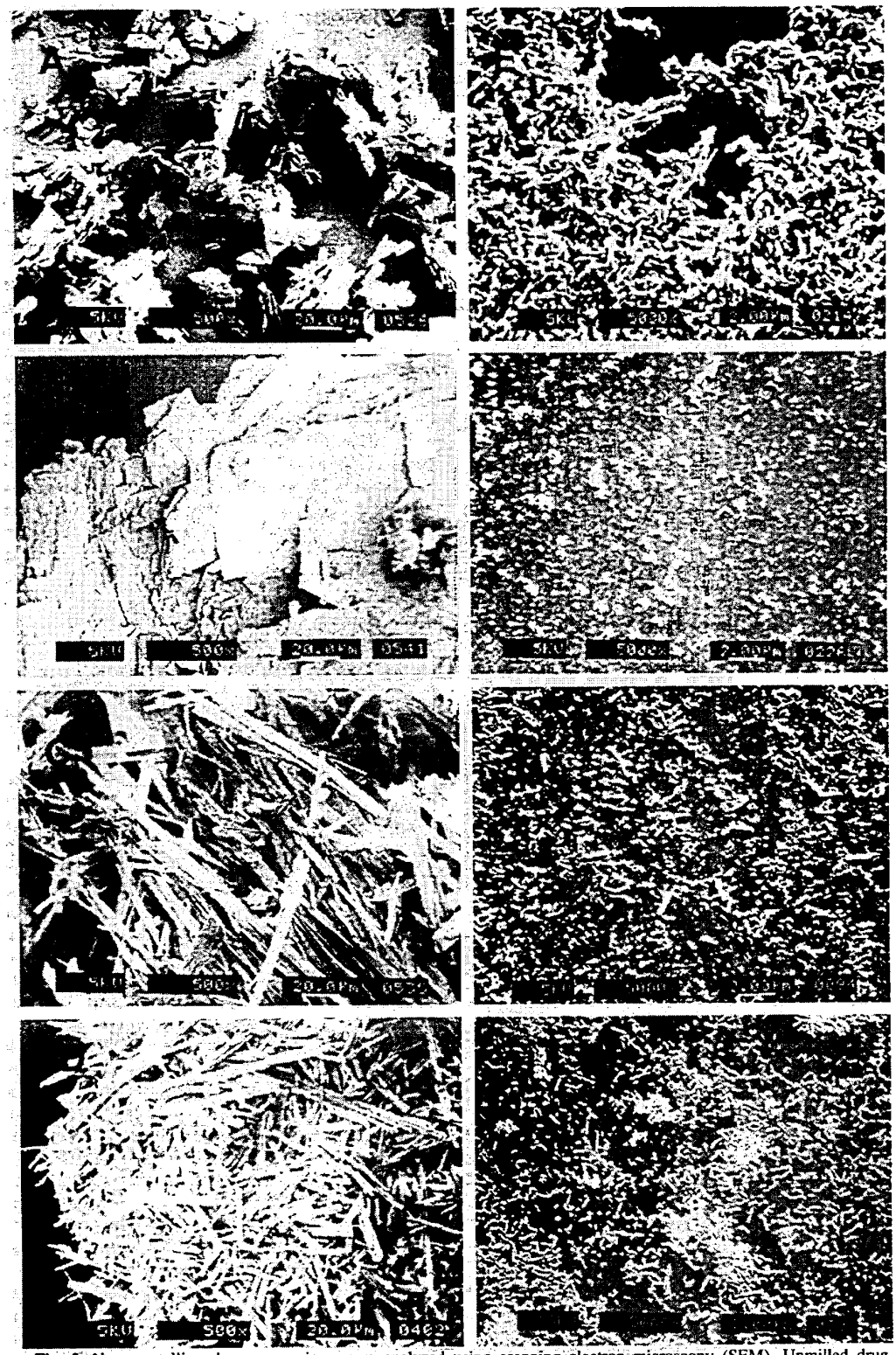

Fig. 3. Nanocrystalline drug suspensions were analyzed using scanning electron microscopy (SEM). Unmilled drug substance was visualized at 500 × magnification and is shown on the left side of the figure as: A) piposulfan; B) camptothecin; C) etoposide; and D) paclitaxel. The corresponding nanocrystalline suspensions (5,000 ×) are shown on the right side of the figure labeled as: E) nanopiposulfan; F) nanocamptothecin; G) nanoetoposide; and H) nanopaclitaxel.

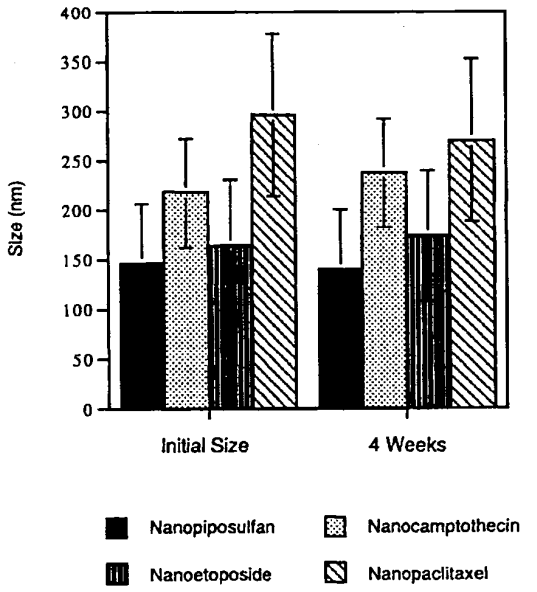

Fig. 4. Physical stability of nanocrystal suspensions were studied at ambient temperature for a four week period. The antitumor agents were roller milled as described in Table 1 and Figure 1. The suspensions were then stored at room temperature for four weeks and re-sized using PCS. The graph compares mean particle size of the preparations immediately after milling with the mean particle size of the suspension following storage at room temperature for one month.

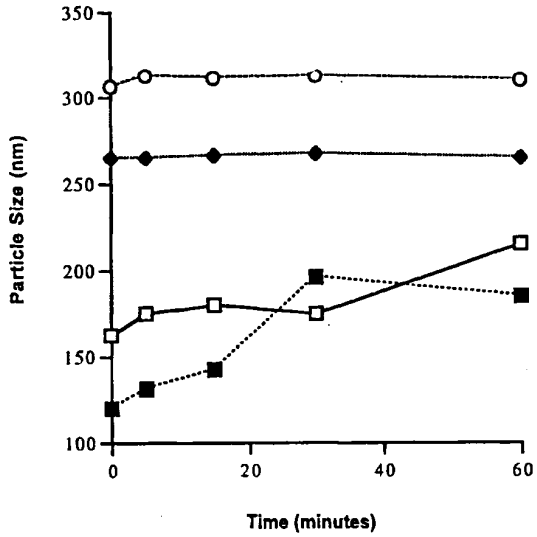

Fig. 5. The physical stability of nanocrystal drug suspensions was studied in the presence of plasma. Nanopiposulfan—□, nanocamptothecin—◆, nanoetoposide—■, and nanopaclitaxel—○, were diluted 1:2 with plasma and incubated for 60 min at 37°C. For particle size analysis samples were diluted with deionized water and sized using PCS. PCS size analysis was confirmed using optical microscopy.

drug development was abandoned, whereas, solubility issues associated with the camptothecins are being aggressively pursued via the identification of water soluble analogs (20–23). The solubility of the anticancer agents chosen for this study range from ~200 ug/ml to less than 4.0 ug/ml (24,25) and as demonstrated in this study can be readily formulated as an aqueous suspension of fine particles using a low energy wet milling process.

The nanoparticle suspensions of piposulfan, camptothecin, etoposide and paclitaxel that are described were generated using a conventional ball mill. As the data show, in the presence of the selected surfactant stabilizer(s) the procedure was effective in producing nanoparticles of pure drug substance. Generally, the higher molecular weight polymeric stabilizers were optimal for effective particle size reduction, shelf stability, and prevention of agglomeration in the presence of blood proteins. For instance, stable suspensions ~250 nm in diameter were obtained for camptothecin, etoposide and paclitaxel using the pluronic block co-polymers F108 and F127. The higher molecular weight Pluronics have been shown to be excellent stabilizers for various colloidal delivery systems (26). In addition, these surfactant coatings have been known to reduce opsonization of particulate drug carriers and enhance delivery of the desired agent to various anatomical targets which would be advantageous for passive delivery to solid tumors (27,28). The pharmacokinetic properties of these nanoparticle drug suspensions are being studied. However, since the technology described in this study is relatively new and the biodistributional properties of colloidal nanocarriers are dictated by a complexity of interactions, it would be surprising if the blood clearance of these nanosuspensions is not rapid. Currently, methodology is being developed so that properties of the nanosuspensions, e.g. size, surface characteristics and shape can be modulated to optimize delivery.

Though the effects of the comminution process on the physical state of the drug was not performed, previous studies using X-ray diffraction have shown that the dispersion process did not change the crystal structure of the compound (14). In this study the electron micrographs of the milled dispersions suggest that the process generates nanocrystalline drug particles. However, comparisons between pre and post processed materials remains to be studied.

For certain drugs such as piposulfan, generating a stable nanocrystalline suspension proved challenging. After screening a series of surfactants and surfactant combinations, the use of a Tween 80 and Span 80 mixture provided optimal physical stabilization. As shown in Figure 1 and 2, using this surfactant combination, particle size of the suspension was reduced to 250 nm in ~4 day period. For piposulfan the surfactant mixture adequately wets the drug substance and provides steric stabilization. However, as judged from the plasma stability data shown in Figure 5, though the preparation does not agglomerate in plasma, the particles apparently interact with plasma proteins resulting in an overall increase in mean particle size of the preparation. This interaction does not appear to compromise the safety and efficacy of the suspension (Table 2).

As shown in Table 2, the efficacy of oncologic agents when formulated as nanoparticles was satisfactory. For etoposide and paclitaxel, novel nanoparticle suspensions were compared to currently used clinical formulations. Etoposide is formulated using polyethylene glycol, Tween 80 and benzyl alcohol while paclitaxel is dissolved in a mixture of Cremophor EL and ethanol. To avoid acute toxicity, both formulations must be

Table II. Efficacy of Nanoparticle Suspensions Against Early Stage Mammary Adenocarcinoma 16C in CH3 Mice[a]

Formulation	Schedule	Total Dose (mg/kg)	Drug Deaths (#deaths/total)	Median Tumor Burden (mg)	T/C %
NanoPiposulfan	BID 1–4	356	0/5	0 (0–234)	0
Comparator I	BID 1–4	360	2/5	32 (0–63)	1.0
No Treatment	NA	NA	0/5	3582 (1352–4678)	NA
NanoCamptothecin	d 1–3	40	0/5	907 (284–2514)	35
Comparator II	d 1–3	20	5/5	NA	NA
	d 1–3	12.5	1/5	969 (352–1224)	38
No Treatment	NA	NA	0/5	2576 (627–3054)	NA
NanoEtoposide	d 1,5	125	0/5	0 (0–88)	0
Comparator III	d 1,3,5	102	5/5	NA	NA
	d 1,3,5	69	4/5	75 (75)	5
	d 1,3,5	45	0//5	171 (0–260)	12
No Treatment	NA	NA	0/5	1387 (0–2016)	NA
NanoPaclitaxel	d 3.6	88	0/5	53 90–196)	3
Comparator IV	BID 3,4,5	100	3/5	158 (138–839)	7
No Treatment	NA	NA	0/5	2674 (0–3043)	NA

[a] Formualtions were injected intravenously using the stated schedule and dosage. Comparator formulations were: a) Comparator I: cyclophosamide in saline; 2) Comparator II: camptothecin in 5% ethanol, 2% POE40; c) Comparator III: etoposide in 7% ethanol, 3% POE40 and d) Comparator IV: paclitaxel in 50% ethanol, 50% Cremophor. Once a day and twice daily injections are denoted by "d" and "BID" respectively.

diluted prior to use and slowly infused. In the present study, these formulations were slowly injected, whereas, nanoparticulate suspensions were administered via a rapid bolus injection. The data show that in the mammary 16C murine tumor model, the performance of nano-etoposide and nano-paclitaxel was comparable or slightly improved over the response elicited by the conventional formulations. In addition, the data clearly show that when administered as a fine particle preparation, dosages could be increased without incidence of acute toxicity, abnormal weight loss, or organ pathology observed during necropsy. Since the surfactants used in the nanocrystal formulations were selected so as to prevent agglomeration in the presence of plasma, the improved acute safety profile of the suspension was anticipated. However, other factors such as an altered pharmacokinetic profile should be considered.

Our data suggest that the ability to formulate water insoluble chemotherapeutic agents as nanocrystal drug suspensions may offer a number of advantages over a more classical approach. First, since these fine particle drug suspensions are well tolerated following intravenous injection, nanoparticles provide a convenient remedy for administering high doses of drug without the risks routinely associated with conventional formulations containing cosolvents. Second, the potential for being able to safely increase the dosage of a particular drug may lead to the identification of new medical indications and usages for the given drug. Lastly, the technology described in this study provides a convenient platform for targeted delivery of water insoluble therapeutic and/or diagnostic agents.

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JX058

Interoffice Memo

31-Aug-1995 09:10am

TO: Gary G Liversidge (LIVERSIDGEGG)
FROM: Gary G Liversidge, NanoSystems, (610) 983-7993

RE: Patents

ELANP0137357

Interoffice Memo

09-Feb-1995 07:22am

TO: J HAWLEY (3=KODAK@*ID\KR25.LTP100@EKX400@KDKMSG)
From: Gary G Liversidge, NanoSystems, (610) 983-7993

RE: Jannsen Cited Patents

Jeff:

I hope you found the information I FAXED you on particle sizing and surface area helpful.

Would it be possible for you to FAX or FED EX me copies of the File Wrappers for the 4 cited patents after you receive the materials from the PTO? I think they may help me prepare for your visit on Thursday, February 16.

If there is anything I can do to help in the interim please do not hesitate to contact me.

Regards

Gary

CC: Eugene R Cooper (COOPERER)

ELANP0137358

Interoffice Memo

07-Feb-1995 11:29am

TO: Eugene R Cooper (COOPERER)
From: Gary G Liversidge, NanoSystems, (610) 983-7993

RE: Crystallinity and Size Measurments of Nanoparticle Dispersio

Please find attached a description of the methodology used to determine X-ray diffraction patterns for a nano-dispersion of Naproxen (2 pages). Similar methodology was used to determine the XRDP for Danazol, Nanoparticulate Danazol suspension. Other examples of XRDP for nanoparticulate iodobenzene analogues are available.

The X-ray diffraction patterns for Danazol pre and post milling are given in Figure 1. The patterns are definitive evidence that Nanoparticulate Danazol Suspension contains crystalline Danazol nanoparticles with no amorphous material present and that they have the same crystalline structure as the material prior to milling.

The X-ray diffraction pattern for Nanoparticulate Naproxen Suspension is given in Figure 2. The pattern is definitive evidence that Nanoparticulate Naproxen Suspension contains crystalline Naproxen nanoparticles with no amorphous material present.

Below is data on particle size measurements for some naproxen and Danazol suspensions prepared and measured by NanoSystems. Please note that the mean particle size by weight averaging is in the 260 nm. size range. Such particles are NOT covered in claim 1 of our invention in US Patent 5,145,687 since the claim covers only particle in which (see column 5 lines 20-40) **"at least 90% of the particles have a weight average particle size of less than about 400 nm.....In particularly preferred embodiments, essentially all of the particles have a size less than 400 nm."** Clearly the examples below are not covered by our invention and scientific data citing only an average particle size from a scientific perspective would in no way suggest, teach or read on our invention. Further citing of an average particle size without defining the method of sizing and measurement taken i.e. weight averaging or number averaging, from a scientific perspective is vague and indefinite making it impossible to take the teaching to reproduce the results cited.
(Continued on next page)

FORMULATION		50% SIZE nm. WEIGHT AVE.	90% SIZE nm. WEIGHT AVE.	LAB NOTE BOOK REF.
40% Naproxen, 6% PVP-K30	268.5	624.6	MRA-5458-056	
10% Danazol, 5% PVP-K17	263.6	563.8	MRA-5458-054	
40% Naproxen, 6% PVP-K30	261.8	710.8	MRA-5458-047	

ELANP0137359

40% Naproxen 6% PVP-K29/32	266.5	519.0	MRA-5458-017

Regards

Gary

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International Journal of Pharmaceutics 125 (1995) 91–97

international journal of pharmaceutics

Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs

Gary G. Liversidge [a,*], Kenneth C. Cundy [b]

[a] *NanoSystems, 1250 S. Collegeville Rd, Collegeville, PA 19426–0900, USA*
[b] *Gilead Science Inc., 353 Lakeside Drive, Foster City, CA 94404, USA*

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Abstract

Danazol is a poorly water soluble compound (10 μg/ml) that demonstrates poor bioavailability. The impact on bioavailability of increasing the area for dissolution by decreasing drug crystal particle size to less than 200 nm and stabilizing the particles to prevent agglomeration in the GI tract has been evaluated. A randomized three-way crossover study was conducted in fasted male beagle dogs to compare absolute oral bioavailability of danazol from three formulations. The three formulations examined were: A, an aqueous dispersion of nanoparticulate danazol (mean particle size 169 nm); B, danazol-hydroxypropyl-β-cyclodextrin (HPB) complex; C, an aqueous suspension of conventional danazol particles (mean particle size 10 μm). The three formulations were administered (200 mg) at 1 week intervals, and a fourth leg was conducted using intravenous danazol-HPB at a dose of 3 mg/kg. Plasma samples were obtained over the course of 24 h and analyzed by SPE-HPLC. Absolute oral bioavailability of each formulation was determined by comparison of oral AUC values to intravenous AUC values in the same dog, normalized to a 20 mg/kg dose. Absolute bioavailabilities of the three formulations were: nanoparticulate danazol, 82.3 ± 10.1%; cyclodextrin complex, 106.7 ± 12.3%; conventional danazol suspension, 5.1 ± 1.9%. The bioavailabilities of nanoparticle dispersion and cyclodextrin complex are not significantly different ($P = 0.05$) suggesting that the nanoparticle dispersion had overcome the dissolution rate limited bioavailabilty observed with conventional suspensions of danazol. This approach should have general applicability to many poorly soluble drugs with dissolution rate-limited absorption.

Keywords: Danazol; Bioavailability; Particle size; Nanoparticle; Poor water solubility; Dissolution; Dog

1. Introduction

The factors that can limit the oral bioavailability of drugs include first-pass metabolism, chemical stability, transport, solubility, and dissolution. These factors are generally addressed in discovery programs through chemical modifications of a molecule to improve bioavailability. However, this approach can result in significant alterations in the biological effect of the pharmacophor. Formulation approaches to modify bioavailability of

* Corresponding author. Tel. 610-983-7993; Fax 610-983-6929.

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a molecule are attractive alternatives but have met with modest success. Poorly soluble compounds tend to be eliminated from the GI tract before they have had opportunity to fully dissolve and be absorbed into the circulation. This results in low and erratic bioavailability and poor dose proportionality. These drawbacks have limited the development of poorly soluble molecules. A generally applicable approach to enhance the bioavailability of poorly water soluble molecules that are dissolution rate limited is presented herein.

The dissolution rate of a drug is a function of its intrinsic solubility and its particle size. Previous studies with a number of poorly soluble drugs have demonstrated that particle size reduction can lead to an increased rate of dissolution and higher oral bioavailability, including progesterone (Hargrove et al., 1989), nitrofurantoin (Watari et al., 1983), estradiol and estrone (Englund and Johansson, 1981), oxfendazole (Shastri et al., 1980) and proquazone (Nimmerfall and Rosenthaler, 1980). The majority of such studies have involved mechanical size reduction to particles larger than 1 μm. Kondo et al. (1993a) have reported a modest doubling in bioavailability for HO-221 when the mean particle size is reduced from 4.15 to 0.45 μm. This size reduction should lead to an increase in specific area and consequently dissolution of approx. 10-fold for the drug particles. Thus, a much greater improvement in bioavailability would be expected for such a change in particle size if dissolution was rate limiting, assuming that the particles remain discrete. If agglomeration of particles occurs in the GI tract then the effective surface area for dissolution will be reduced and concomitantly bioavailability. In subsequent publications Kondo et al. (1993b) reported that the 0.45 μm formulation of HO-221 was approx. 5–20% bioavailable, showed fed vs fasted variability and was not dose proportional, whereas in Kondo et al. (1994) coprecipitates with HPMCP (hydroxypropyl methylcellulose phthalate) were almost totally bioavailable. The data from these publications (Kondo et al., 1993b, 1994) are suggestive that HO-221 has dissolution rate limited bioavailability.

The above-cited studies demonstrate that there is still considerable potential for substantially enhancing bioavailability by particle size reduction in the submicron range. The present study was designed to evaluate the applicability of this approach to danazol, which has a low water solubility (10 μg/ml) and poor bioavailability. Accurate evaluation of this approach required comparison of a nanoparticulate danazol dispersion with a suspension of conventional danazol particles in the presence of the same stabilizing agent. The stabilizing agent was intended to prevent agglomeration of the nanoparticles both in the formulation and following addition to simulated gastric and intestinal fluids. A measure of the maximum oral bioavailability achievable by particle size reduction can be provided by a cyclodextrin-danazol complex, in which danazol is dispersed on a molecular level. Hydroxpropyl-β-cyclodextrin was chosen as the complexing agent, since this is the least toxic of available cyclodextrins. Dog was chosen as the species for bioavailability determinations, since this is the accepted model for preclinical bioequivalence studies, and data were available on the oral availability of the commercial danazol dosage form (Danocrine) in the same species.

2. Materials and methods

2.1. Materials

Danazol (17-α-pregna-2,4-dien-20-yno[2,3-*d*]isoxazol-17-ol) was obtained in micronized form (mean particle size, 10 μm; particle size range, 5–30 μm) from Sterling Drug Inc., New York, NY. Polyvinylpyrrolidone K-15 (PVP) was purchased from GAF, Wayne, NJ. Hydroxpropyl-β-cyclodextrin (HPB) was obtained from Pharmatec, Alachua, FL. Zirconium oxide grinding spheres 0.85–1.18 mm diameter were purchased from Zircoa Inc., Solon, OH.

2.2. Preparation of formulations

An intravenous formulation of danazol was prepared as by dissolving 10 mg/ml danazol in 50% w/w HPB, and sterilizing by filtration.

Three oral formulations of danazol were prepared as follows:

1. A hydroxypropyl-β-cyclodextrin (HPB)/danazol complex containing 10 mg/ml danazol in 50% w/w in HPB.
2. An aqueous suspension of conventional danazol particles containing 5% w/w danazol and 1.5% w/w PVP.
3. A nanoparticulate dispersion of danazol was prepared using a ball milling process. A 600 ml cylindrical vessel (inside diameter 7.6 cm) was filled approximately halfway with zirconium oxide grinding spheres. The following ingredients were added directly to the glass vessel: danazol, 10.8 g; polyvinylpyrrolidone K-15, 3.24 g; high purity water, 201.96 g. 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. After 5 days of ball milling, the slurry was separated from the grinding media through a screen and evaluated for particle size by sedimentation field flow fractionation and SEM and under light microscopy for particle agglomeration.

2.3. Bioavailability studies

Animal experiments were conducted in accordance with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals and Institutional Animal Care and Use Committee.

The three oral formulations of danazol were administered in a randomized three-way crossover study to fasted male beagle dogs with 1 week between doses. An intravenous leg was conducted in all five dogs on the fourth week. The doses were:

1. Aqueous dispersion of nanoparticulate danazol (169 nm), 4.0 ml (200 mg) orally.
2. Hydroxypropyl-β-cyclodextrin/danazol complex, 20 ml (200 mg) orally.
3. Aqueous suspension of conventional danazol particles, 4.0 ml (200 mg) orally.
4. Hydroxypropyl-β-cyclodextrin/danazol complex, sterilized by filtration, 3 mg/kg intravenously.

Five dogs received each of the three oral formulations during the first 3 weeks of the protocol, with 1 week between administrations. Dogs were fasted for 12 h prior to oral dosing. The cephalic vein of each dog was cannulated and formulations were administered by oral gavage. Blood samples were withdrawn from the cephalic cannula at 0 (pre-medication), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after administration and collected in heparinized tubes. Plasma (0.5 ml) was obtained immediately and rapidly frozen (dry ice/acetone) prior to analysis. Food was returned to each animal at 12 h post administration.

All five dogs received the intravenous formulation in the fourth week. Dogs were fasted as for oral administration and cannulated in the cephalic vein. The intravenous formulation was administered via the cephalic cannula and blood samples were withdrawn at 0 (pre-medication), 2, 4, 8, 15, 30 min and 1, 2, 4, 8, 12 and 24 h after administration, and collected into heparinized tubes. Plasma (0.5 ml) was obtained immediately and rapidly frozen (dry ice/acetone) prior to analysis. Food was returned to each animal at 12 h post administration.

2.4. Bioanalytical methods

Plasma samples were prepared by solid-phase extraction prior to analysis. Plasma (250 μl) was mixed with 25 μl water and 50 μl of internal standard (testosterone propionate, 220 μg/ml in methanol). Samples were mixed by vortex and applied to 1 ml C18 SPE cartridges (J.T. Baker) prepared by application of 1 column volume each of methanol and water. Following application of samples, the columns were washed with 0.5 ml water and 0.5 ml 10% acetonitrile. Danazol and internal standard were eluted in 0.5 ml acetonitrile and eluent was evaporated to dryness under nitrogen. Samples were reconstituted in 100 μl of mobile phase (0.01 M ammonium phosphate, pH 6.8/60% acetonitrile) and transferred to WISP mini-vials for HPLC analysis. Recovery of danazol and testosterone propionate by this method was > 85%. Standard curves were linear over the

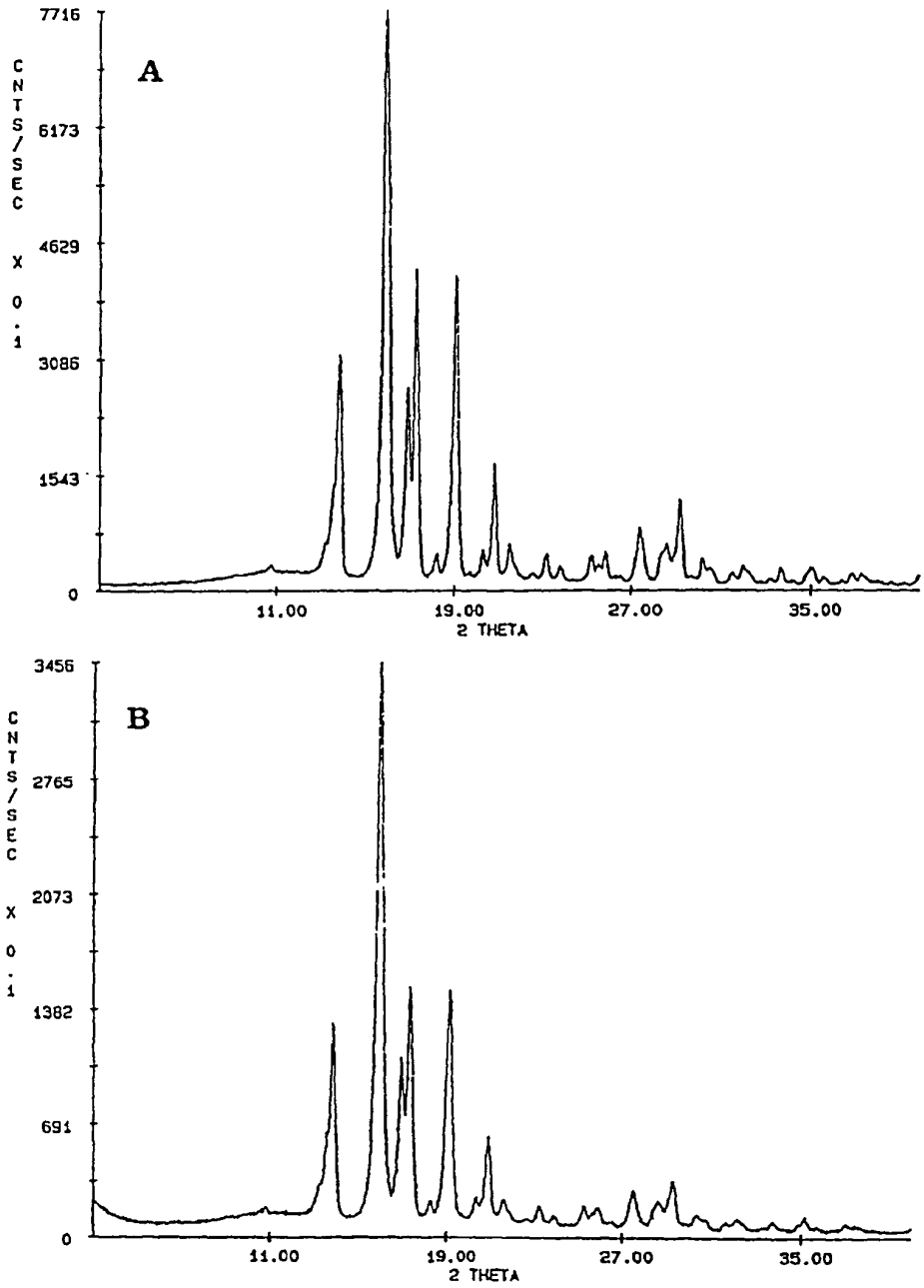

Fig. 1. X-ray diffraction patterns: (A) danazol before milling; (B) aqueous nanoparticulate danazol dispersion.

range 20 to 4000 ng/ml. The minimum quantifiable level of danazol in dog plasma was 10 ng/ml.

Samples were analyzed by HPLC using a published method (Nygard et al., 1987). The column was a Zorbax C8 (3 cm × 4 mm) with a Zorbax C8 guard cartridge. The mobile phase was A: 0.01 M ammonium phosphate, pH 6.8/40% acetonitrile, and B: 0.01 M ammonium phosphate, pH 6.8/60% acetonitrile, with a linear gradient from 50% B to 100% B over 7 min. The flow rate was 2.5 ml/min with a run time of 15 min.

3. Results and discussion

The use of particle size reduction to increase the surface area for dissolution and thereby increase bioavailability of poorly water soluble molecules has met with modest success, particularly in the submicron size range (Kondo et al., 1993b). Decreasing particle size increases the surface-to-volume ratio and size is inversely proportional to specific surface area for spherical particles (Schott, 1985), e.g., a 10-fold reduction in mean particle size results in a 10-fold increase in specific surface area. Thus, for drugs with dissolution rate limited bioavailability a 10-fold reduction in particle size would be anticipated to increase dissolution rate by 10-fold and subsequently increase bioavailability by 10-fold. The modest gains in bioavailability observed with particle size reduction have been attributed to agglomeration of particles in the milieu of the GI tract, resulting in a decrease in the effective surface area for dissolution. Agglomeration is of particular concern for particles in the submicron range (Higuchi et al., 1985) necessitating the use of stabilizers.

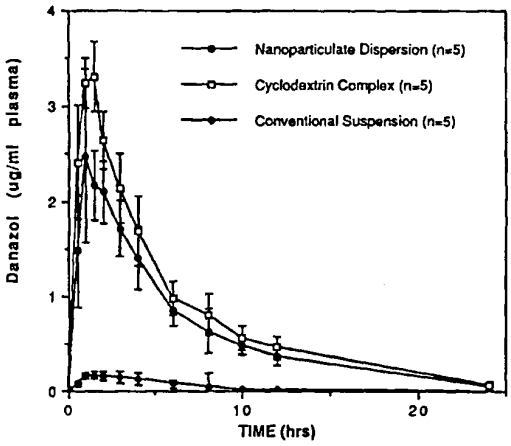

Fig. 2. Effect of formulation on mean ± SE plasma concentrations of danazol following oral administration of three formulations to fasted male beagle dogs.

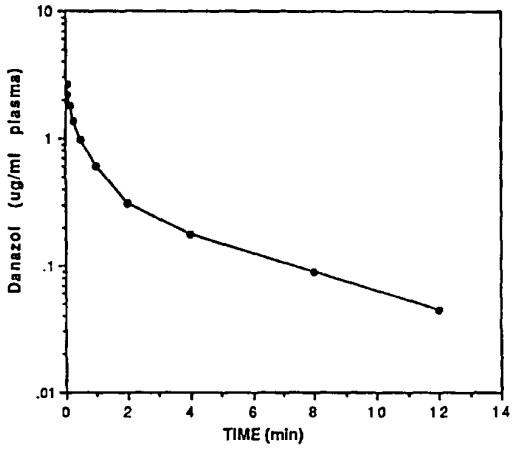

Fig. 3. Mean plasma concentrations of danazol following intravenous administration of cyclodextrin/danazol complex to fasted male beagle dogs (SE values lie within mean data point).

Stabilization of a nanoparticulate dispersion can be achieved by steric or charge stabilization methods. The variation in pH during transit through the GI tract may preclude the use of a charge stabilizing suspending agent, such as sodium lauryl sulfate. The low pH of the stomach can result in neutralization of cationic surfactant stabilizer's charge leading to irreversible agglomeration of nanoparticles. Nanoparticles of danazol stabilized with sodium lauryl sulfate agglomerate when exposed to simulated gastric fluid. Generally, anionic surfactant stabilizers are too toxic to be considered for oral application. Charged surfactant stabilizer performance can be

dramatically impacted by the ionic strength of the medium. However, dispersions prepared with steric stabilizers would not be expected to demonstrate pH and ionic strength dependent stability.

A nanoparticulate formulation of danazol (5% w/w) stabilized with PVP (1.5% w/w) was prepared as outlined in the methods section. The resultant formulation was sized using a sedimentation field flow fractionator and had a number average particle diameter of 84.9 nm. and a weight average particle diameter of 169.1 nm. The particles varied in size from 26 to 340 nm. The crystal structure was determined by X-ray diffraction to be unchanged by the dispersion process (see Fig. 1). SEM of the formulation supported the size range reported above. The formulation did not flocculate or agglomerate when added to simulated gastric fluid or intestinal fluid USP. The formulation was physically and chemically stable for at least 6 months at room temperature.

The concentration of 1.5% w/w PVP was found to be critical in conferring stability of the nanoparticles. A nanoparticulate formulation of danazol (5% w/w) stabilized with PVP (1.0% w/w) was prepared as outlined in section 2. The resultant formulation was observed under light microscopy. The slurry was partially aggregated with aggregates of up to 10 μm in diameter. The amount of stabilizer (PVP 1%) was apparently insufficient to confer stability.

The mean plasma levels obtained for the three oral formulations are presented in Fig. 2. The mean plasma levels obtained for the intravenous administration are presented in Fig. 3.

Model independent pharmacokinetic parameters C_{max} and t_{max} are presented in Table 1 for the oral formulations. Values of the area under individual plasma time curves were calculated by computer using NONLIN84 analytical methods. The area under the curve for all animals was normalized to a 20 mg/kg dose using the actual dose administered and the weight of each dog at the time of administration, as follows:

$$\text{AUC for 20 mg/kg dose} = \frac{\text{AUC} \times \text{dog weight (kg)} \times 20}{\text{actual dose (mg)}}$$

Normalized AUC values are presented in Table 1. Absolute oral bioavailabilities of each formulation were calculated using the AUC values from intravenous administration in the same dog. The absolute oral bioavailabilities of the three oral formulations are presented in Table 1. The nanoparticulate dispersion and cyclodextrin complex were both significantly different from the conventional danazol suspension (unpaired Student's t-test). However, the nanoparticle dispersion and cyclodextrin complex are not significantly different at $P = 0.05$. The cyclodextrin complex given orally was not significantly different at $P = 0.05$ from when given intravenously.

The size reduction of danazol crystals from a mean weight average particle size of 10 μm to 169 nm should result in an approx. 59-fold increase in specific surface area based on the assumption that crystals are essentially spherical. SEM evaluation of the crystals supports this assumption. Thus, if discreet nanoparticles are maintained in the GI tract a significant enhancement in bioavailability of up to 59-fold compared to a conventional dispersion would be anticipated, subject to a boundary condition of 100% bioavailability assuming no permeation rate limitation or metabolic or chemical instability losses. The bioavailability data obtained with the dana-

Table 1
Pharmacokinetic parameters following oral and intravenous administration of danazol formulations to fasted male beagle dogs ($n = 5$)

Formulation	C_{max} (μg/ml)	t_{max} (h)	AUC [a] (μg h ml^{-1})	Absolute bioavailability
Cyclodextrin oral	3.94 ± 0.14	1.2 ± 0.2	20.4 ± 1.9	106.7 ± 12.3
Nanoparticle dispersion	3.01 ± 0.80	1.5 ± 0.3	16.5 ± 3.2	82.3 ± 10.1
Conventional suspension	0.20 ± 0.06	1.7 ± 0.4	1.0 ± 0.4	5.1 ± 1.9
Cyclodextrin i.v.			19.8 ± 0.6	100

[a] Based on NONLIN84 AUC values normalized to a dose of 20 mg/kg.

zol/cyclodextrin complex suggest that the influence of permeation, metabolic and chemical instabilities on bioavailability of danazol are minor. The cyclodextrin complex is a molecular dispersion of danazol and represents a formulation in which there is no dissolution step to limit bioavailability. This formulation may be considered the standard to which other oral formulations should be compared. The bioavailability of the nanoparticle dispersion and cyclodextrin complex are not significantly different ($P = 0.05$) suggesting that the nanoparticle dispersion had overcome the dissolution rate limited bioavailabilty observed with conventional suspensions of danazol.

The approach of reducing particle size to the low nanometer range in the presence of a steric stabilizer for enhancing oral bioavailability is anticipated to have general applicability to many poorly soluble drugs with dissolution rate-limited absorption. The specific use of a steric stabilizer (e.g., PVP) to stabilize the nanoparticulate dispersion against agglomeration in gastric and intestinal environments is a key aspect in preserving the benefits of particle size reduction in vivo, i.e., greatly increased available surface area for dissolution.

4. Conclusions

An aqueous nanoparticle dispersion of danazol (5% w/w) and PVP (1.5% w/w) was prepared using wet milling techniques to achieve a weight average particle size of 169 nm. The formulation did not agglomerate or flocculate when added to simulated gastric or intestinal fluid.

The absolute bioavailability in fasted male beagle dogs of the nanoparticulate danazol formulation ($82.3 \pm 10.1\%$), danazol-hydroxypropyl-β-cyclodextrin complex ($106 \pm 12.3\%$) and an aqueous suspension of conventional danazol particles ($5.1 \pm 1.9\%$) were determined. The nanoparticle dispersion and cyclodextrin complex were not significantly different ($P = 0.05$) suggesting that the nanoparticle dispersion has overcome the dissolution rate limited bioavailability observed with conventional suspensions of danazol.

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The United States of America



The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

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If the application for this patent was filed on or after December 12, 1980, maintenance fees are due three years and six months, seven years and six months, and eleven years and six months after the date of this grant, or within a grace period of six months thereafter upon payment of a surcharge as provided by law. The amount, number and timing of the maintenance fees required may be changed by law or regulation. Unless payment of the applicable maintenance fee is received in the Patent and Trademark Office on or before the date the fee is due or within a grace period of six months thereafter, the patent will expire as of the end of such grace period.

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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**

[75] Inventors: Gary G. Liversidge; Elaine Liversidge, both of West Chester; Pramod P. Sarpotdar, Malvern, all of Pa.

[73] Assignee: Eastman Kodak Company, Rochester, N.Y.

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[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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Primary Examiner—Thurman K. Page

Assistant Examiner—William E. Benston, Jr.

Attorney, Agent, or Firm—Arthur H. Rosenstein

[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.

	$T_{\frac{1}{2}}$ 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% nonaqueous 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	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	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 Stability 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

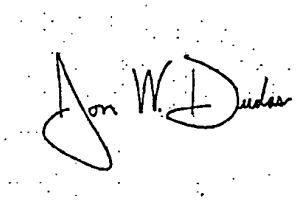
PATENT NO. : 5,399,363
DATED : March 21, 1995
INVENTOR(S) : Gary G. Liversidge, Elaine Liversidge and Pramod P. Sarpotdar

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

Form PTO-377C (Rev. 2/97)

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Acting Commissioner of Patents and Trademarks

Attest

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

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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, immuriological 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 sphere 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 6 to 340 nm. The amount and type of surface modifier was sufficient to provide colloidal stability to agglomertion 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 Ultrasoni Power Supply, Horn Diameter=0.5 inch (1.27 cm Power setting=2). The slurry was sized under a micro scope. An Olympus BH-2 optical microscope equippe with phase contrast illumination was used to observ the size and condition of the dispersion.

A drop of the above dilute slurry was placed betwee a microscope slide and glass cover slip and observe microscopically at high magnification (1,000 times) an compared to the slurry similarly diluted with wate only (no surface modifier). The unmodified dispersio exhibited extensive particle agglomeration. The partic size of the unmodified dispersion was more than 1 microns and the unmodified dispersion exhibited n Brownian Motion. Brownian motion is the oscillator or jiggling motion exhibited by particles in a liquid th fall in the size range of less than about 1 micron. Th Lecithin modified particles exhibited rapid Brownia motion. The thus observed dispersion had the charac teristics and appearance consistent with a number ave age particle size of less than 400 nm. Furthermore, it expected that additional milling would lead to furthe particle size reduction.

EXAMPLE 7

Alkyl Aryl Polyether Sulfonate Modified Steroid A

Example 6 was repeated except that the Lecithin wa 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 wa replaced with gum acacia (available from Eastma Kodak Co.) Similar results were observed.

EXAMPLE 9

Sodium Lauryl Sulfate Modified Steroid A

Example 6 was repeated except that the Lecithin wa replaced with sodium lauryl sulfate (available as Dupo nol ME from DuPont, Inc.). Similar results were ob served.

EXAMPLE 10

Steroid A Modified with a Dioctylester of Sodium Sulfosuccinic Acid

Example 6 was repeated except that the Lecithin wa replaced with Aerosol OT (available from America Cyanamid Chemical Products, Inc.). Similar result 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 wa replaced with Pluronic F68 (available from BASI 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 pre pared by ball milling with zirconium oxide grindin

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.

* * * * *

PTO 377B (9–90)

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APPLICATION NUMBER: *07/647,105*
FILING DATE: *January 25, 1991*
PATENT NUMBER: *5,145,684*
ISSUE DATE: *September 08, 1992*

By Authority of the
Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office

D. Montgomery

D. W. MONTGOMERY
Certifying Officer

PART (/) OF (/) PART(S)

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
07/647,105	01/25/91	424	489	152	BENSTON

GARY G. LIVERSIDGE, WEST CHESTER, PA; KENNETH C. CUNDY, POTTSTOWN, PA; JOHN J. BISHOP, ROCHESTER, NY; DAVID A. CZEKAI, HONEOYE FALLS, NY.

5145684

CONTINUING DATA********
VERIFIED

FOREIGN/PCT APPLICATIONS********
VERIFIED

FOREIGN FILING LICENSE GRANTED 04/02/91

Foreign priority claimed	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
35 USC 119 conditions met	PA	0	19	3	$ 750.00	PRF-0123/90
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WILLIAM J. DAVIS
PAT. DEPT.
STERLING DRUG, INC.
81 COLUMBIA TURNPIKE
RENSSELAER, NY 12144

J. Jeffrey Hawley
Eastman Kodak, Patent Legal Staff
343 State Street
Rochester, NY 14650

SURFACE MODIFIED DRUG NANOPARTICLES

U.S. DEPT. of COMM.-Pat. & TM Office — PTO-436L (rev. 10-78)

S OF APPLICATION
D SEPARATELY

NOTICE OF ALLOWANCE MAILED	PREPARED FOR ISSUE 1-31-92	CLAIMS ALLOWED
Jan 28, 1992	William E. Benston SR Assistant Examiner / D. Gassaway Docket Clerk	Total Claims 20 / Print Claim 1

ISSUE FEE		THURMAN K. PAGE SUPERVISORY PATENT EXAMINER ART UNIT 152 1502 Primary Examiner	DRAWING		
If Due	Date Paid		Sheets Drwg.	Figs. Drwg.	Print Fig.
1300.00	4-16-92		4	4	4

ISSUE CLASSIFICATION		ISSUE BATCH NUMBER
Class	Subclass	
424	489	W10

Label

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PTO-436

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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	:	Examiner W. Benston, Jr.
Nanoparticles	:	
	:	
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:

~~10.~~ 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,

Arthur H. Rosenstein
Attorney for Applicants
Registration No. 24,352

(215) 640-8802
WJD:mm

PAGES A 527 - A 547

REDACTED IN THEIR ENTIRETY

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

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[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

Case No. 06-438-GMS
DX-015

ABRX 0000020

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 Copolymer 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.

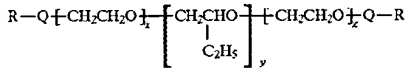
Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics™.

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:

~~(PEO)–(PBO)$_n$–(PEO)–(PBO)–(PEO)$_n$–~~ and ~~(PEO)–(PBO)–(PEO)–(PBO)$_n$–~~

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:



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.

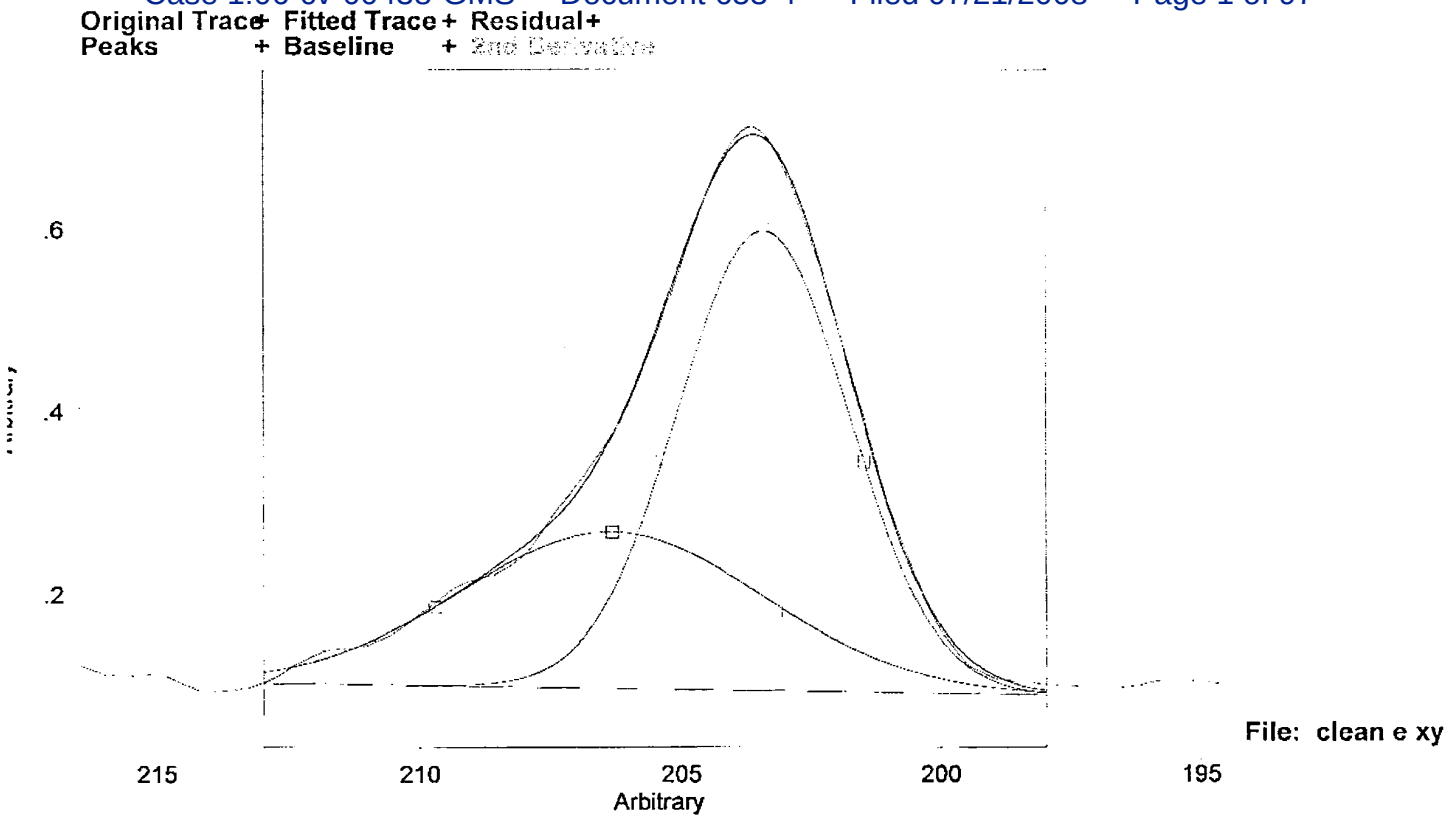
19. The method of claim 17 wherein said drug is an organic therapeutic substance.

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

* * * * *

PAGES A 558 - A 830

REDACTED IN THEIR ENTIRETY



Munson Exhibit E

FWHH = 1.6

Requested Peaks = 2

Case No. 06-438-GMS
DX-169

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:50

Peak #1: Gaussian
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High Limit:	212.99397	None	None
Standard Error:	.0229197886	.000881383882	.030641102

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Area: 2.071834

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Standard Error:	.000516525315	.00159643169	.00398459085

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Statistics Summary:
Solution AT A MINIMUM

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Fitted Equations:	3
Total Parameters:	8
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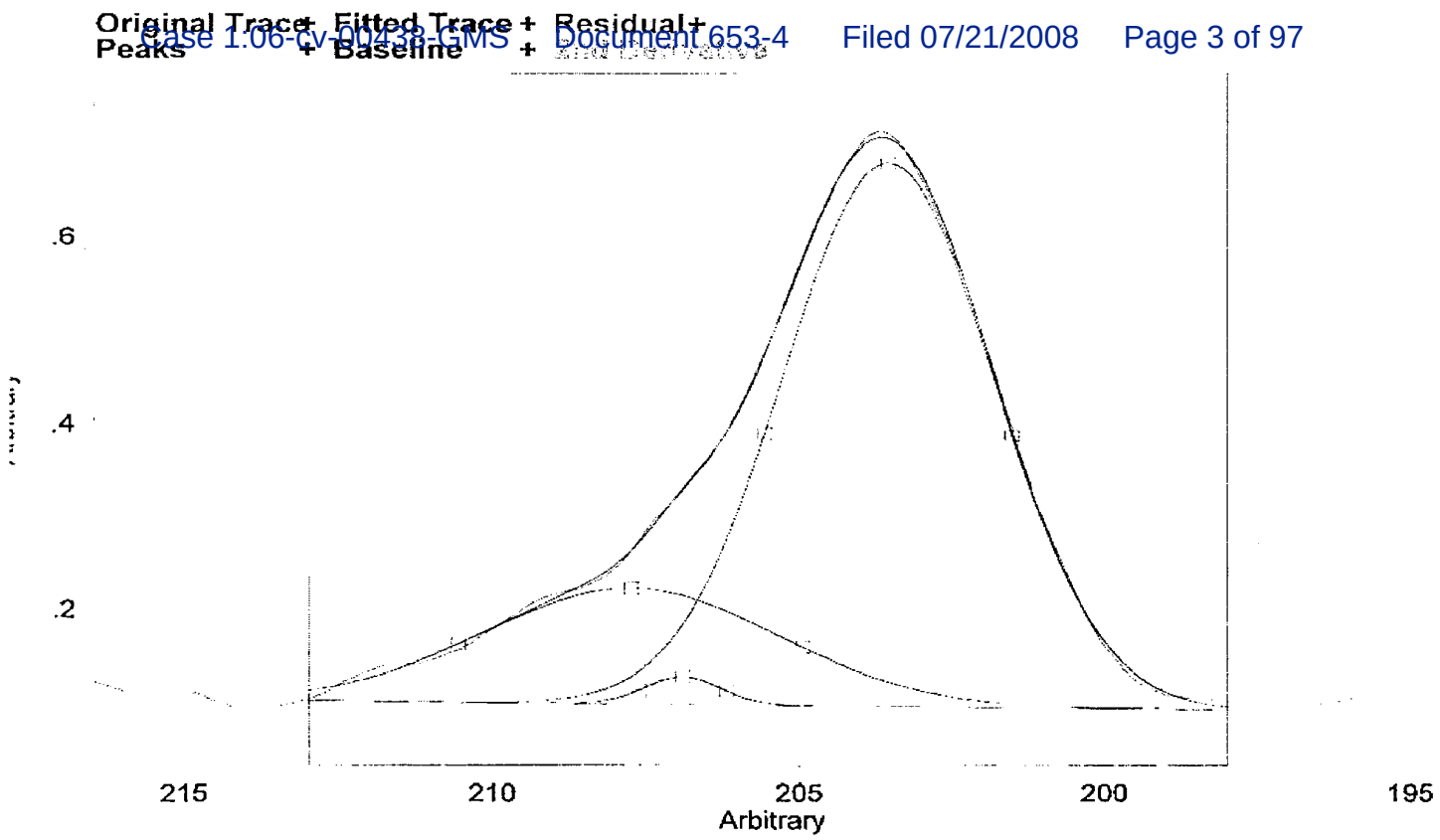

Munson Exhibit E

FWHH = 1.6

Requested Peaks = 3

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:51

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Peak #3: Gaussian
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High Limit:	212.994	None	None
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Baseline: Linear

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Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

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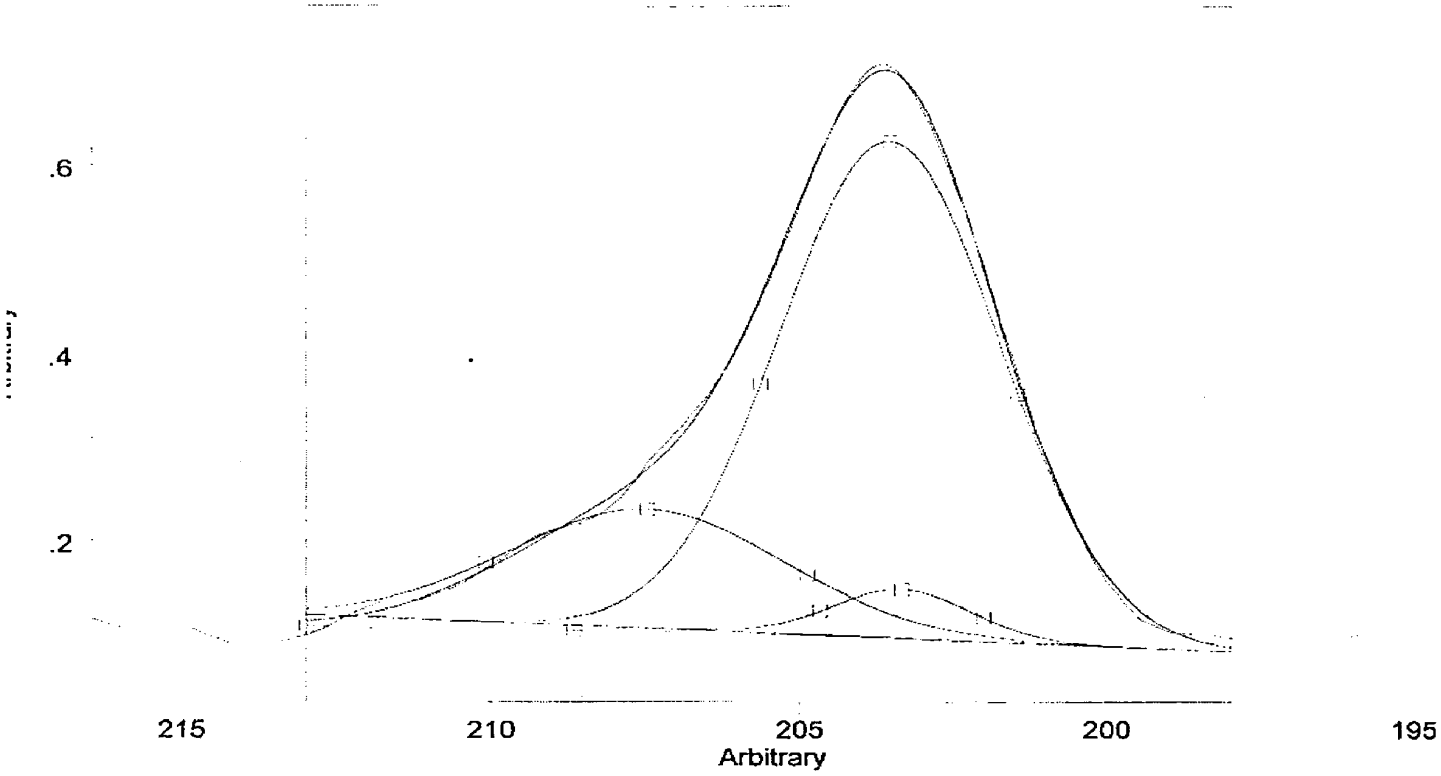

Munson Exhibit E

FWHH = 1.6

Requested Peaks = Max

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:52

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Standard Error:	0	0	0

Baseline: Linear

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Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

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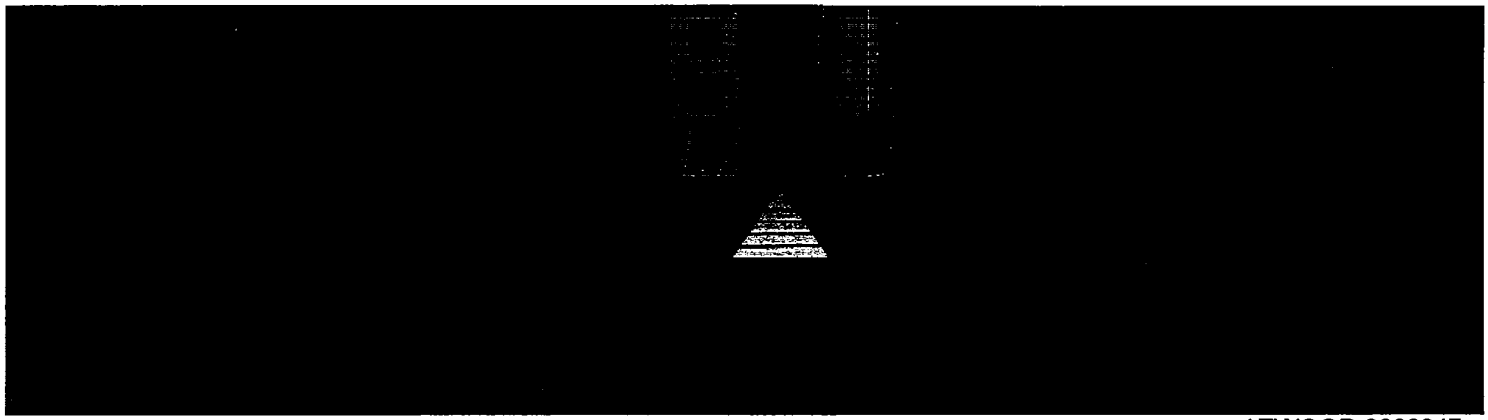

ATWOOD 0000347

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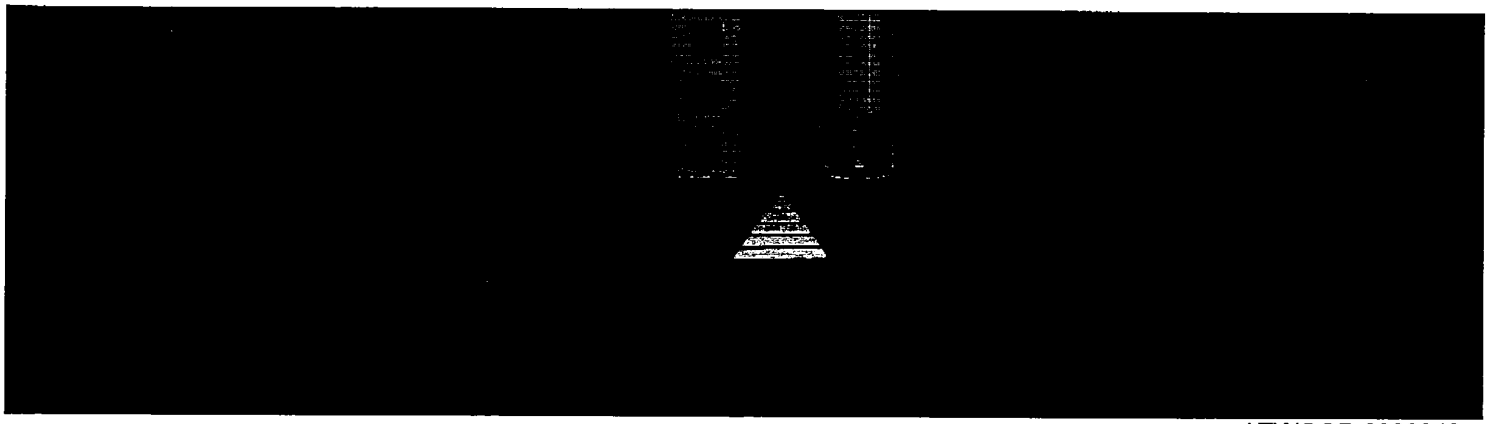

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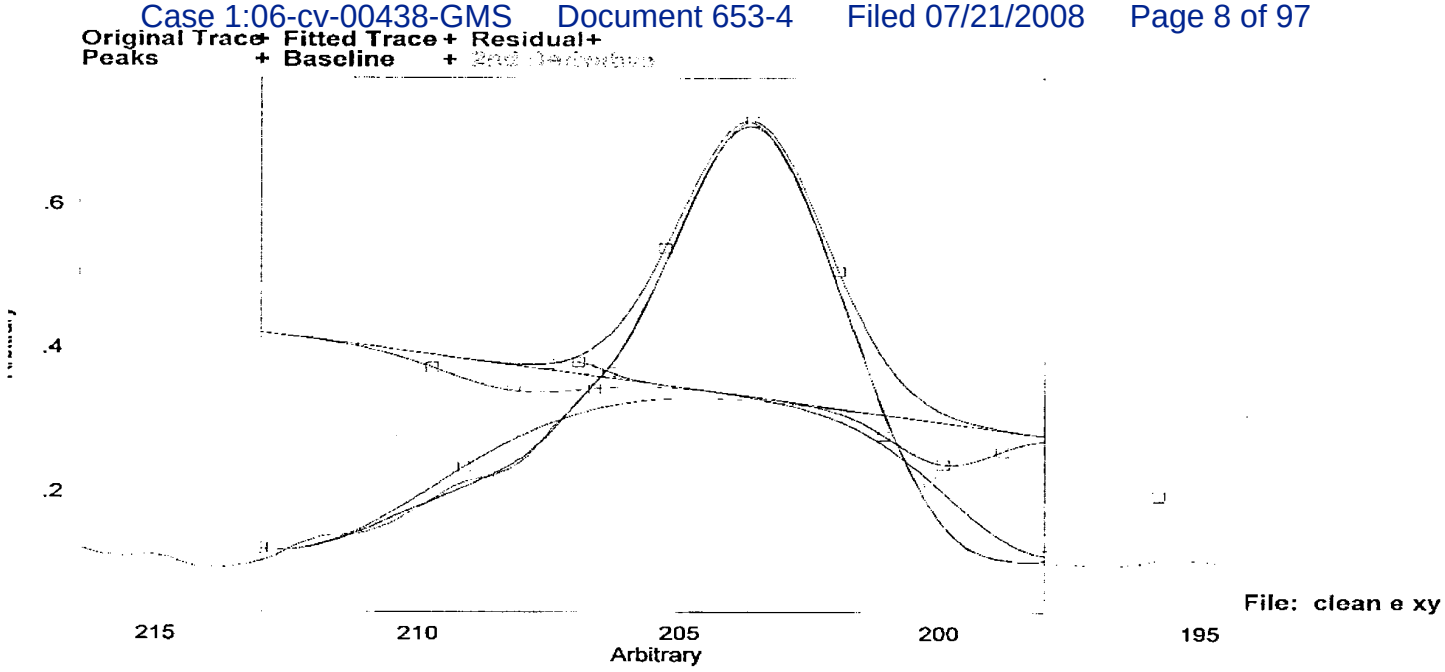
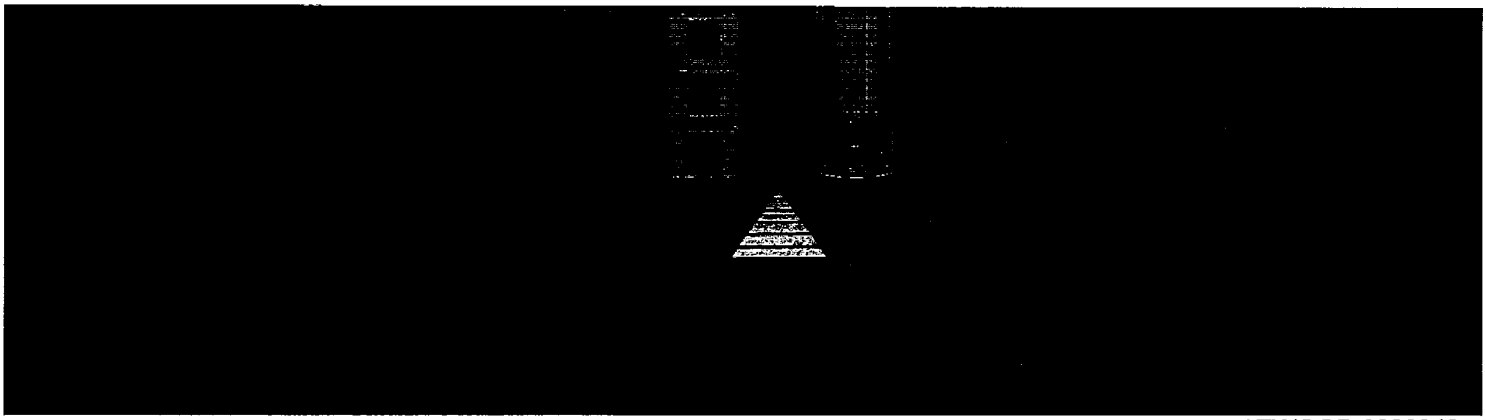


Munson Exhibit E

FWHH = 0.8

Requested Peaks = Max



ATWOOD 0000349

Peak Report

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Date: 10/29/2007 16:54

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High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Peak #3: Gaussian
Area: .02042147

	Center X	Height	Width
Value:	206.952296	.0160832779	1.19283467
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.00224009221	.0000107453263	0

Peak #4: Gaussian
Area: 1.363821

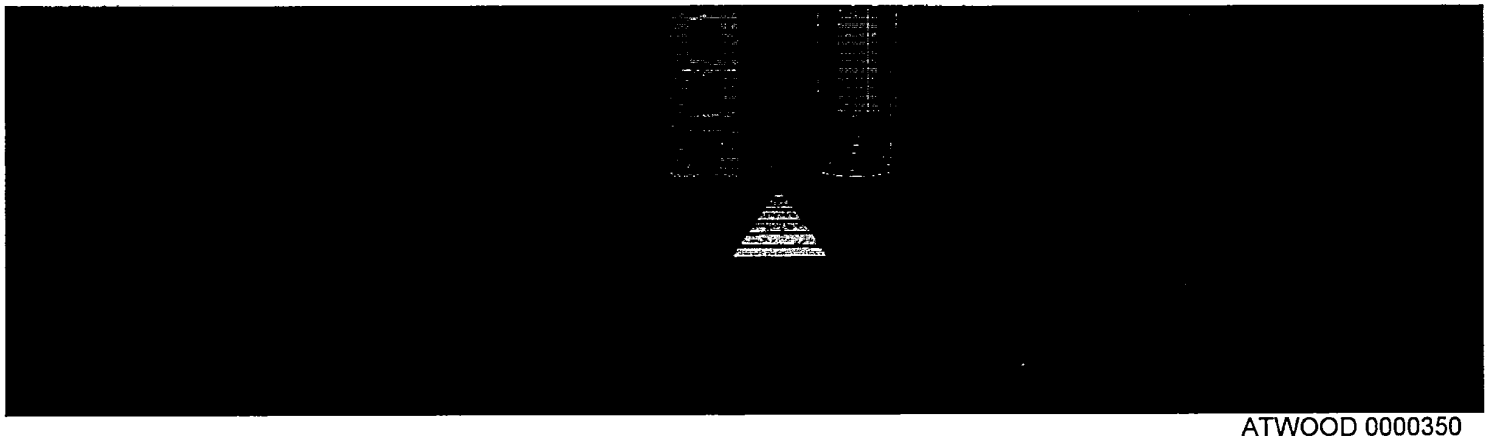
	Center X	Height	Width
Value:	203.620252	.38327701	3.34292895
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: -.1384858

	Center X	Height	Width
Value:	199.938132	-.0582822404	2.27988813
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: -.3925984

	Center X	Height	Width
Value:	197.999977	-.16582354	4.38828201
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0



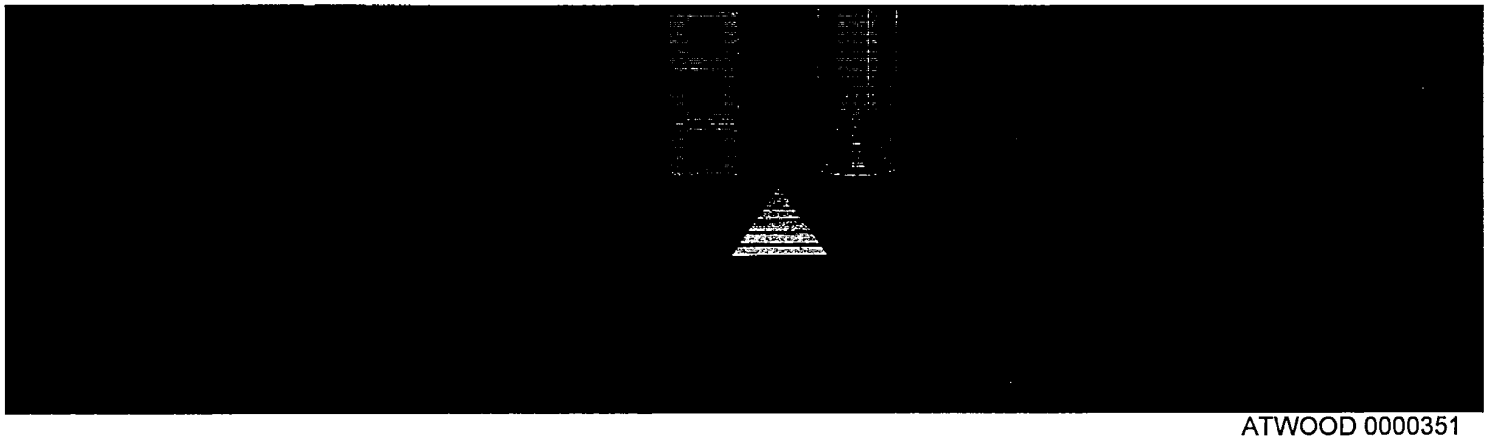
ATWOOD 0000350

Baseline: Linear

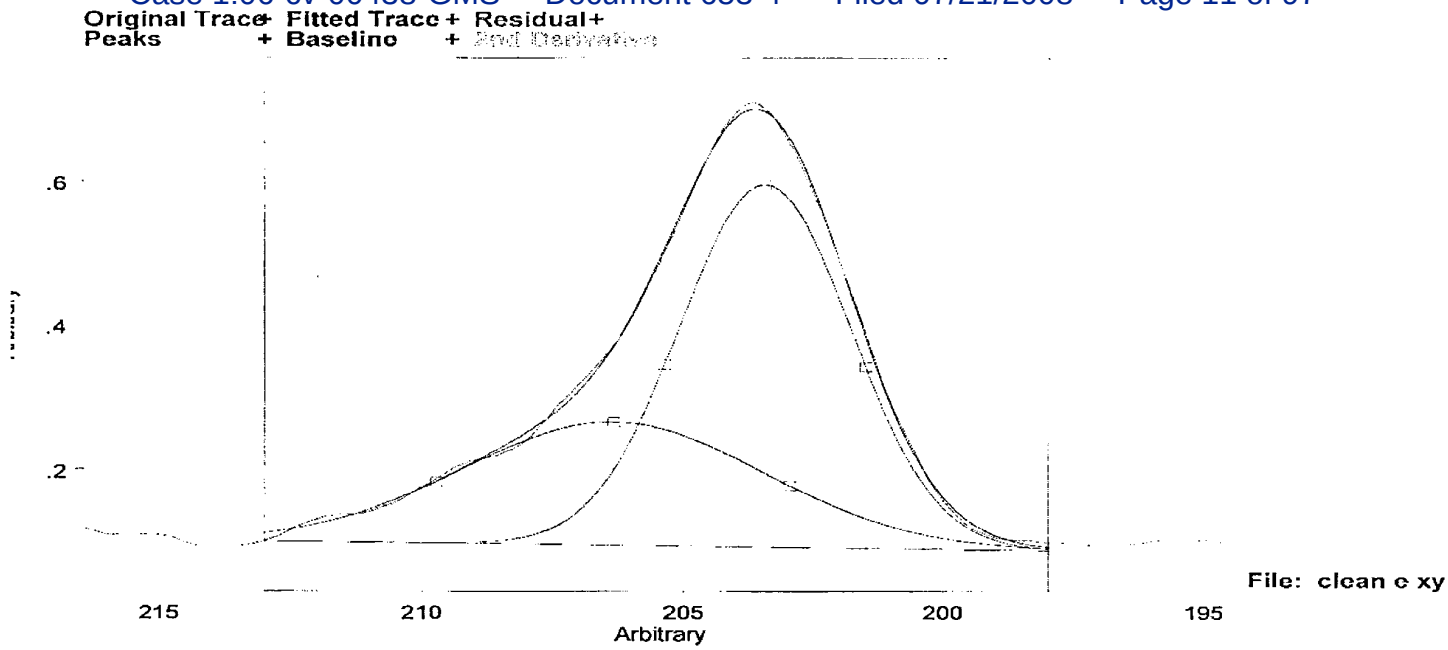
	Offset	Linear
Value:	-1.63151456	.00962116528
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	7
Total Parameters:	20
Fitted Parameters:	19
Fixed Parameters:	1
Reduced Chi ²:	48.51191
RMS Noise:	.00057
Correlation (R²):	1.000114
Standard Error:	.003914468



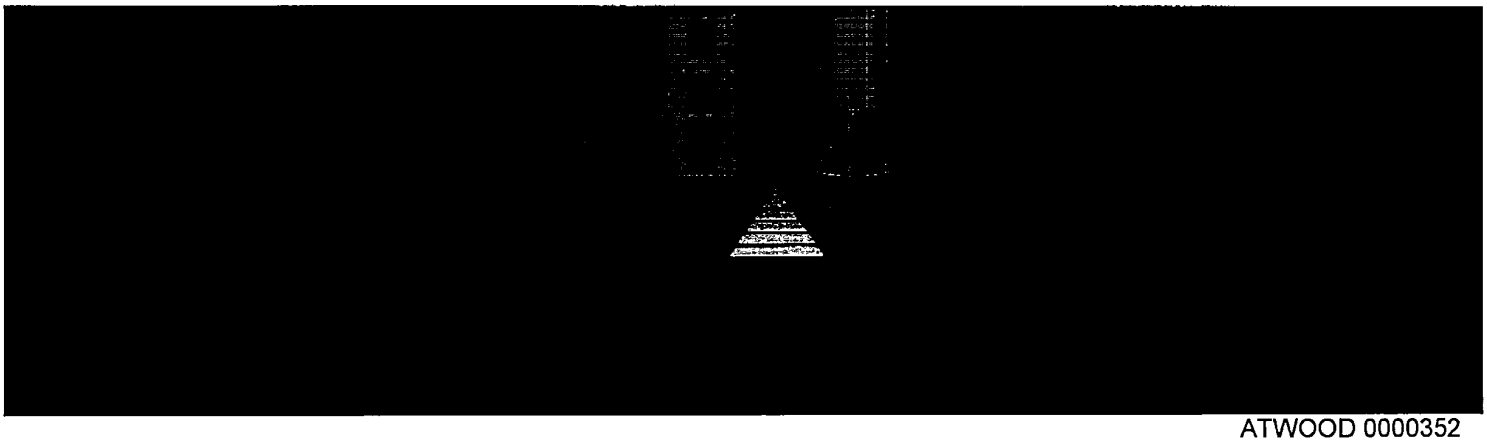
ATWOOD 0000351



Munson Exhibit E

FWHH = 0.8

Requested Peaks = 2



ATWOOD 0000352

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:55

Peak #1: Gaussian
Area: 1.217423

	Center X	Height	Width
Value:	206.338274	.170932824	6.77311437
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: 2.071859

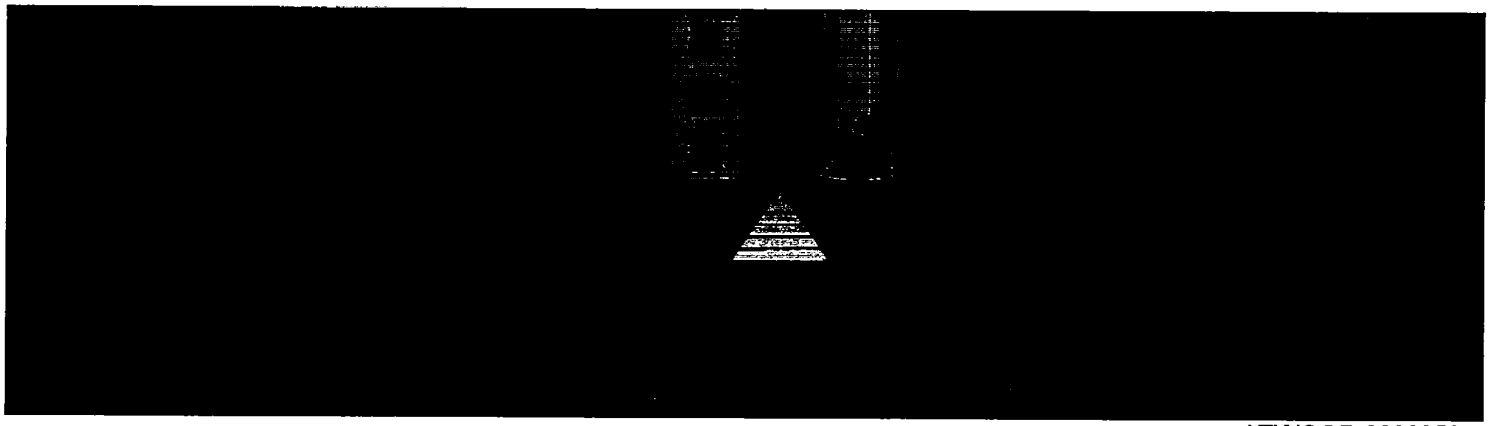
	Center X	Height	Width
Value:	203.427434	.50320806	3.86967252
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Baseline: Linear

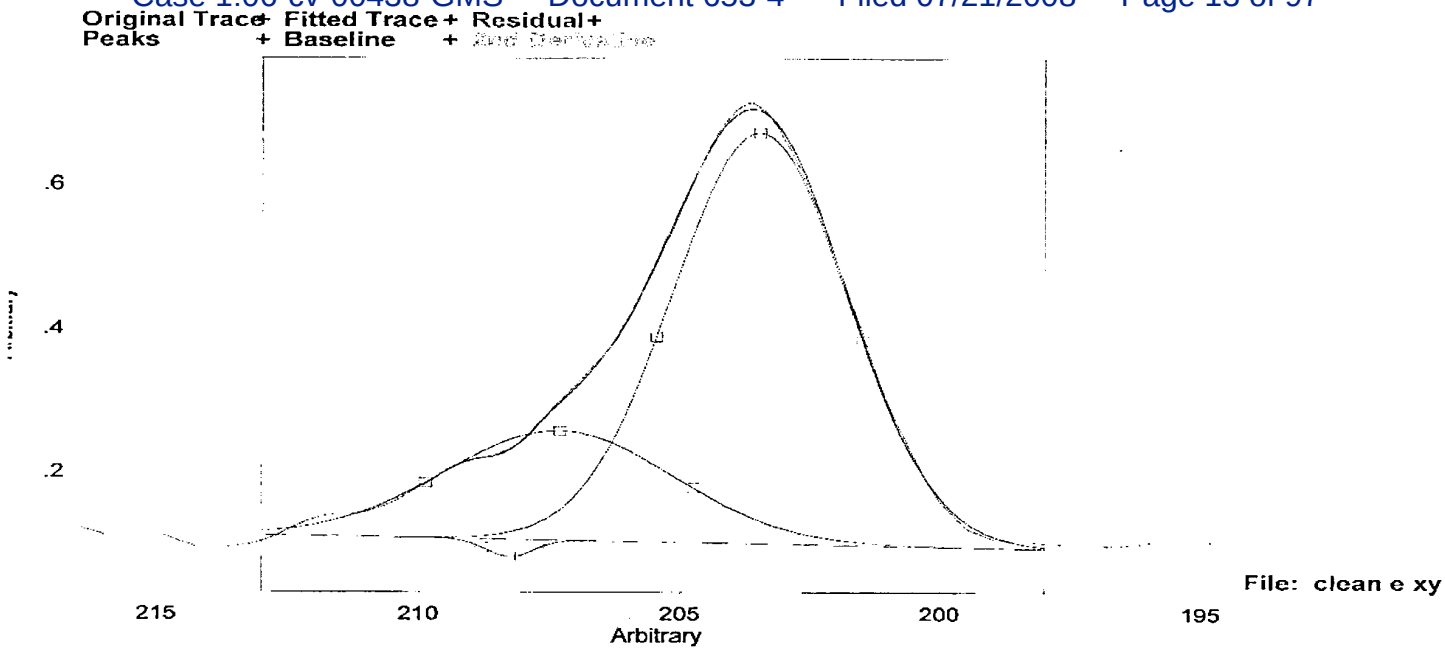
	Offset	Linear
Value:	-.0835348327	.000859330806
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	.00224009221	.0000107453263

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	85.08482
RMS Noise:	.00057
Correlation (R^2):	.9992916
Standard Error:	.005226879



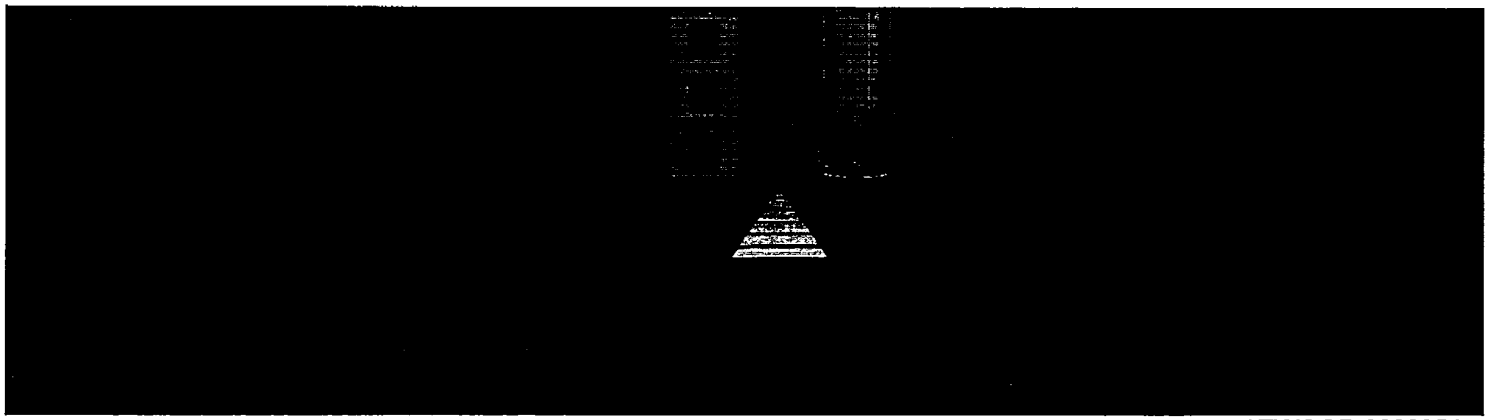
ATWOOD 0000353



Munson Exhibit E

FWHH = 0.8

Requested Peaks = 3



ATWOOD 0000354

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:56

Peak #1: Gaussian
Area: .02731721

	Center X	Height	Width
Value:	208.257148	-.024089527	1.06530971
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: .8210624

	Center X	Height	Width
Value:	207.318696	.151545945	5.11271154
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Peak #3: Gaussian
Area: 2.40246

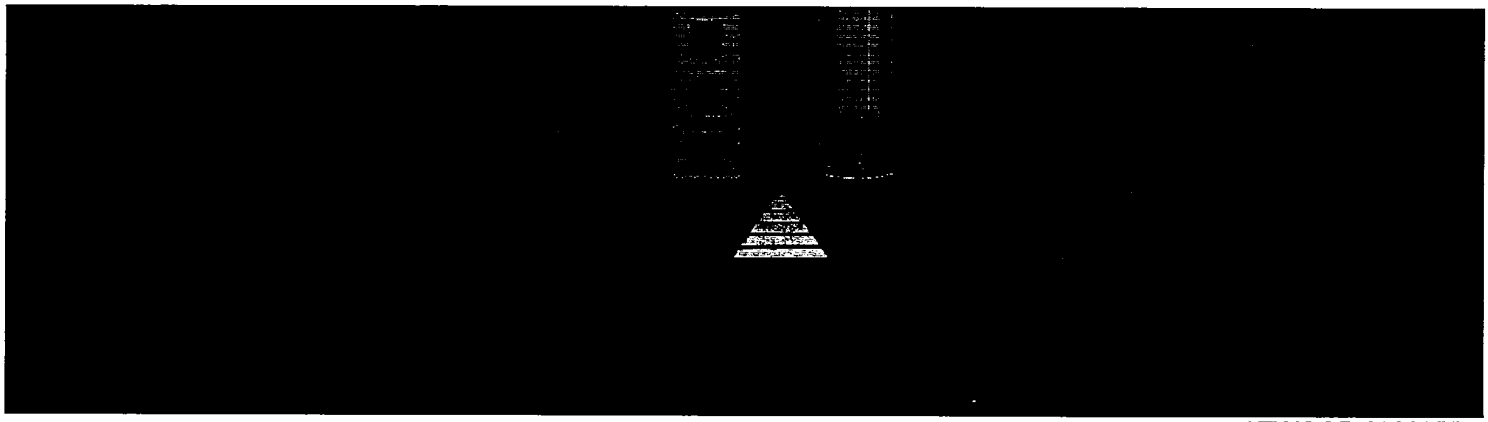
	Center X	Height	Width
Value:	203.477547	.570722148	3.95662777
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.00224009221	.0000107453263	0

Baseline: Linear

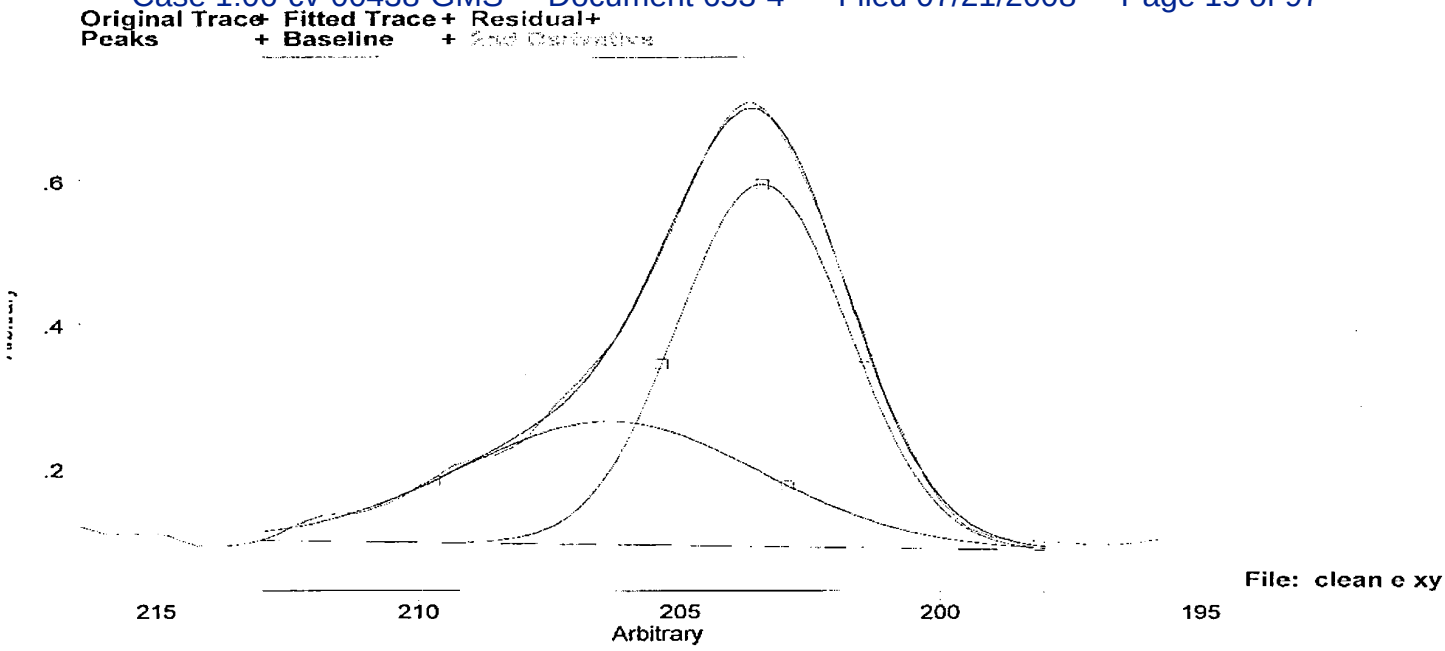
	Offset	Linear
Value:	-.1598329	.00126078719
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	11
Fixed Parameters:	0
Reduced Chi ²:	56.29934
RMS Noise:	.00057
Correlation (R^2):	.9995333
Standard Error:	.004242294



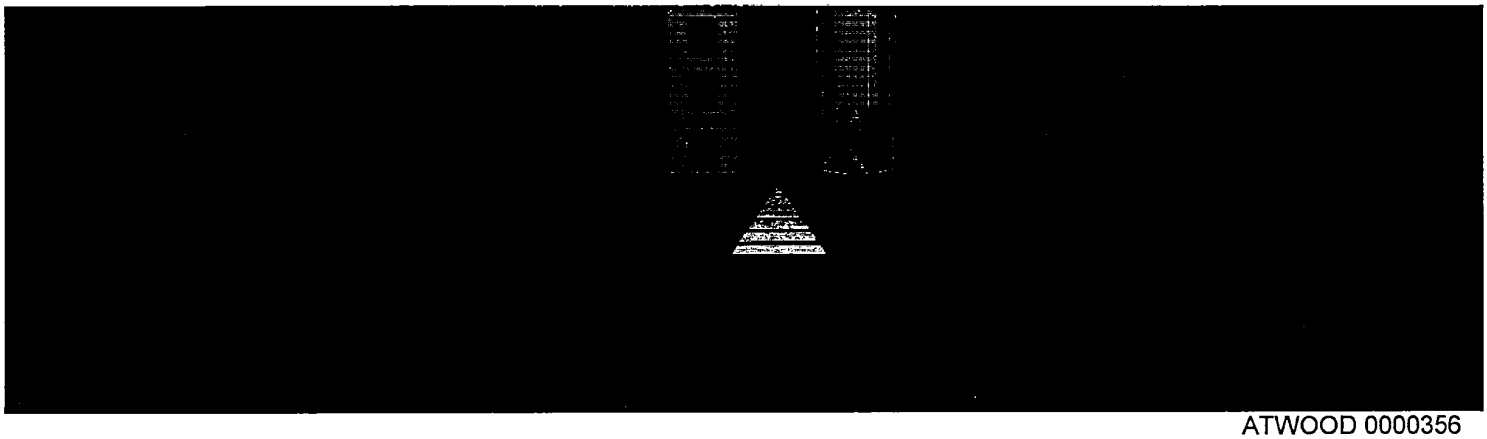
ATWOOD 0000355



Munson Exhibit E

FWHH = 3.0

Requested Peaks = 3 (2 found)



ATWOOD 0000356

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:58

Peak #1: Gaussian			
Area: 1.217455			
	Center X	Height	Width
Value:	206.338211	.170935247	6.77320241
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

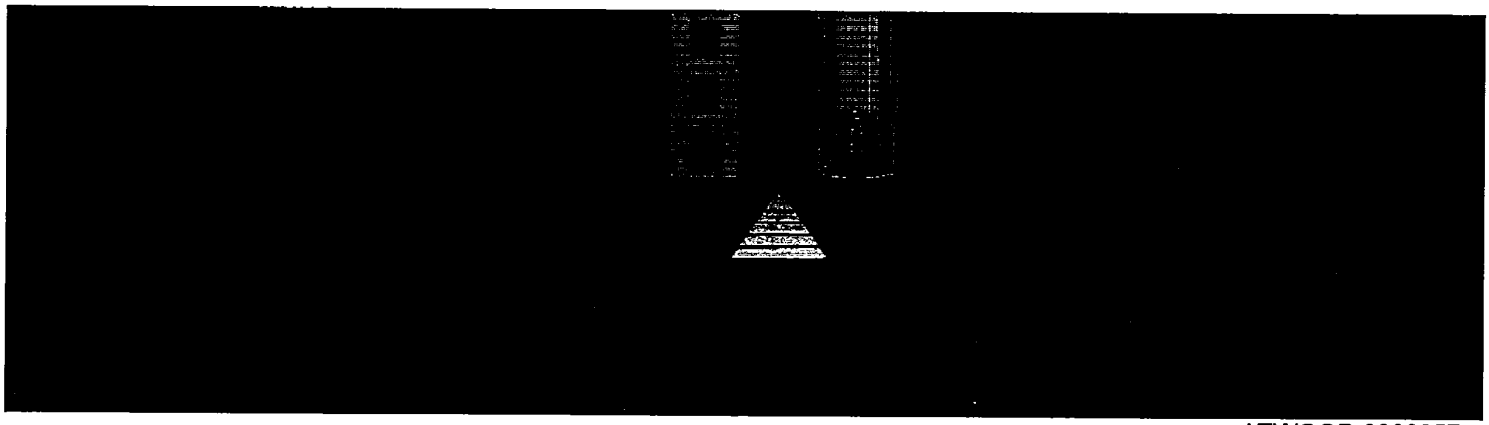
Peak #2: Gaussian			
Area: 2.071835			
	Center X	Height	Width
Value:	203.427433	.503203608	3.86966206
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Baseline: Linear

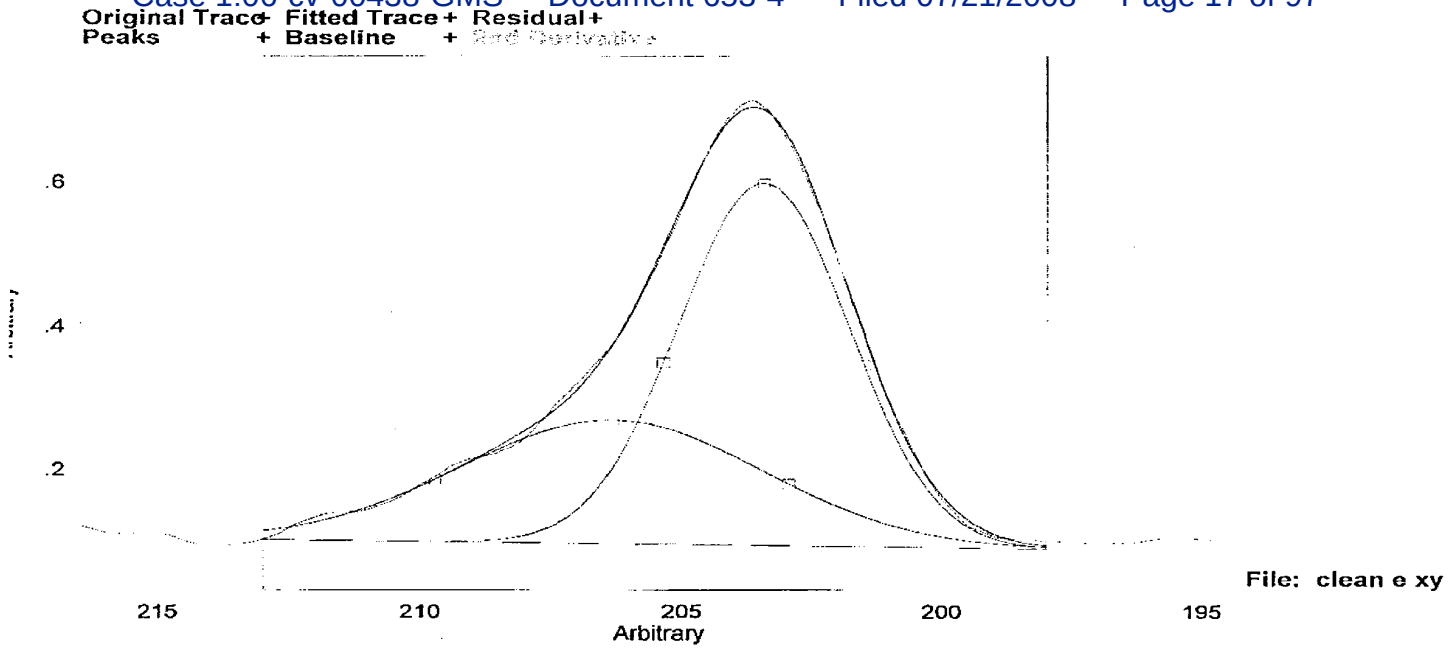
	Offset	Linear
Value:	-.0835371943	.00085933955
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	.00224009221	.0000107453263

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	85.08482
RMS Noise:	.00057
Correlation (R^2):	.9992916
Standard Error:	.005226879



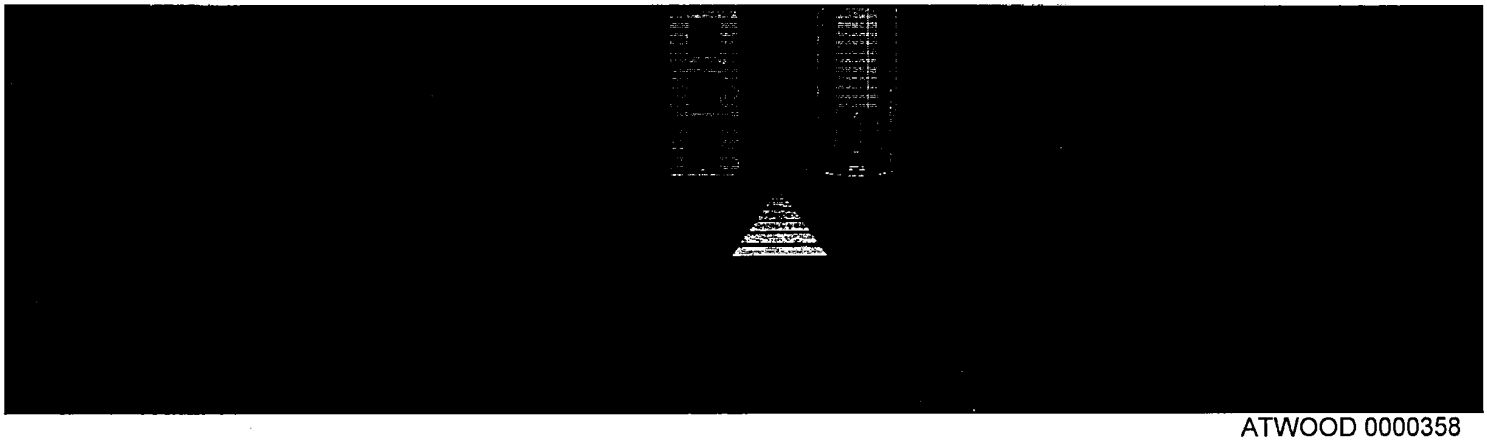
ATWOOD 0000357



Munson Exhibit E

FWHH = 3.0

Requested Peaks = 2 (2 found)



ATWOOD 0000358

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 16:59

Peak #1: Gaussian
Area: 1.217455

	Center X	Height	Width
Value:	206.338211	.170935247	6.77320241
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: 2.071835

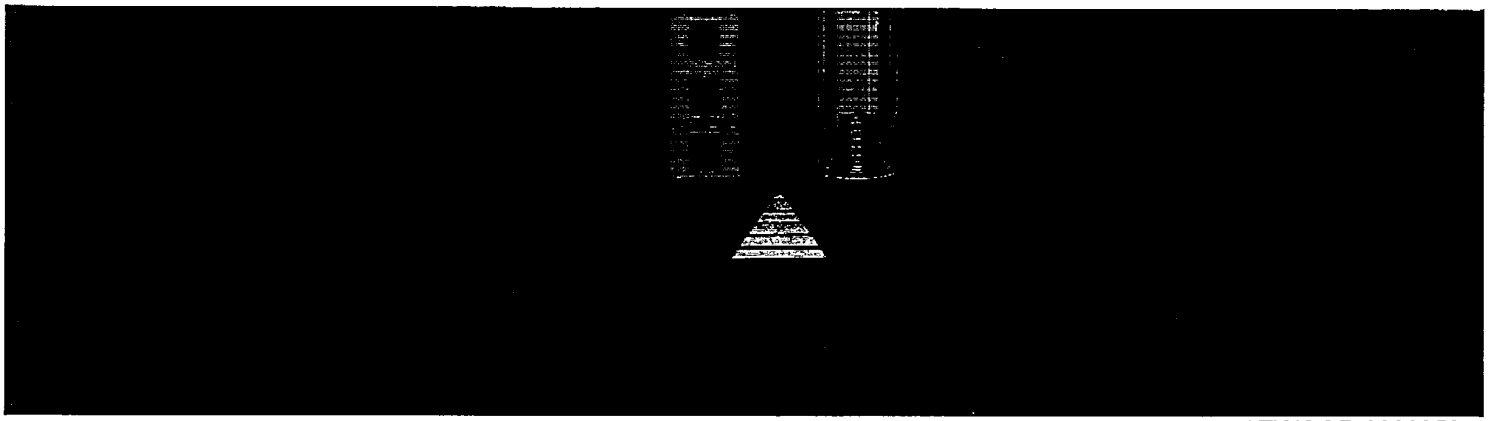
	Center X	Height	Width
Value:	203.427433	.503203608	3.86966206
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Baseline: Linear

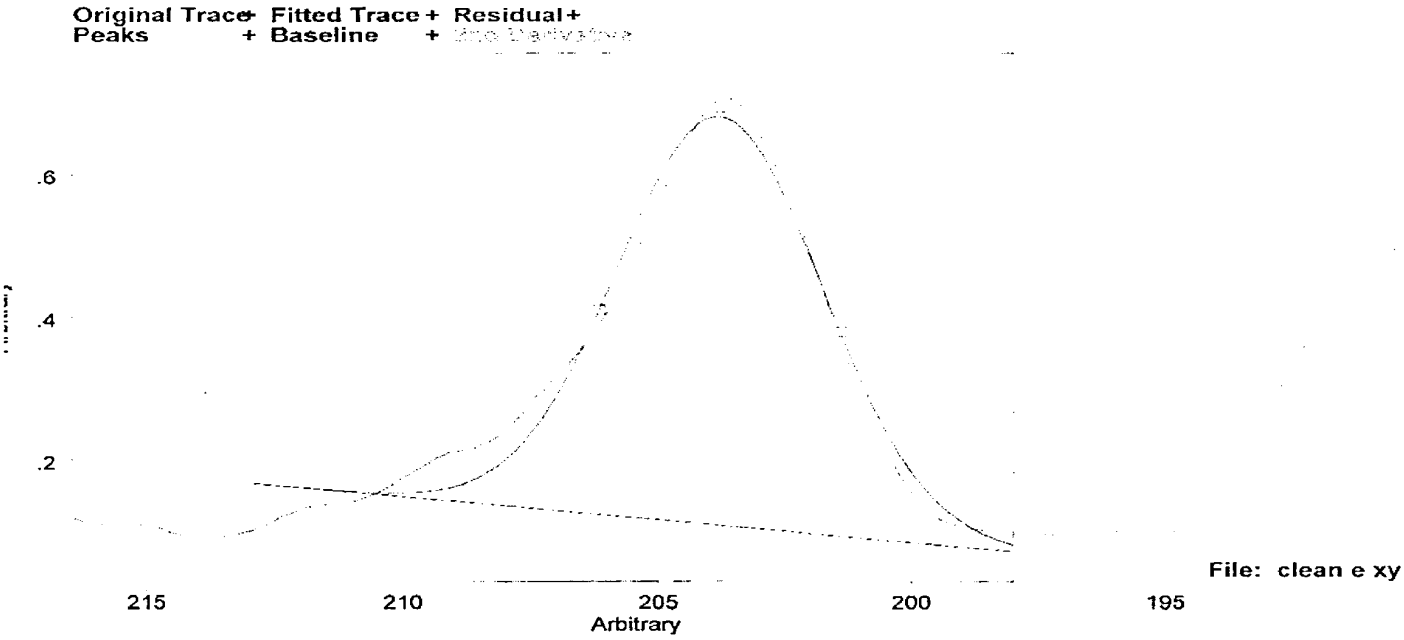
	Offset	Linear
Value:	-.0835371943	.00085933955
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	.00224009221	.0000107453263

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	85.08482
RMS Noise:	.00057
Correlation (R^2):	.9992916
Standard Error:	.005226879



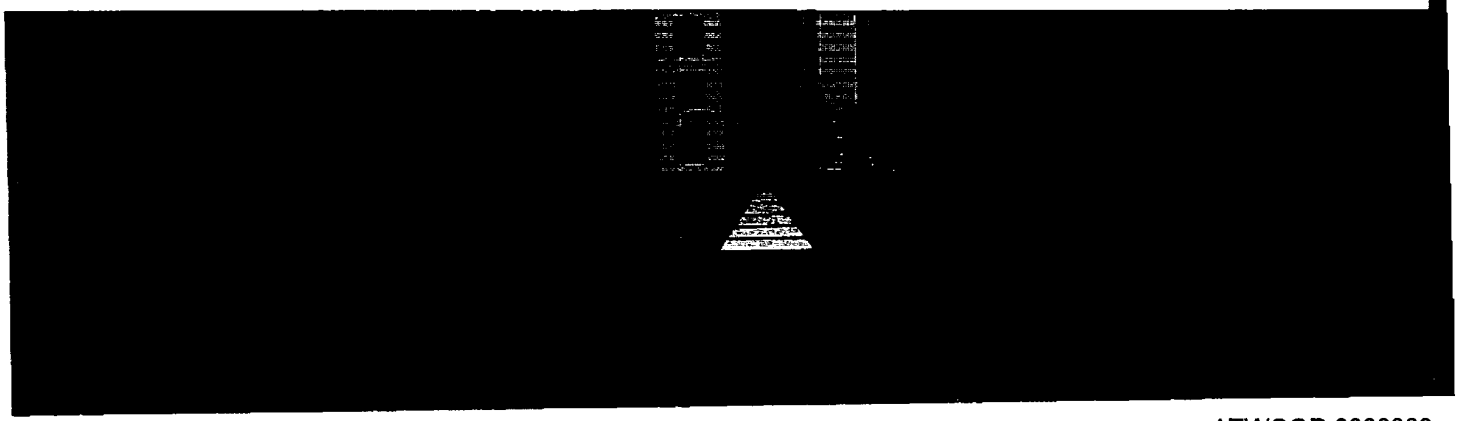
ATWOOD 0000359



Munson Exhibit E

FWHH = 5.0

Requested Peaks = Max (1 found)



ATWOOD 0000360

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:00

Peak #1: Gaussian
Area: 2.895926

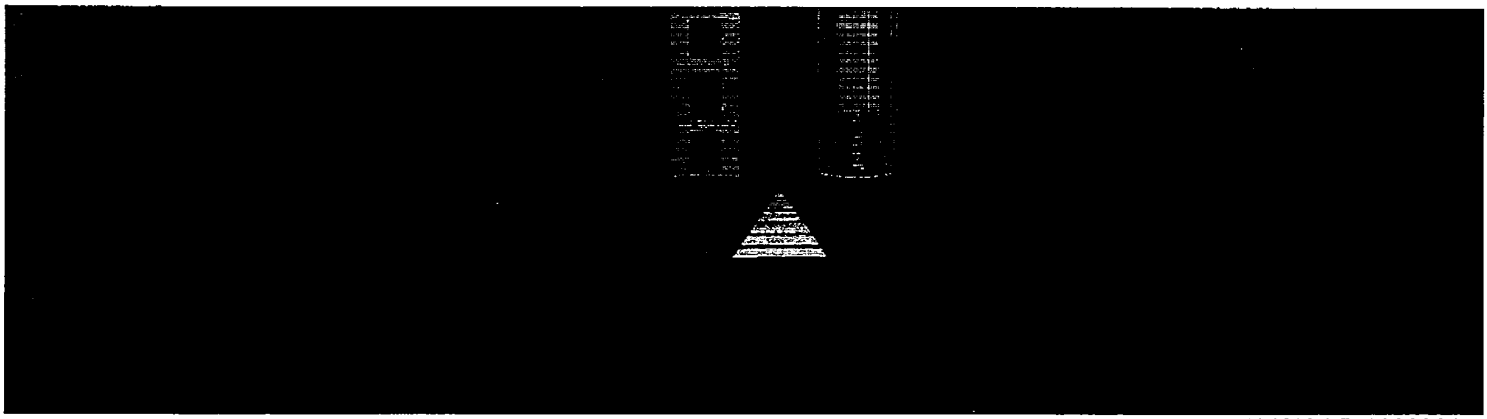
	Center X	Height	Width
Value:	203.786801	.573475326	4.75333394
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Baseline: Linear

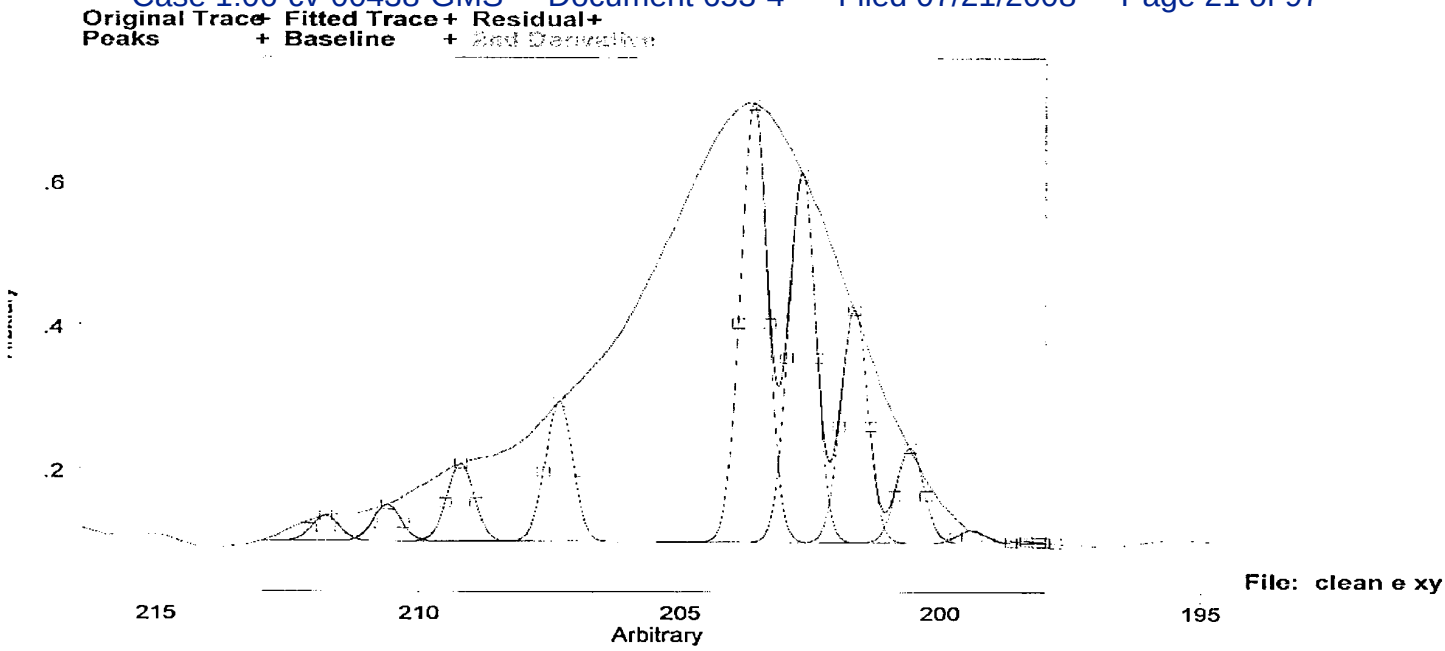
	Offset	Linear
Value:	-1.20841735	.00646087677
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	.000516525315	.00159643169

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	2
Total Parameters:	5
Fitted Parameters:	5
Fixed Parameters:	0
Reduced Chi ²:	2572.028
RMS Noise:	.00057
Correlation (R^2):	.9784897
Standard Error:	.02880164



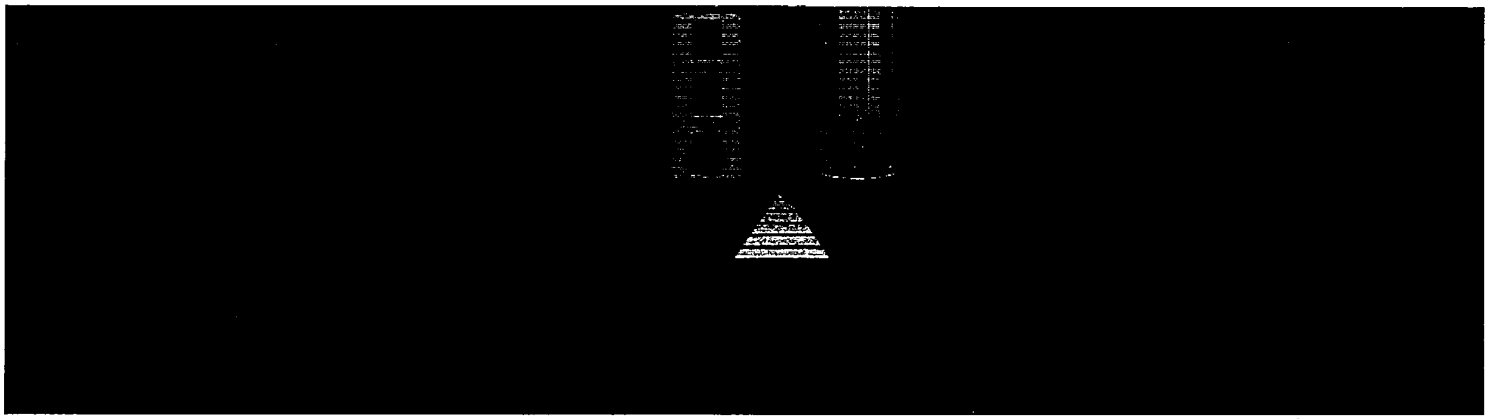
ATWOOD 0000361



Munson Exhibit E

FWHH = 0.6

Requested Peaks = Max (non-refined)



ATWOOD 0000362

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:02

Peak #1: Gaussian
Area: .0222634

	Center X	Height	Width
Value:	211.783971	.0348584775	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: .03212949

	Center X	Height	Width
Value:	210.617972	.0503060607	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Peak #3: Gaussian
Area: .06859686

	Center X	Height	Width
Value:	209.209973	.107404087	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.00224009221	.0000107453263	0

Peak #4: Gaussian
Area: .1240714

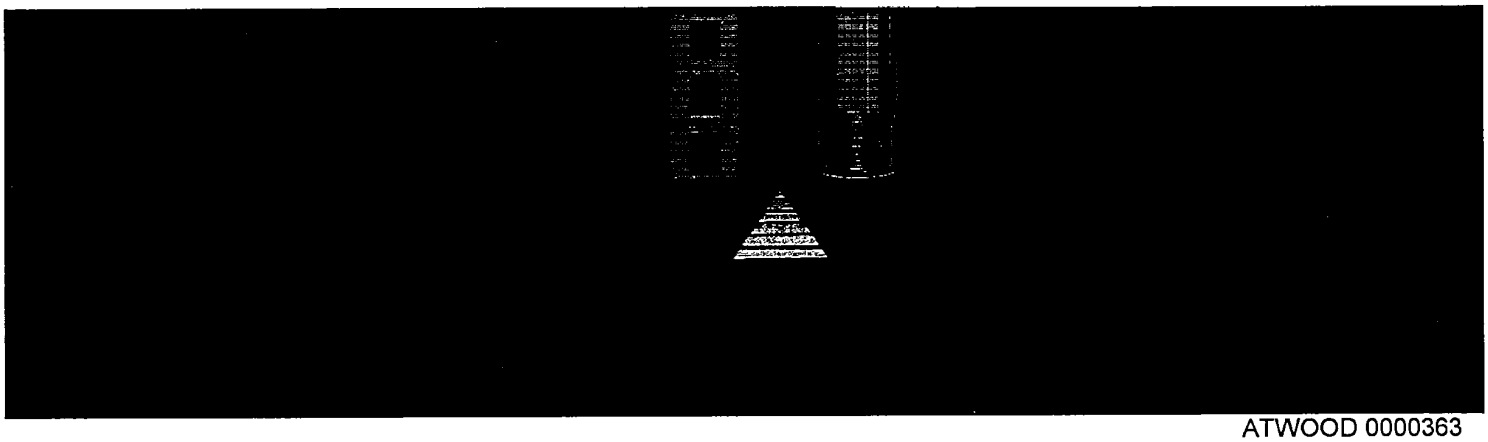
	Center X	Height	Width
Value:	207.339974	.194262167	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: .3893307

	Center X	Height	Width
Value:	203.599976	.609586291	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: .3276036

	Center X	Height	Width
Value:	202.675977	.512938385	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0



ATWOOD 0000363

Peak #7: Gaussian
Area: .2066483

	Center X	Height	Width
Value:	201.663978	.323555241	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #8: Gaussian
Area: .0840791

	Center X	Height	Width
Value:	200.585979	.131645068	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #9: Gaussian
Area: .01124948

	Center X	Height	Width
Value:	199.397979	.0176136283	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #10: Gaussian
Area: .001582443

	Center X	Height	Width
Value:	198.34198	.00266543142	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

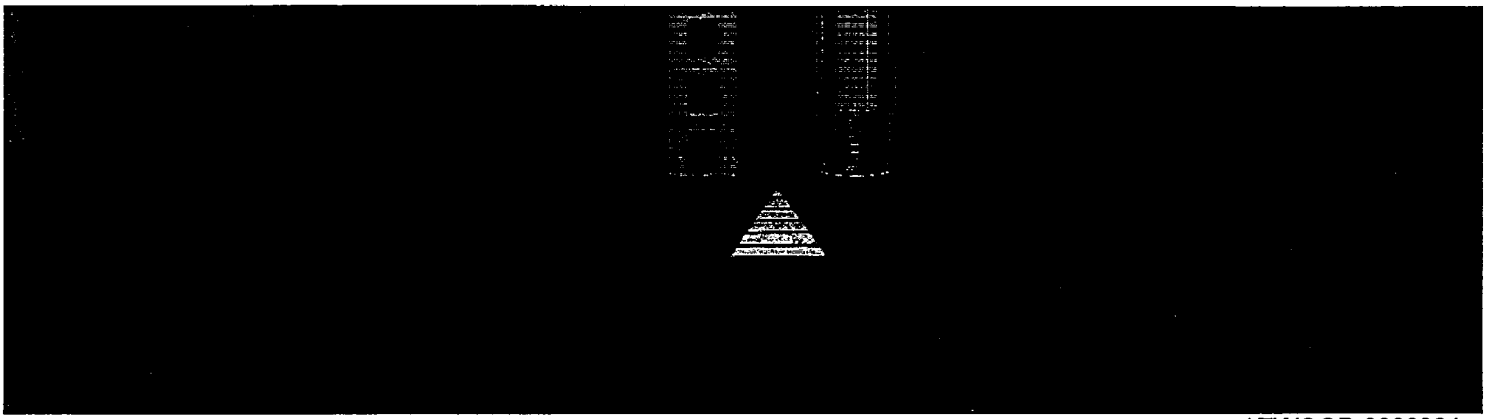
Peak #11: Gaussian
Area: .0003163907

	Center X	Height	Width
Value:	198.09998	.000709428901	.6
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Baseline: Linear

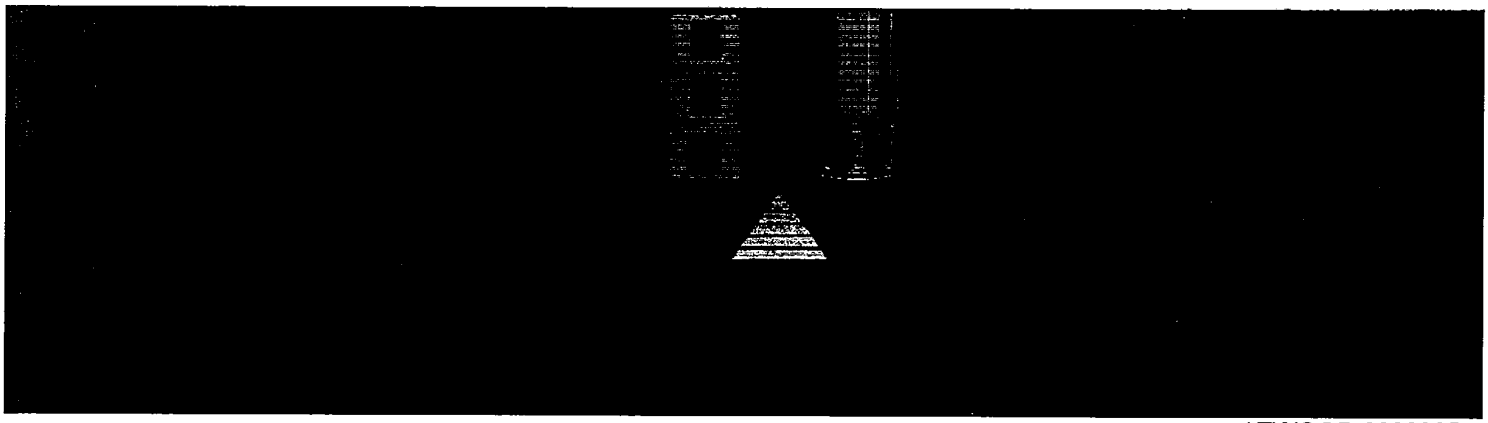
	Offset	Linear
Value:	.0618003184	.000179731105
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM
Total Data Points: 683
Fitted Region: 212.994 to 197.99

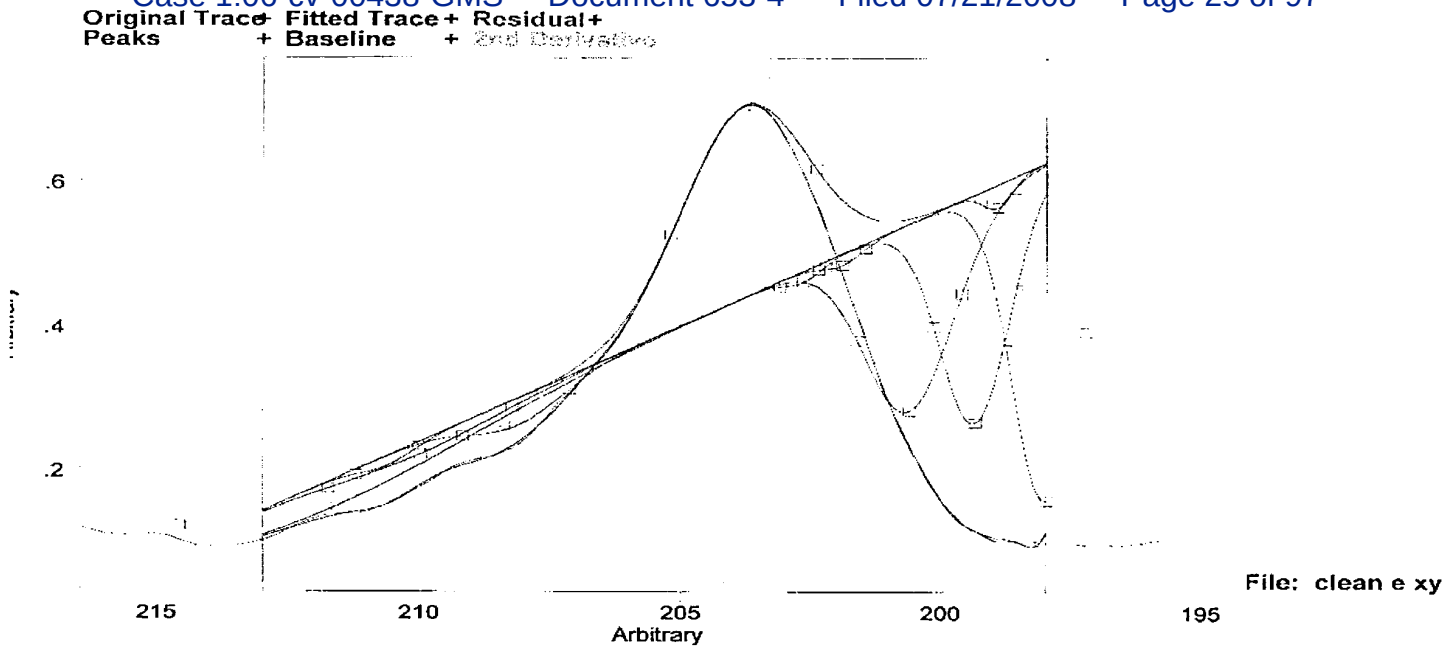


ATWOOD 0000364

Fitted Equations:	12
Total Parameters:	5
Fitted Parameters:	5
Fixed Parameters:	0
Reduced Chi ²:	135783
RMS Noise:	.00057
Correlation (R^2):	.8999104
Standard Error:	.2025229



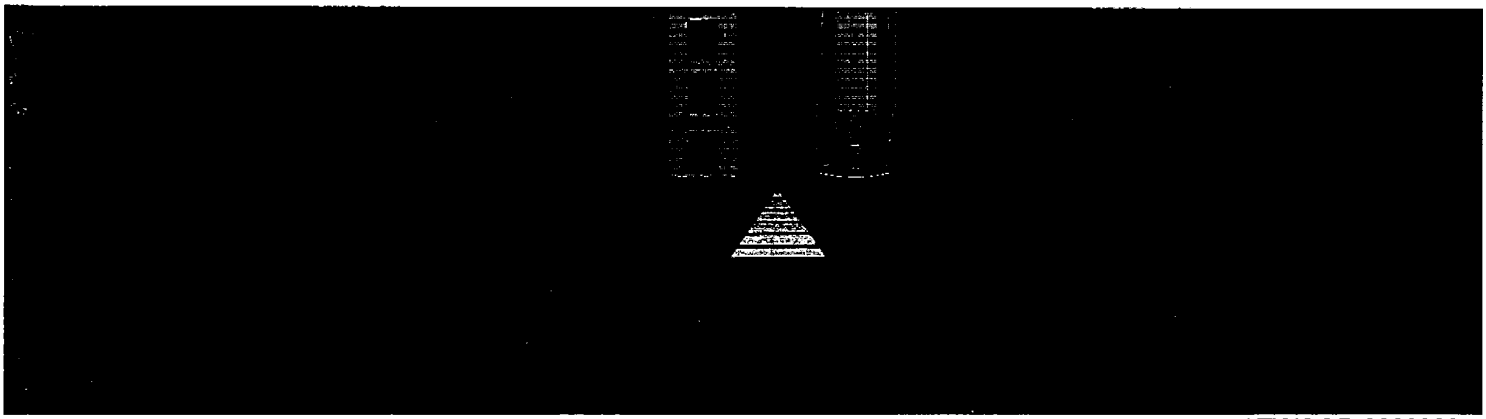
ATWOOD 0000365



Munson Exhibit E

FWHH = 0.6

Requested Peaks = 0



ATWOOD 0000366

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:03

Peak #1: Gaussian
Area: -.1787285

	Center X	Height	Width
Value:	211.567667	-.0390659786	6.0476677
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: -.01785974

	Center X	Height	Width
Value:	210.623617	-.0139066291	1.20648469
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Peak #3: Gaussian
Area: -.06576419

	Center X	Height	Width
Value:	209.994455	-.0179748391	3.51533127
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.00224009221	.0000107453263	0

Peak #4: Gaussian
Area: -.08146617

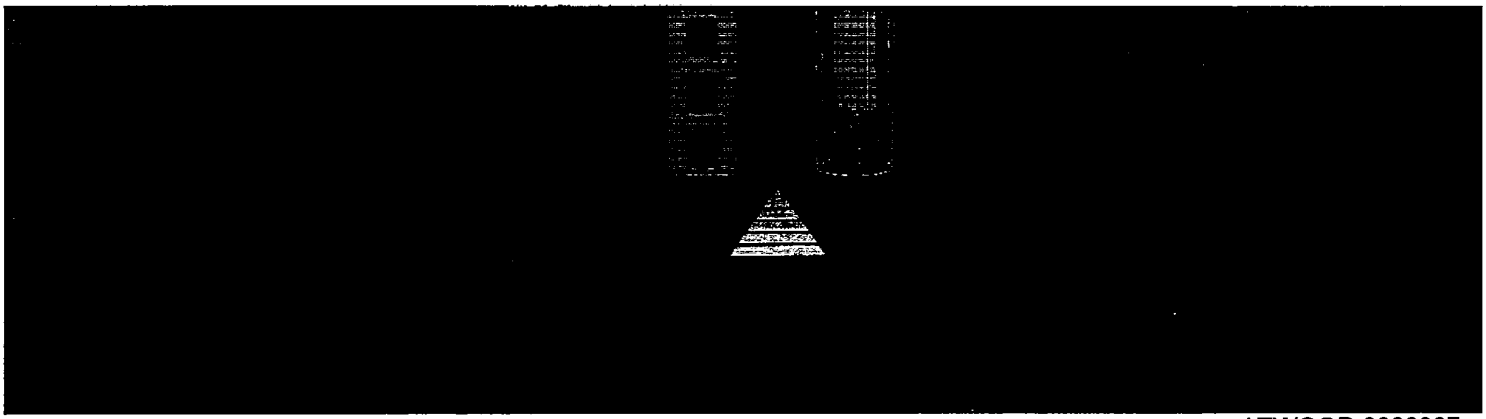
	Center X	Height	Width
Value:	208.163732	-.0377328709	2.02826754
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: .7950733

	Center X	Height	Width
Value:	203.809884	.267011547	2.79733892
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: -.01393636

	Center X	Height	Width
Value:	202.674443	-.0150416129	.870407611
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0



ATWOOD 0000367

Peak #7: Gaussian
Area: -.01524599

	Center X	Height	Width
Value:	201.913965	-.0158194186	.905384199
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #8: Gaussian
Area: -.5591131

	Center X	Height	Width
Value:	200.616021	-.259855464	2.02339815
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #9: Gaussian
Area: -.5269588

	Center X	Height	Width
Value:	199.355864	-.31677002	1.59498225
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

Peak #10: Gaussian
Area: : -.02306136

	Center X	Height	Width
Value:	198.923141	-.0324961948	.666923881
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	0	0	0

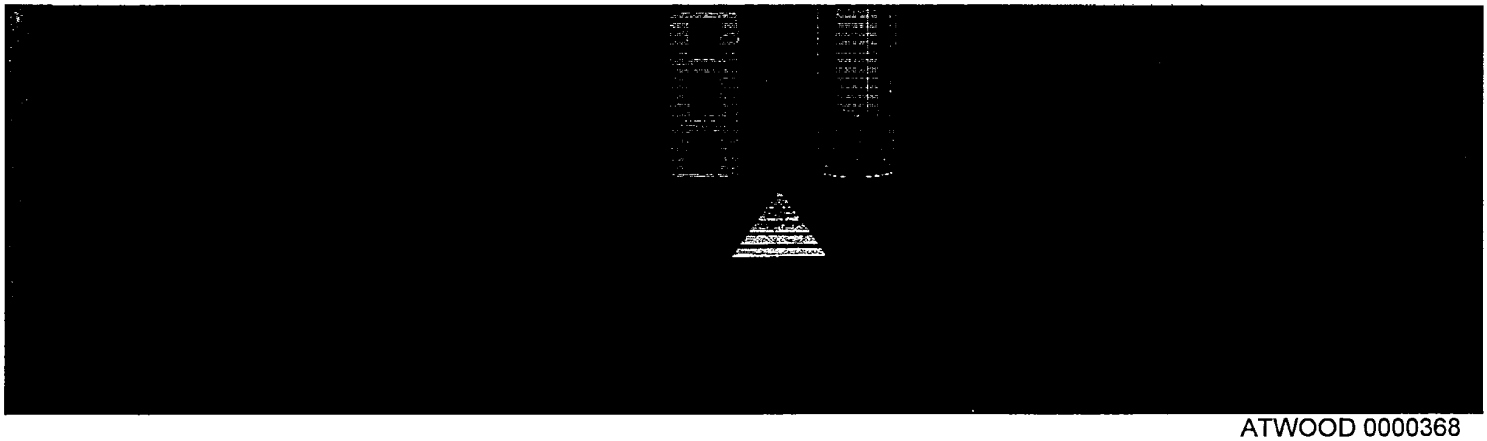
Peak #11: Gaussian
Area: : -.3936065

	Center X	Height	Width
Value:	197.99	-.470089554	1.5318486
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	197.99	None	None
Standard Error:	0	0	0

Baseline: Linear

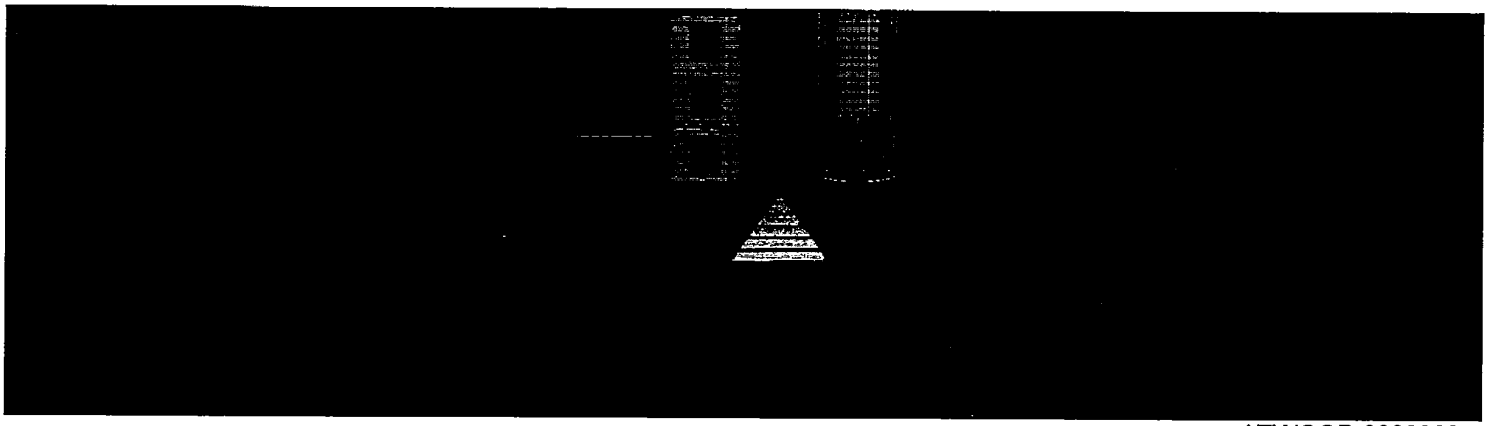
	Offset	Linear
Value:	7.02576299	-.0323245955
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM
Total Data Points: 683
Fitted Region: 212.994 to 197.99

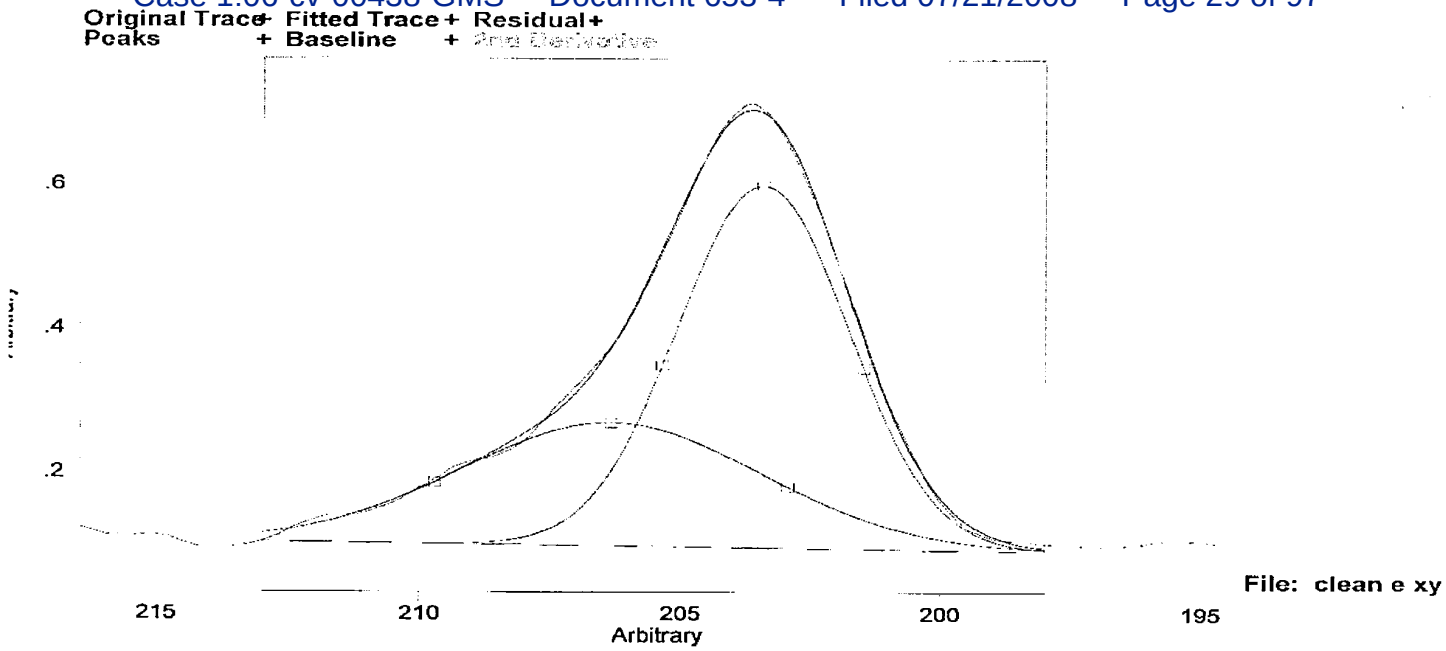


ATWOOD 0000368

Fitted Equations:	12
Total Parameters:	35
Fitted Parameters:	34
Fixed Parameters:	1
Reduced Chi ²:	17.32924
RMS Noise:	.00057
Correlation (R^2):	1.001802
Standard Error:	.002313005



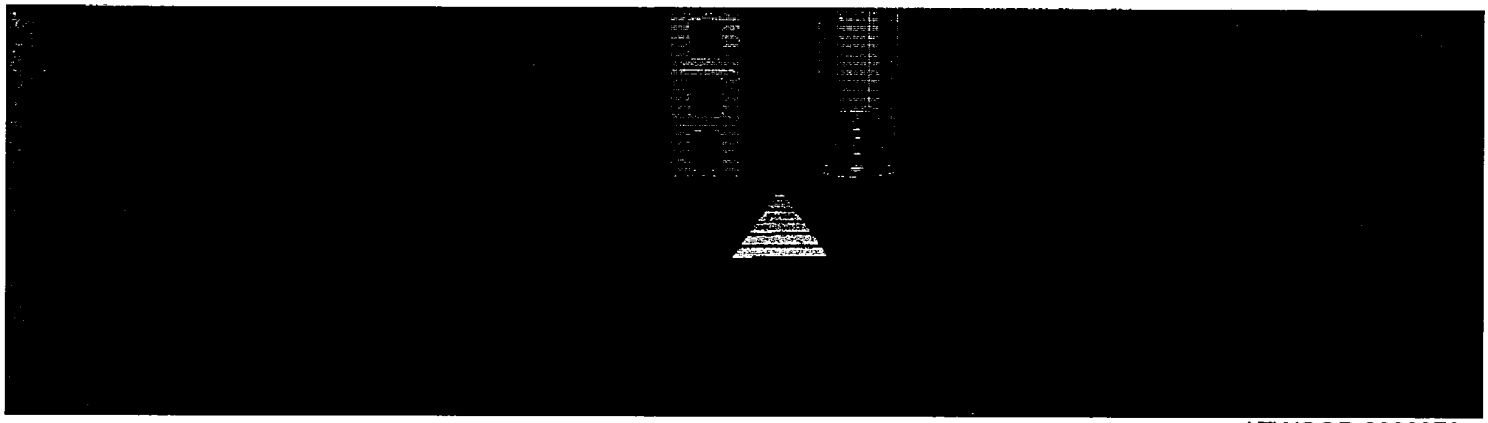
ATWOOD 0000369



Munson Exhibit E

FWHH = 0.6

Requested Peaks = 2



ATWOOD 0000370

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:03

Peak #1: Gaussian
Area: 1.217305

	Center X	Height	Width
Value:	206.338503	.170924037	6.772791
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: 2.071946

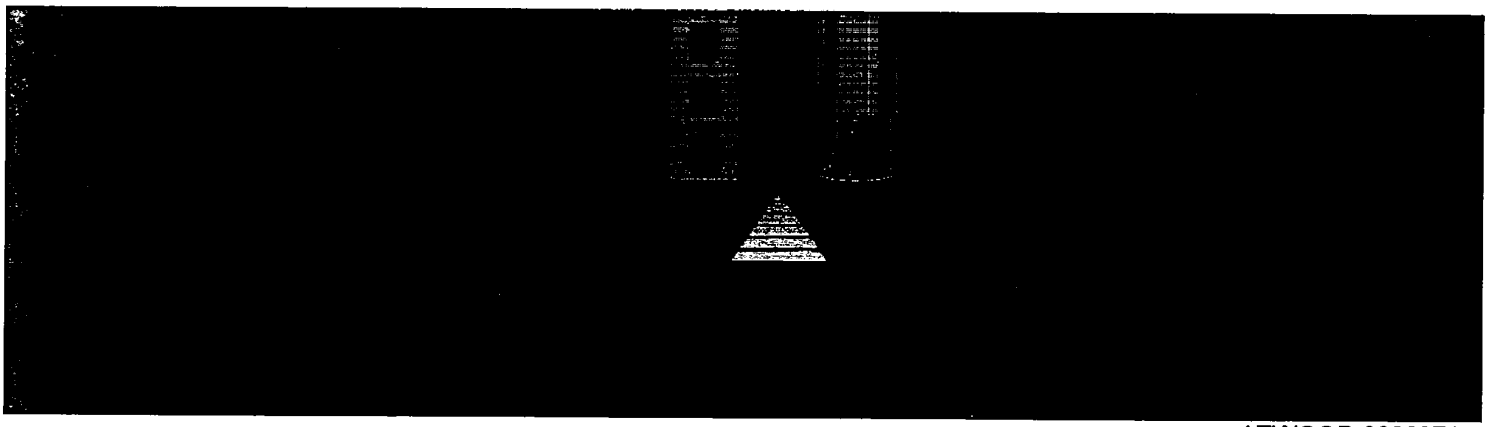
	Center X	Height	Width
Value:	203.427434	.503224192	3.86971023
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Baseline: Linear

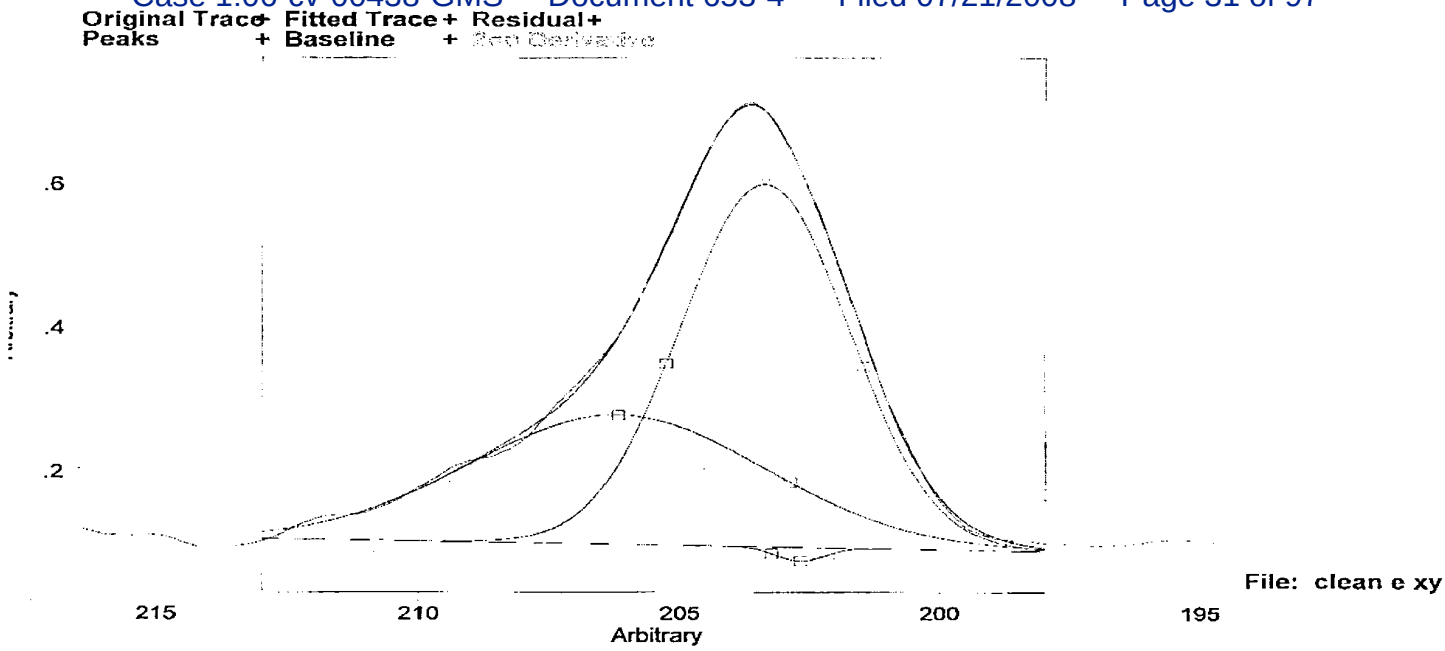
	Offset	Linear
Value:	-.0835273194	.000859304482
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	.00224009221	.0000107453263

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	85.08482
RMS Noise:	.00057
Correlation (R^2):	.9992915
Standard Error:	.005226879



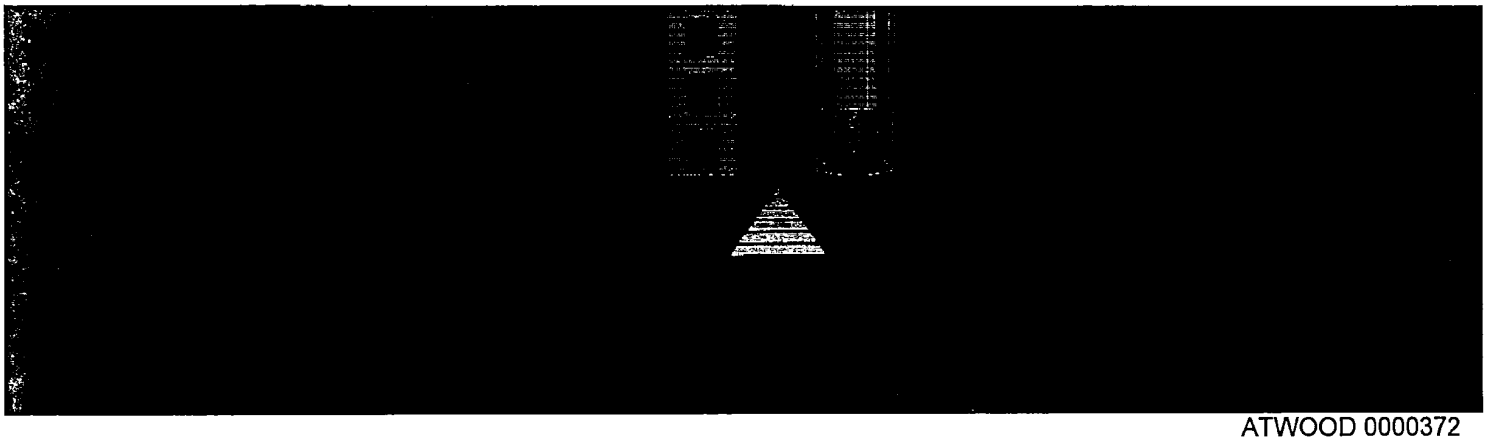
ATWOOD 0000371



Munson Exhibit E

FWHH = 0.6

Requested Peaks = 3



ATWOOD 0000372

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:04

Peak #1: Gaussian
Area: 1.249677

	Center X	Height	Width
Value:	206.204324	.178852292	6.62794299
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.0229197886	.000881383882	.030641102

Peak #2: Gaussian
Area: 2.034223

	Center X	Height	Width
Value:	203.385919	.503701355	3.79543492
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.000516525315	.00159643169	.00398459085

Peak #3: Gaussian
Area: -.02222742

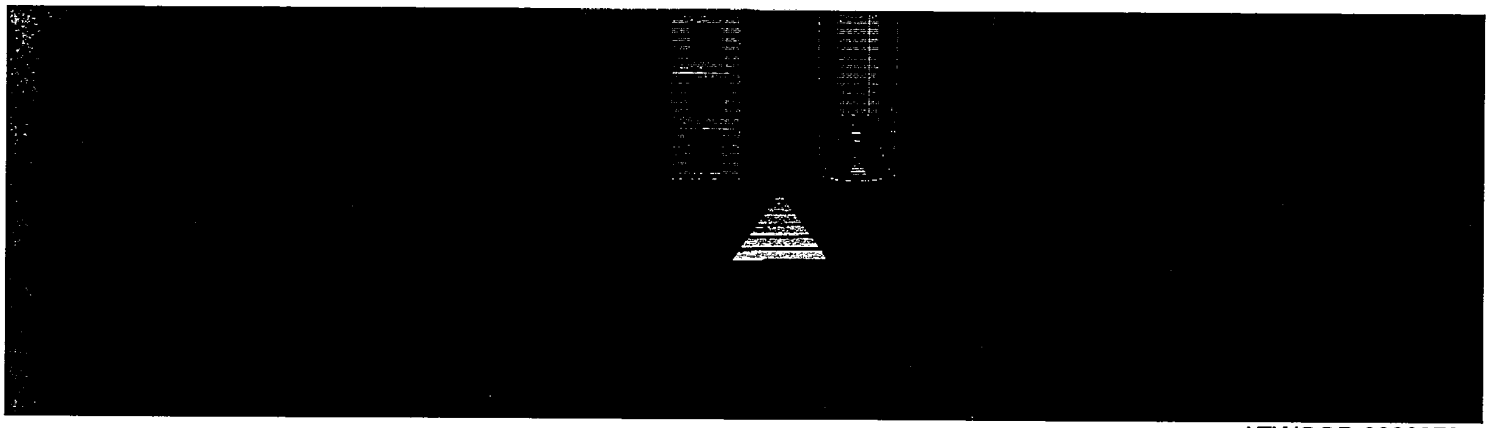
	Center X	Height	Width
Value:	202.694529	-.019110287	1.09267162
Fixed:	NO	NO	NO
Low Limit:	197.99	None	.022
High Limit:	212.994	None	None
Standard Error:	.00224009221	.0000107453263	0

Baseline: Linear

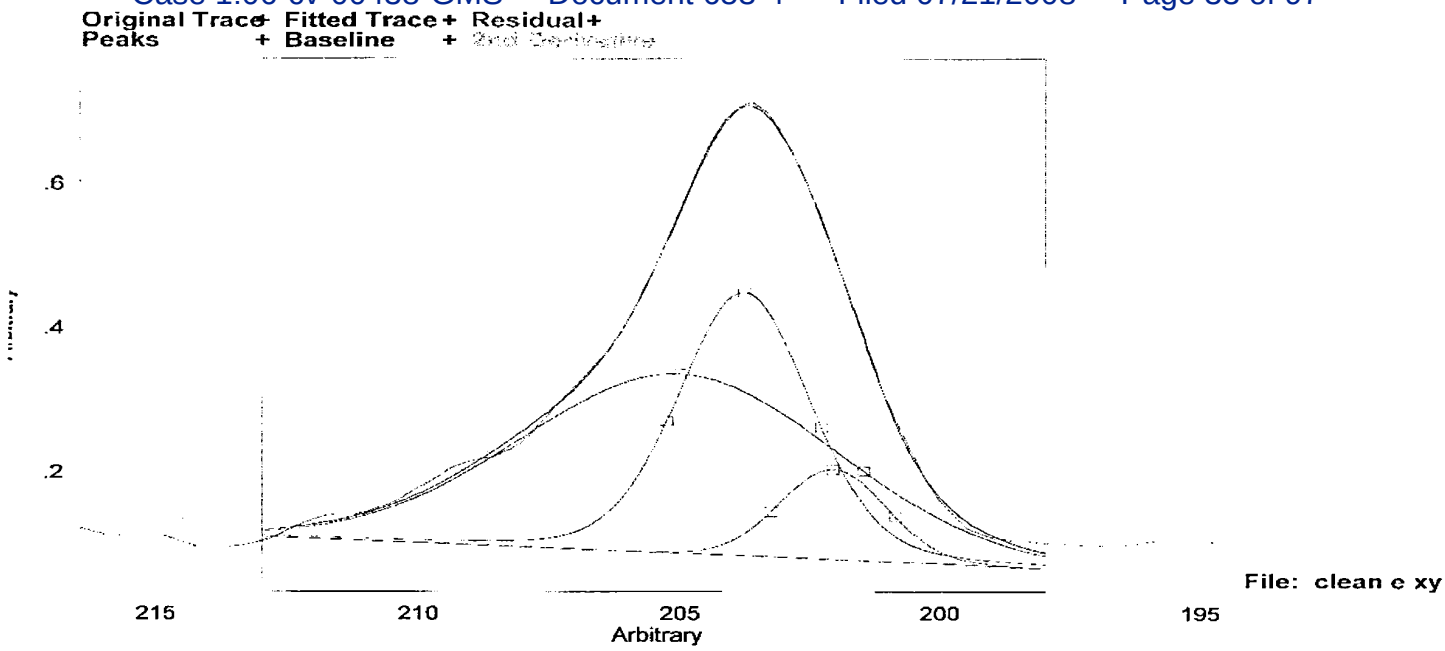
	Offset	Linear
Value:	-.130109397	.00109492757
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	11
Fixed Parameters:	0
Reduced Chi ²:	68.12739
RMS Noise:	.00057
Correlation (R^2):	.9994353
Standard Error:	.004666701



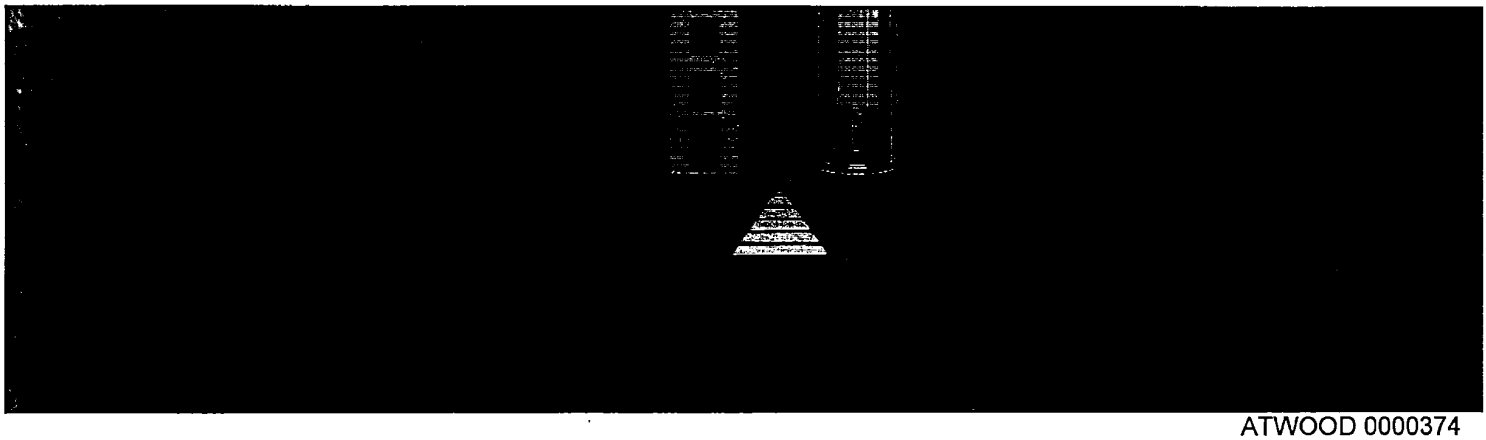
ATWOOD 0000373



Munson Exhibit E

FWHH = 0.6

Requested Peaks = 3 G&L



ATWOOD 0000374

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:06

Peak #1: Mixed G+L
Area: 1.858084

	Center X	Height	Width	Lorentz %
Value:	205.031783	.248941791	7.11028954	0
Fixed:	NO	NO	NO	NO
Low Limit:	197.99	None	.022	0
High Limit:	212.994	None	None	0
Standard Error:	.0229197886	.000881383882	.030641102	0

Peak #2: wMixed G+L
Area: 1.227069

	Center X	Height	Width	Lorentz %
Value:	203.780872	.36601953	2.98254092	.19820278
Fixed:	NO	NO	NO	NO
Low Limit:	197.99	None	.022	0
High Limit:	212.994	None	None	1
Standard Error:	.000516525315	.00159643169	.00398459085	0

Peak #3: wMixed G+L
Area: .3210491

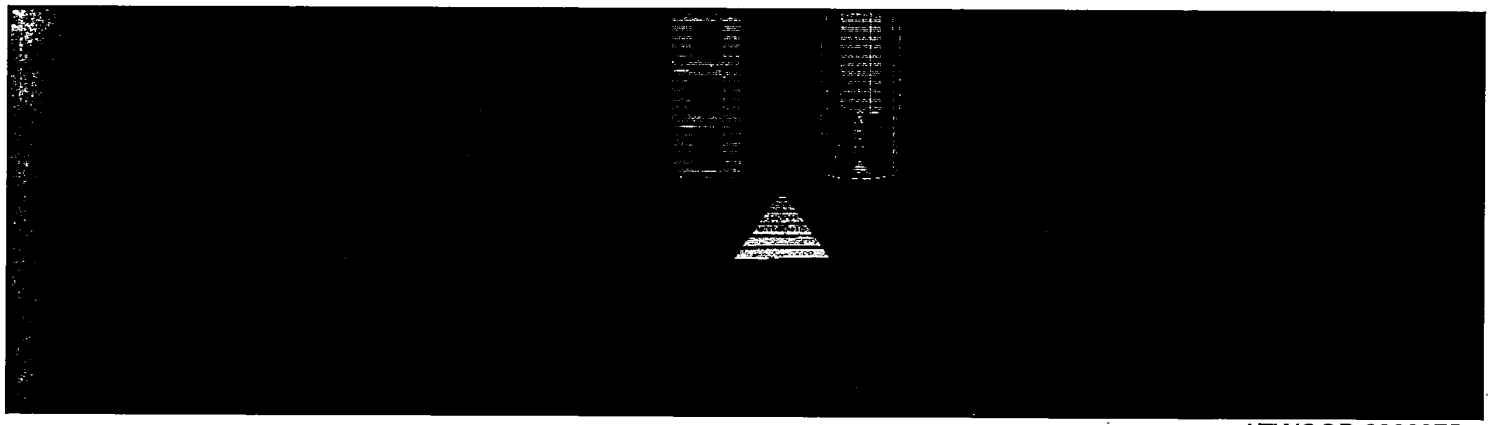
	Center X	Height	Width	Lorentz %
Value:	202.062965	.12529336	2.40726837	0
Fixed:	NO	NO	NO	NO
Low Limit:	197.99	None	.022	0
High Limit:	212.994	None	None	0
Standard Error:	.00224009221	.0000107453263	0	0

Baseline: wLinear

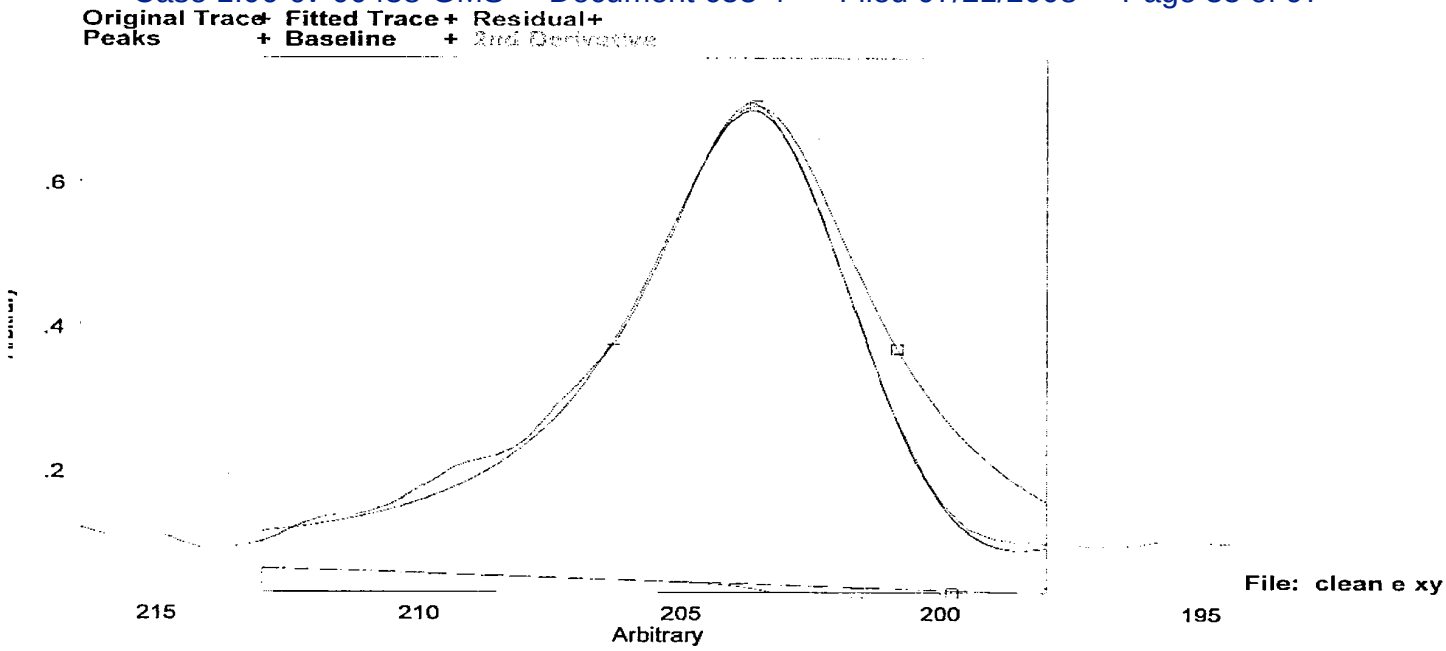
	Offset	Linear
Value:	-.511771597	.00290212619
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	0	0

Statistics Summary:

Solution AT A MINIMUM	
Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	4
Total Parameters:	14
Fitted Parameters:	12
Fixed Parameters:	2
Reduced Chi ²:	112.8092
RMS Noise:	.00057
Correlation (R^2):	1.006049
Standard Error:	.006000645



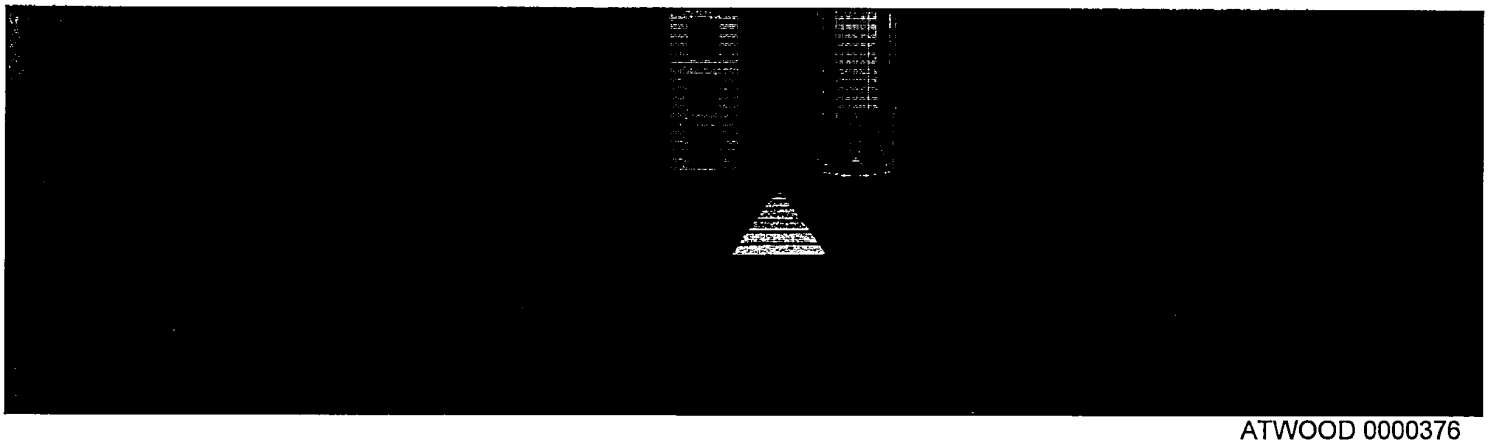
ATWOOD 0000375



Munson Exhibit E

FWHH = 0.6

Requested Peaks = 3 (G&L)



ATWOOD 0000376

Peak Report

Original File: clean e xy data.spc

Date: 10/29/2007 17:07

Peak #1: Mixed G+L
Area: 4.357824

	Center X	Height	Width	Lorentz %
Value:	203.576158	.66406271	5.45177185	1
Fixed:	NO	NO	NO	NO
Low Limit:	197.99	None	.022	1
High Limit:	212.994	None	None	1
Standard Error:	.0229197886	.000881383882	.030641102	0

Peak #2: Mixed G+L
Area: -.4315874

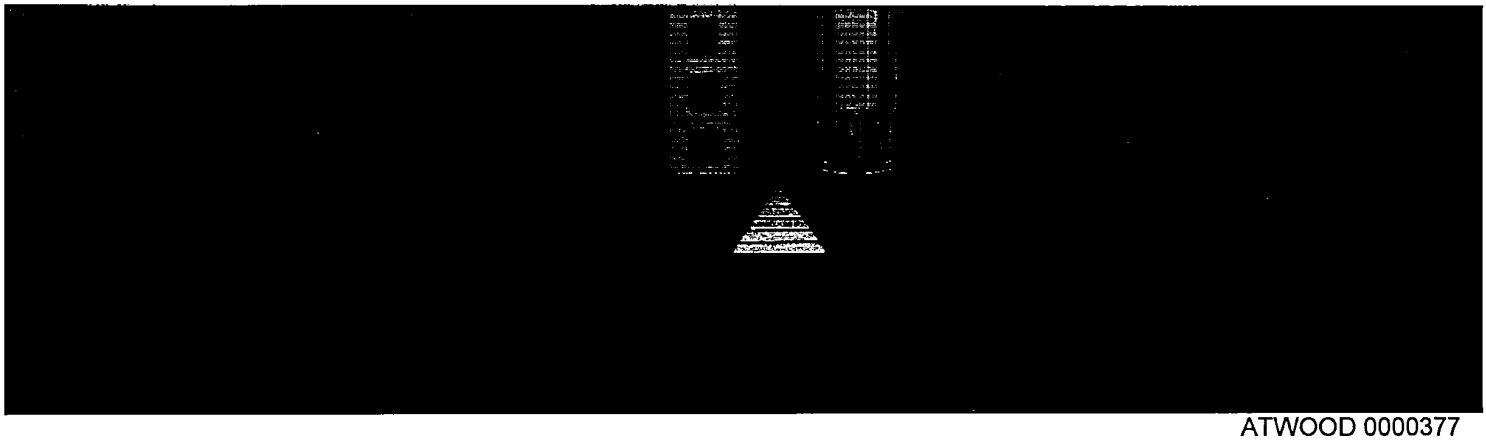
	Center X	Height	Width	Lorentz %
Value:	199.79292	-.125363285	3.68165724	0
Fixed:	NO	NO	NO	NO
Low Limit:	197.99	None	.022	0
High Limit:	212.994	None	None	0
Standard Error:	.000516525315	.00159643169	.00398459085	0

Baseline: Linear

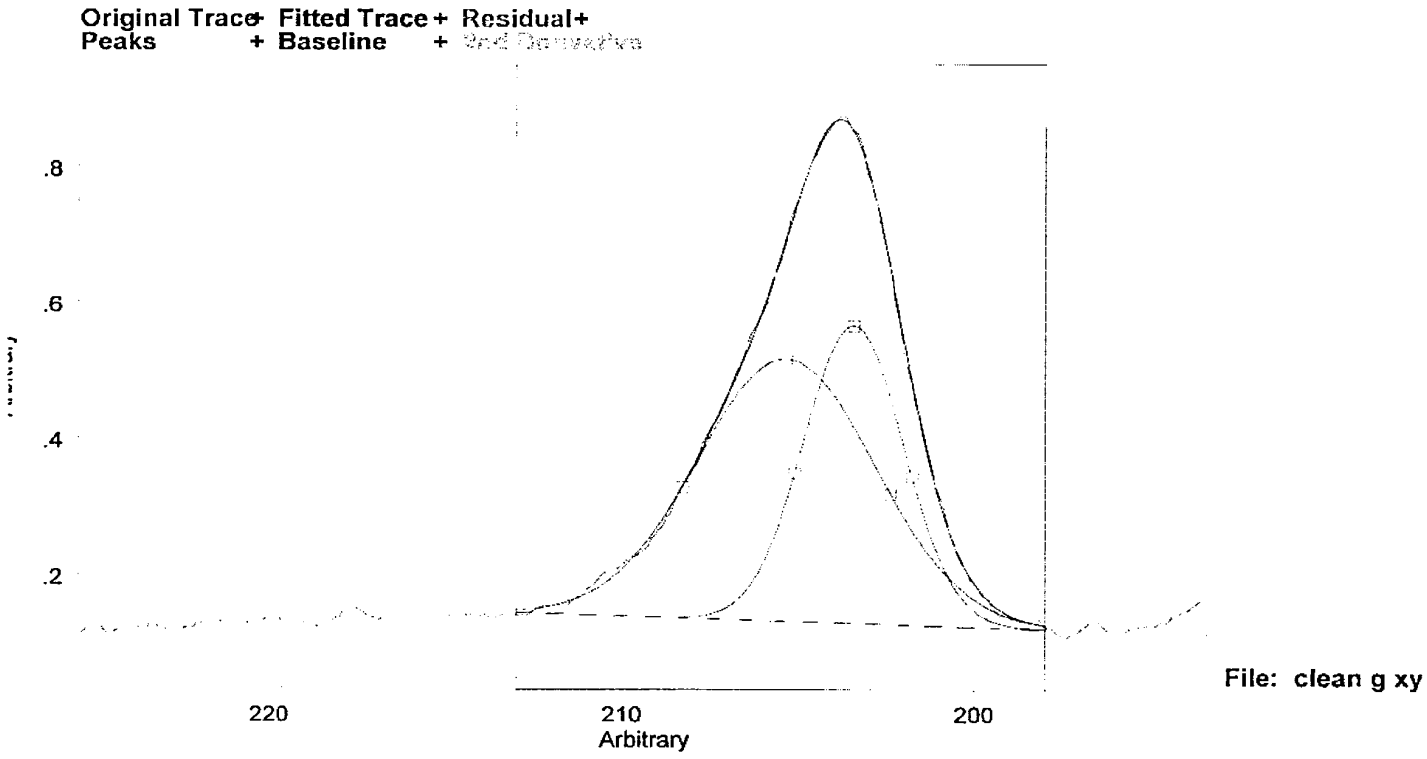
	Offset	Linear
Value:	-.418707444	.00225737564
Fixed:	NO	NO
Low Limit:	-8.58657103	-.0407708964
High Limit:	9.39306812	.0407708964
Standard Error:	.00224009221	.0000107453263

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	683
Fitted Region:	212.994 to 197.99
Fitted Equations:	3
Total Parameters:	10
Fitted Parameters:	8
Fixed Parameters:	2
Reduced Chi ²:	317.7749
RMS Noise:	.00057
Correlation (R^2):	1.033839
Standard Error:	.01010127



ATWOOD 0000377



Munson Exhibit G

FWHH = 1.6

Requested Peaks = 2

ATWOOD 0000378

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 13:53

Peak #1: Gaussian
Area: 2.419912

	Center X	Height	Width
Value:	205.340421	.384561678	5.9285622
Fixed:	NO	NO	NO
Low Limit:	198.007963	None	.0319999847
High Limit:	213.015956	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.555982

	Center X	Height	Width
Value:	203.430258	.437469989	3.34156906
Fixed:	NO	NO	NO
Low Limit:	198.007963	None	.0319999847
High Limit:	213.015956	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

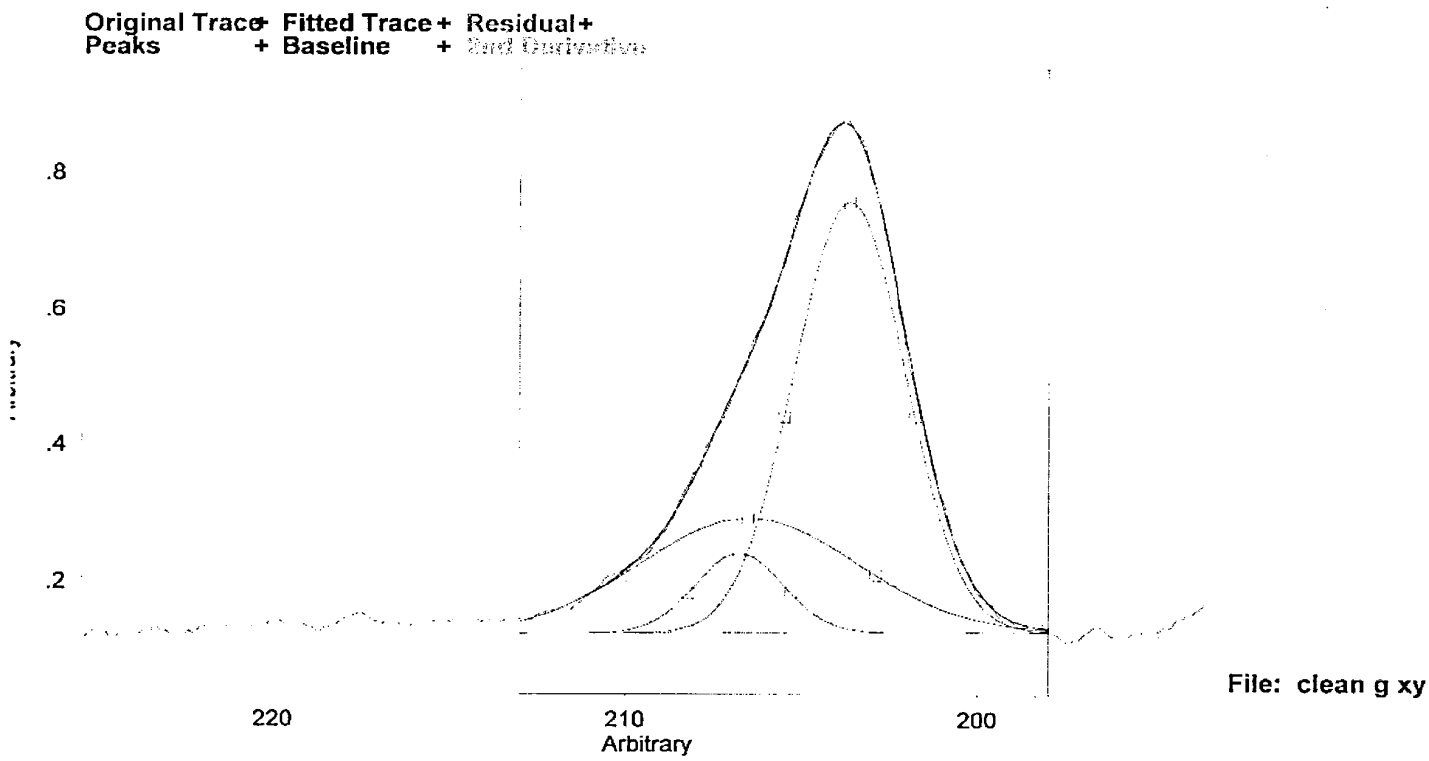
Baseline: BLinear

	Offset	Linear
Value:	-.21427097	.00167892202
Fixed:	NO	NO
Low Limit:	-10.545517	-.0500790979
High Limit:	11.5413636	.0500790979
Standard Error:	.0120330443	.0000572157583

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	5.52221
RMS Noise:	.00242
Correlation (R^2):	.9994788
Standard Error:	.005638244

ATWOOD 0000379



Munson Exhibit G

FWHH = 1.6

Requested Peaks = 3

ATWOOD 0000380

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 13:54

Peak #1: Gaussian
Area: .3642986

	Center X	Height	Width
Value:	206.763658	.116543839	2.93654122
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.267225

	Center X	Height	Width
Value:	206.50685	.168000579	7.22863466
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Peak #3: Gaussian
Area: 2.499542

	Center X	Height	Width
Value:	203.634285	.632487169	3.71320172
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0120330443	.0000572157583	0

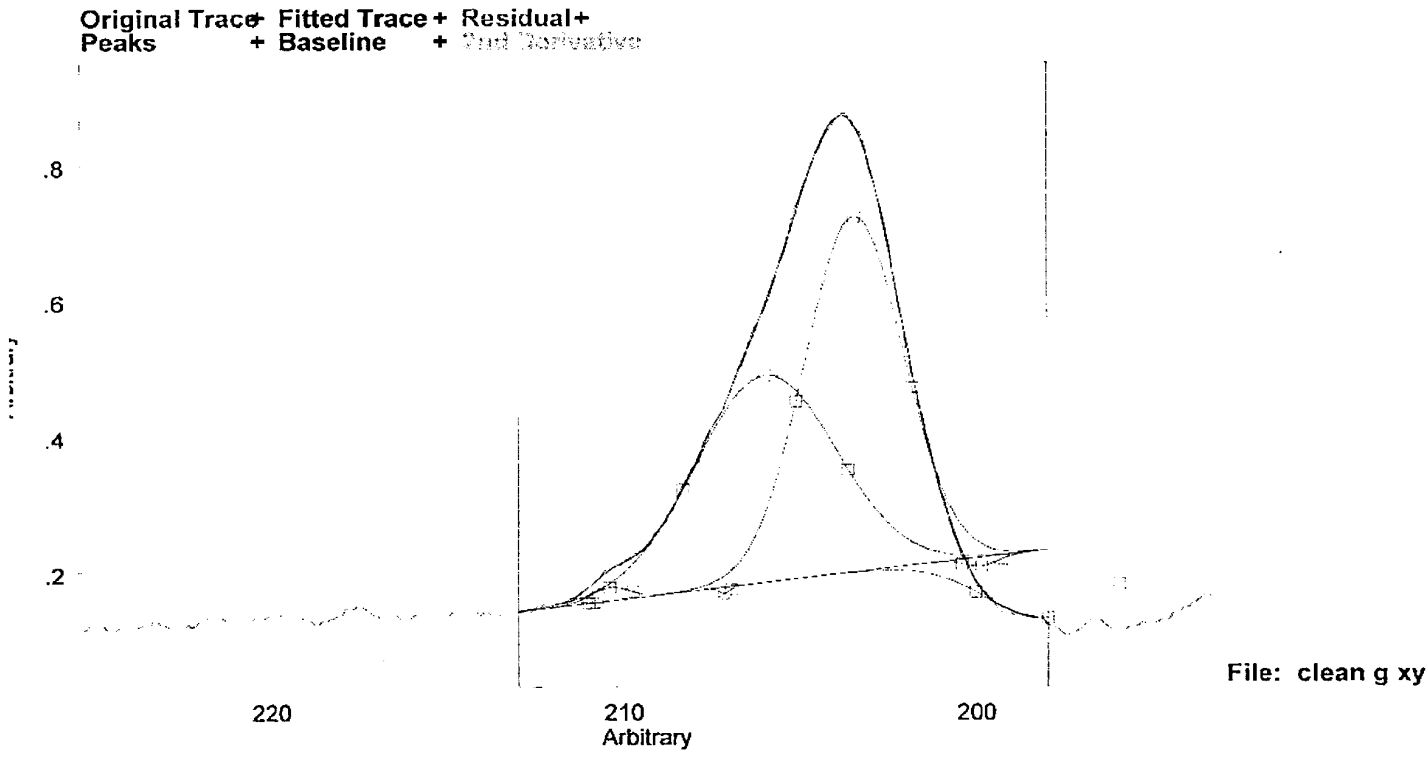
Baseline: BLinear

	Offset	Linear
Value:	.164859881	-.00021625274
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	11
Fixed Parameters:	0
Reduced Chi ²:	3.686064
RMS Noise:	.00242
Correlation (R^2):	.9997641
Standard Error:	.004591496

ATWOOD 0000381



Munson Exhibit G
FWHH = 1.6

Requested Peaks = Max

ATWOOD 0000382

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 13:55

Peak #1: Gaussian
Area: -.00011186

	Center X	Height	Width
Value:	210.902652	-.000452213468	.232380128
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: .02707281

	Center X	Height	Width
Value:	210.425398	.019581003	1.29887339
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Peak #3: Gaussian
Area: 0 -.003789781

	Center X	Height	Width
Value:	207.148882	-.00904207213	.393743955
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0120330443	.0000572157583	0

Peak #4: Gaussian
Area: 1.523332

	Center X	Height	Width
Value:	205.9858	.304493078	4.70100903
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: 1.837288

	Center X	Height	Width
Value:	203.463869	.522773162	3.30180454
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: -.01583131

	Center X	Height	Width
Value:	199.865344	-.0132804267	1.11992093
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #7: EGaussian
Area: -.2224198

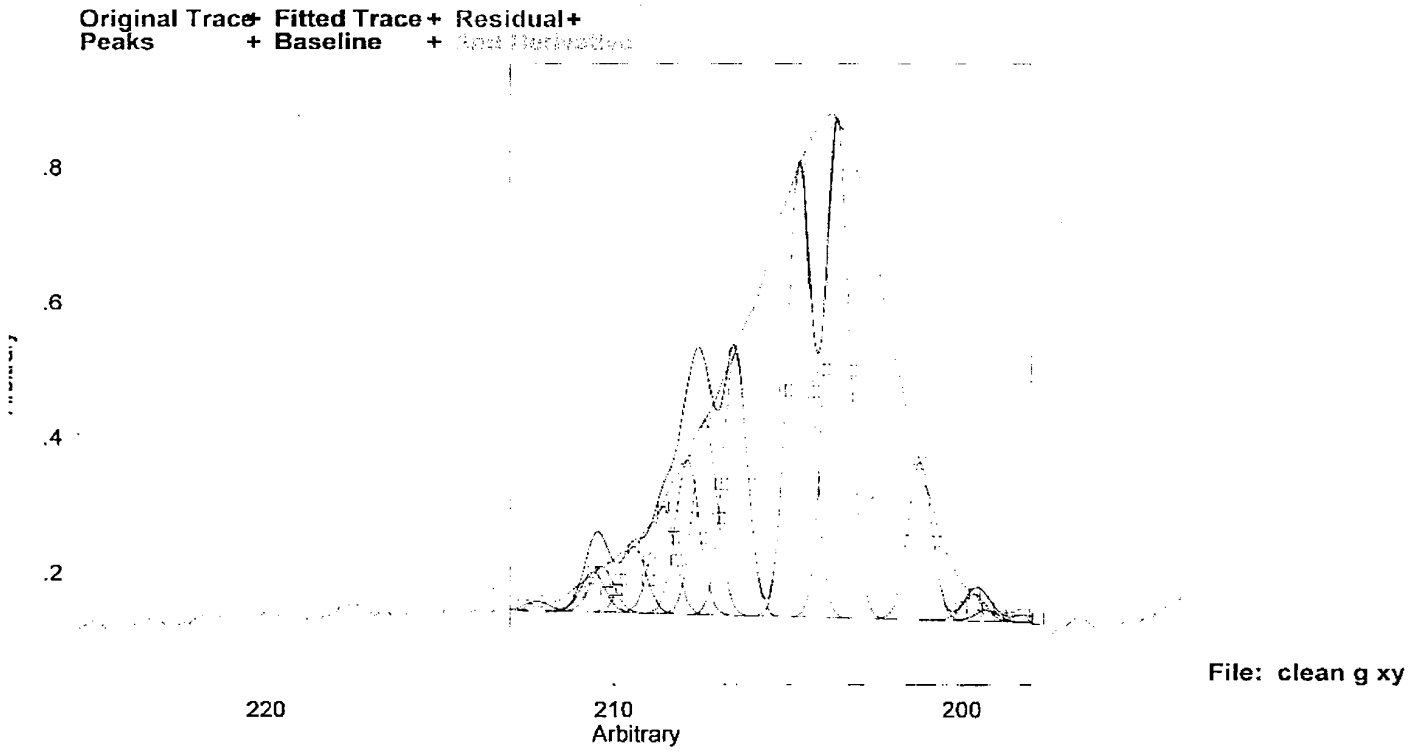
	Center X	Height	Width
Value:	198.008	-.101374549	4.06220083
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	198.008	None	None
Standard Error:	0	0	0

Baseline: ELinear

	Offset	Linear
Value:	1.43826939	-.00609217591
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	0	0

Statistics Summary:
Solution has CONVERGED

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	8
Total Parameters:	23
Fitted Parameters:	22
Fixed Parameters:	1
Reduced Chi ^{2}:	2.524301
RMS Noise:	.00242
Correlation (R^2):	.9967392
Standard Error:	.003753843



Munson Exhibit G

FWHH = 0.8

Requested Peaks = Max

ATWOOD 0000385

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 13:56

Peak #1: Gaussian
Area: .01136716

	Center X	Height	Width
Value:	212.247957	.0135097125	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: .05062933

	Center X	Height	Width
Value:	210.615957	.0594538513	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Peak #3: Gaussian
Area: .05710061

	Center X	Height	Width
Value:	210.359958	.0670530563	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0120330443	.0000572157583	0

Peak #4: Gaussian
Area: .08320304

	Center X	Height	Width
Value:	209.431958	.0977050455	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: .1349576

	Center X	Height	Width
Value:	208.599958	.15848024	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: .193511

	Center X	Height	Width
Value:	207.895959	.227239291	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

ATWOOD 0000386

Peak #7:	Gaussian		
Area:	.2442149		
	Center X	Height	Width
Value:	207.383959	.286780756	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #8:	Gaussian		
Area:	.3308527		
	Center X	Height	Width
Value:	206.519959	.388519207	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #9:	Gaussian		
Area:	.5710178		
	Center X	Height	Width
Value:	204.66396	.670544222	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #10:	Gaussian		
Area:	.6272901		
	Center X	Height	Width
Value:	203.575961	.736624663	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #11:	Gaussian		
Area:	.1981129		
	Center X	Height	Width
Value:	201.207962	.232643287	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #12:	Gaussian		
Area: :	.03351613		
	Center X	Height	Width
Value:	199.639963	.0393578915	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #13:	Gaussian		
Area: :	.01346094		
	Center X	Height	Width
Value:	199.287963	.0158079607	.8

Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Peak #14: Gaussian
Area: : .007241248

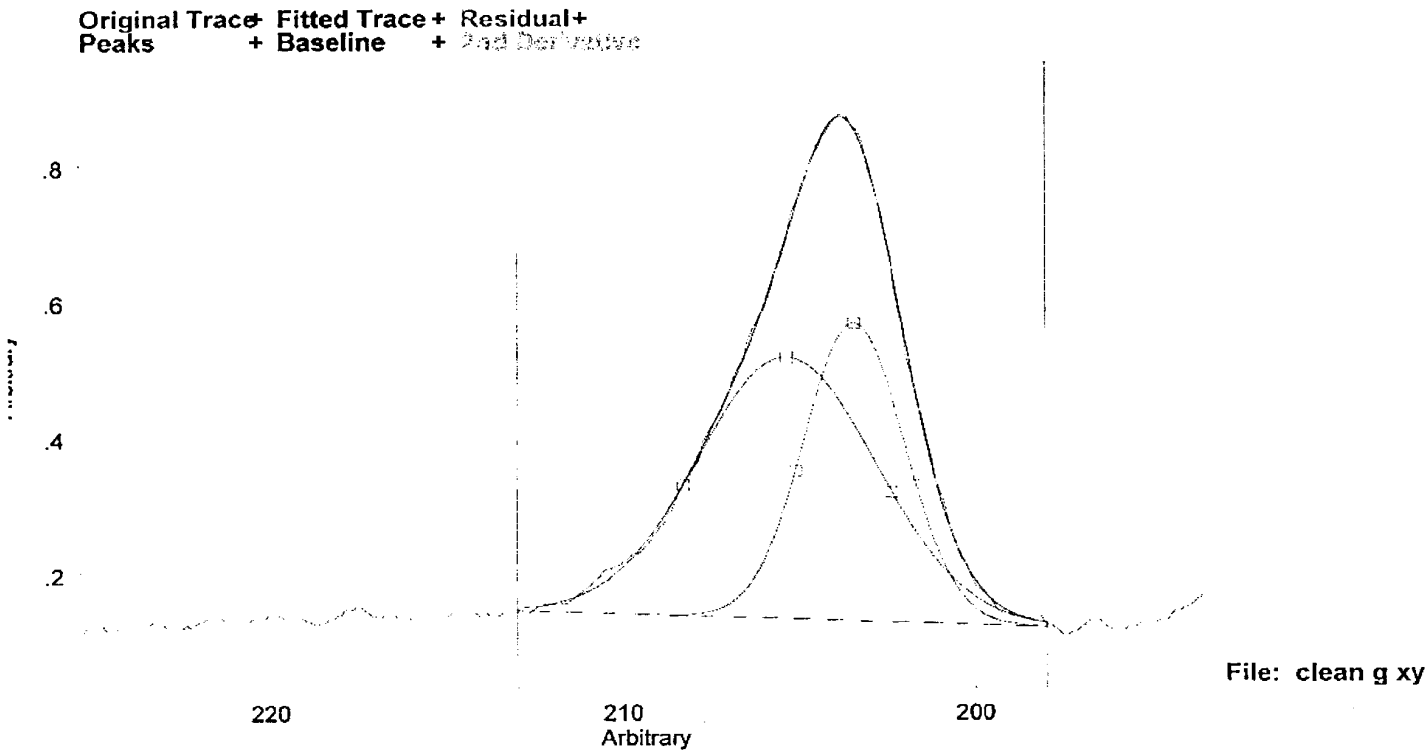
	Center X	Height	Width
Value:	198.231963	.0109691019	.8
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	0	0	0

Baseline: Linear

	Offset	Linear
Value:	-.108531784	.00116491128
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	15
Total Parameters:	23
Fitted Parameters:	22
Fixed Parameters:	1
Reduced Chi ²:	8090.002
RMS Noise:	.00242
Correlation (R^2):	.7348678
Standard Error:	.2032978



Munson Exhibit G

FWHH = 0.8

Requested Peaks = 2

Peak Report
Original File: clean g xy datsa.spc

Date: 10/29/2007 14:00

Peak #1: Gaussian
Area: 2.419913

	Center X	Height	Width
Value:	205.340461	.384561663	5.9285649
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.555983

	Center X	Height	Width
Value:	203.430297	.437470012	3.34157072
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

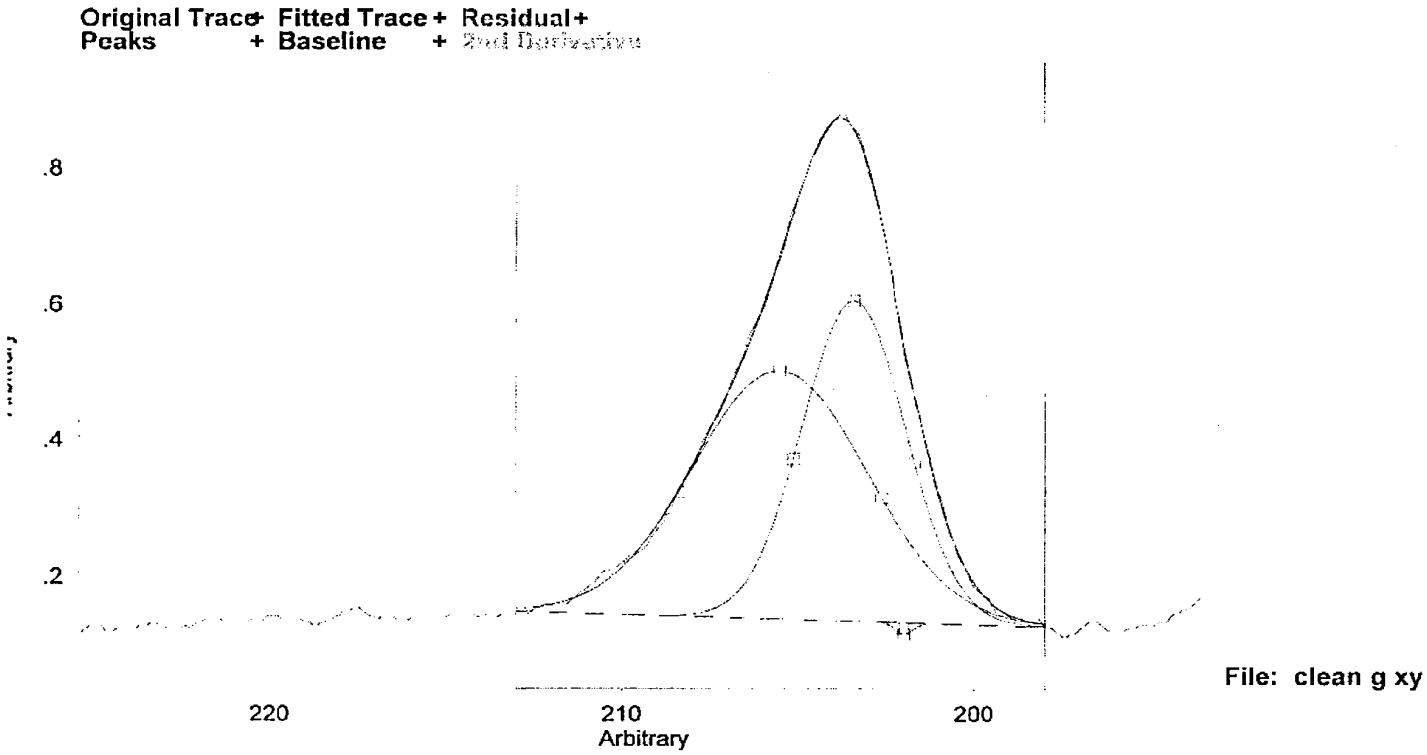
Baseline: Linear

	Offset	Linear
Value:	-.214270812	.00167892093
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	.0120330443	.0000572157583

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ^{2}:	5.52221
RMS Noise:	.00242
Correlation (R^2):	.9994788
Standard Error:	.005638244

ATWOOD 0000390



Munson Exhibit G

FWHH = 0.8

Requested Peaks = 3

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 14:00

Peak #1: Gaussian
Area: 2.233216

	Center X	Height	Width
Value:	205.514124	.363793053	5.77954795
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.729172

	Center X	Height	Width
Value:	203.40718	.470580965	3.45237155
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Peak #3: Gaussian
Area: -.01133273

	Center X	Height	Width
Value:	201.980639	-.0173537568	.613492126
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0120330443	.0000572157583	0

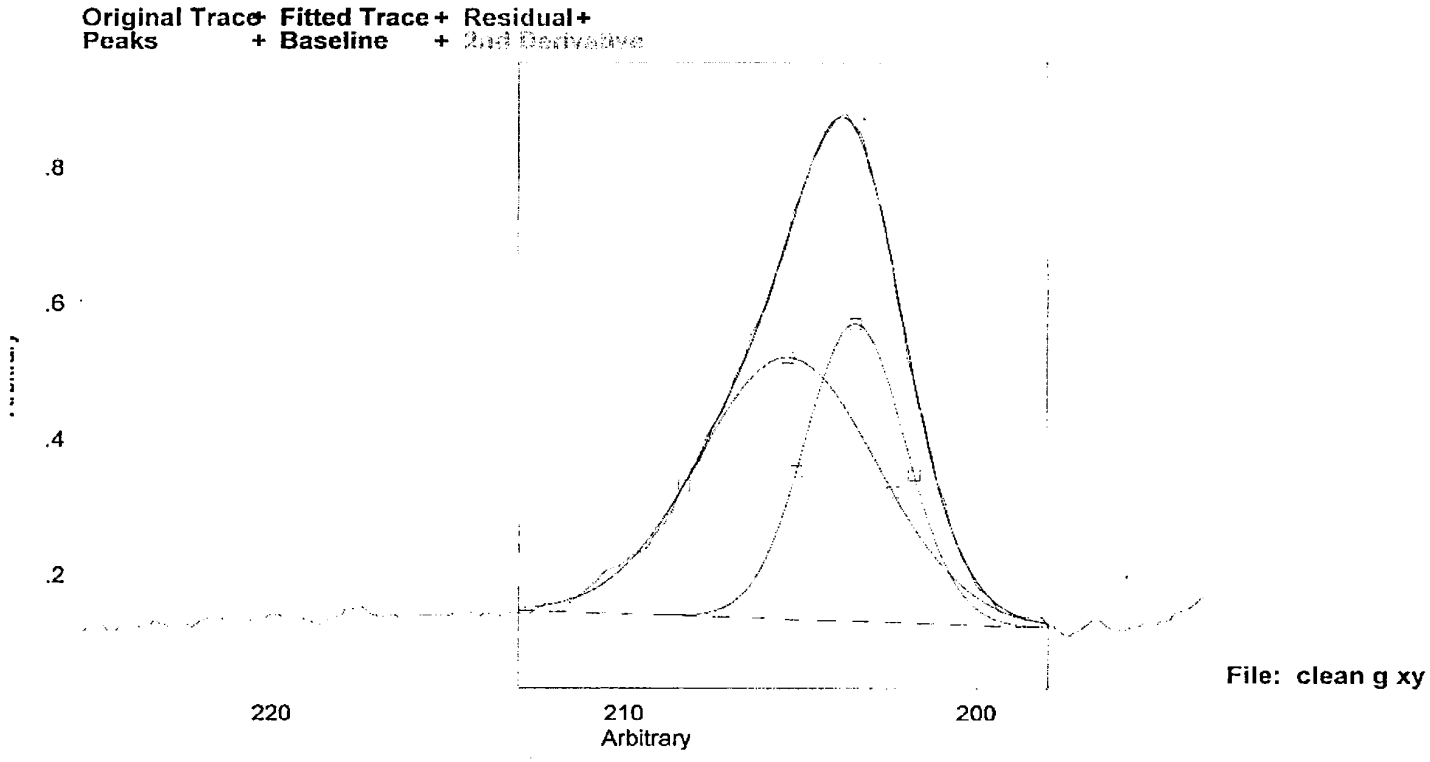
Baseline: -Linear

	Offset	Linear
Value:	-.177560135	.0015083226
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	11
Fixed Parameters:	0
Reduced Chi ²:	4.804243
RMS Noise:	.00242
Correlation (R^2):	.9995461
Standard Error:	.005241858

ATWOOD 0000392



Munson Exhibit G

FWHH = 3.0

Requested Peaks = 3 (2 found)

ATWOOD 0000393

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 14:03

Peak #1: Gaussian
Area: 2.419912

	Center X	Height	Width
Value:	205.340462	.38456158	5.92856427
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.555984

	Center X	Height	Width
Value:	203.430297	.437470132	3.34157104
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

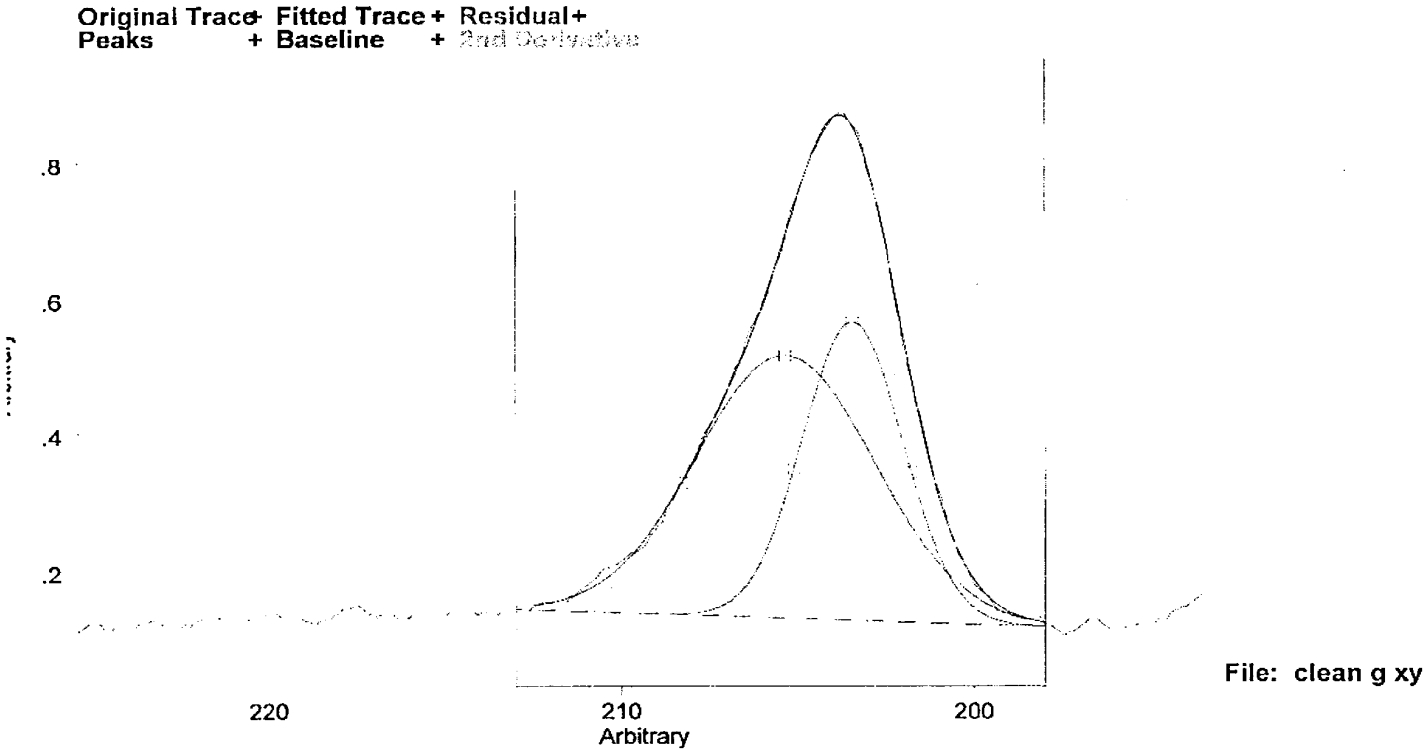
Baseline: Linear

	Offset	Linear
Value:	-.214270493	.00167891944
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	.0120330443	.0000572157583

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	5.52221
RMS Noise:	.00242
Correlation (R^2):	.9994788
Standard Error:	.005638244

ATWOOD 0000394



Munson Exhibit G

FWHH = 3.0

Requested Peaks = Max (2 found)

ATWOOD 0000395

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 14:04

Peak #1: Gaussian
Area: 2.419912

	Center X	Height	Width
Value:	205.340462	.38456158	5.92856427
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.555984

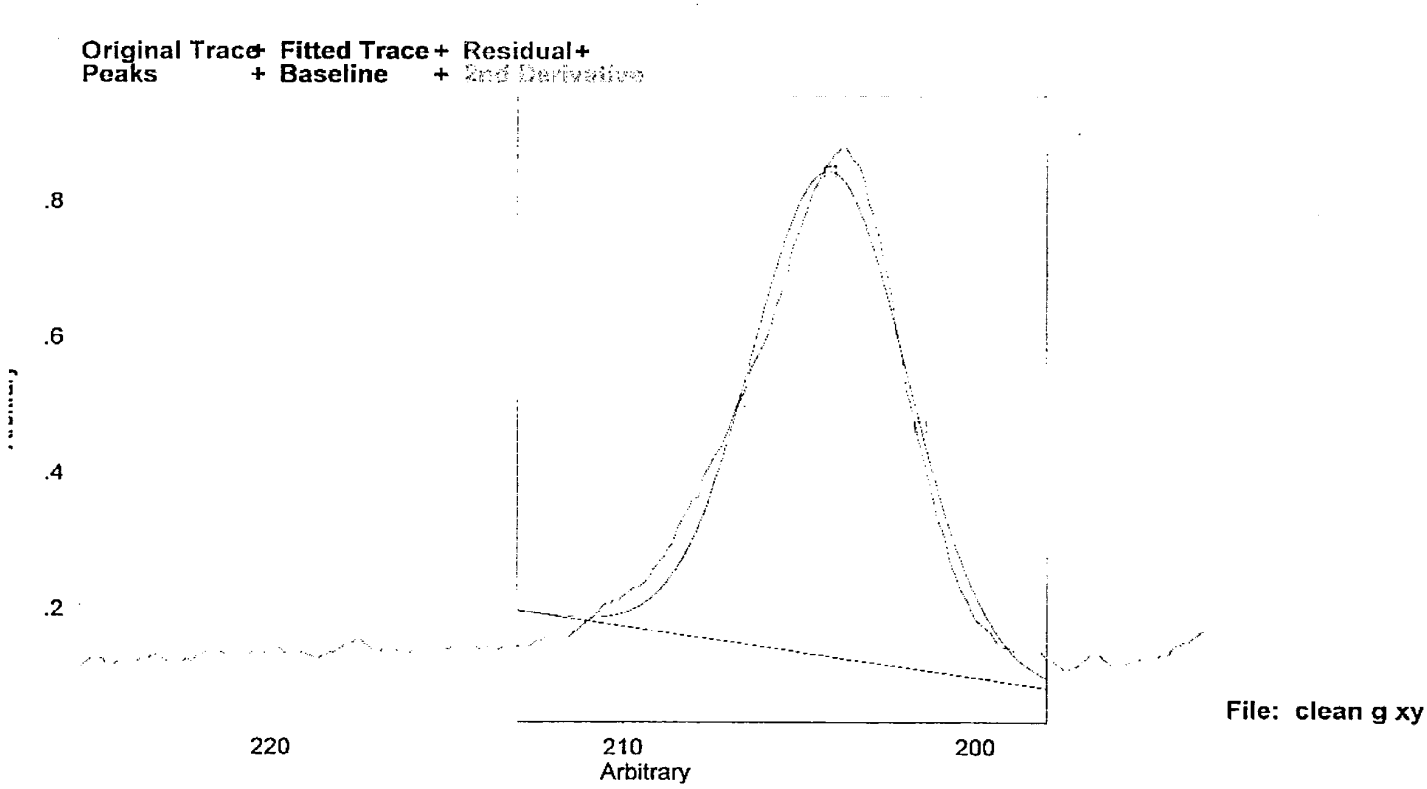
	Center X	Height	Width
Value:	203.430297	.437470132	3.34157104
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Baseline: Linear

	Offset	Linear
Value:	-.214270493	.00167891944
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	.0120330443	.0000572157583

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	5.52221
RMS Noise:	.00242
Correlation (R^2):	.9994788
Standard Error:	.005638244



Munson Exhibit G

FWHH = 5.0

Requested Peaks = Max (1 found)

ATWOOD 0000397

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 14:06

Peak #1: Gaussian
Area: 3.899349

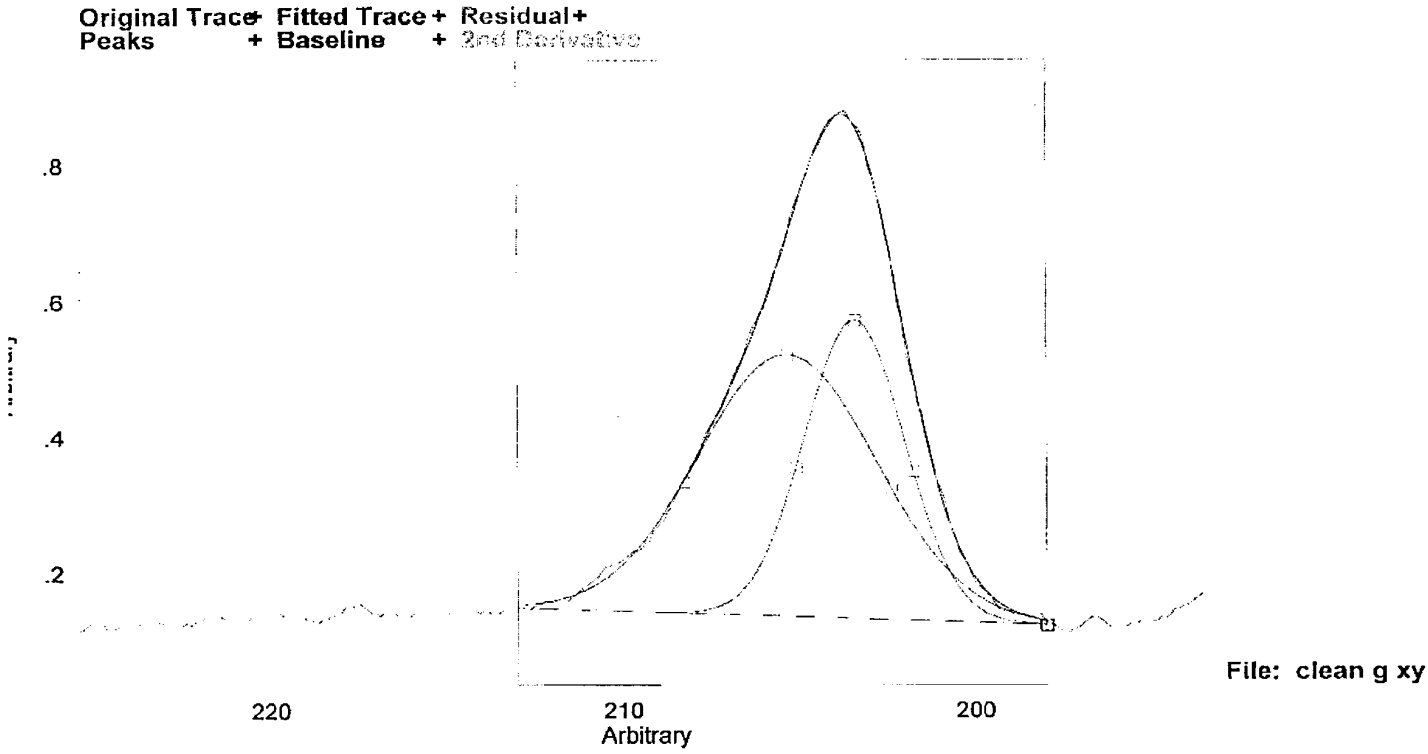
	Center X	Height	Width
Value:	204.133897	.713763049	5.14474959
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Baseline: Linear

	Offset	Linear
Value:	-1.41731835	.00755765083
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	.00297024171	.00406108421

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	2
Total Parameters:	5
Fitted Parameters:	5
Fixed Parameters:	0
Reduced Chi ²:	223.2172
RMS Noise:	.00242
Correlation (R^2):	.9787947
Standard Error:	.03596307



Munson Exhibit G

FWHH = 0.6

Requested Peaks = 3 (2 found)

ATWOOD 0000399

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 14:11

Peak #1: Gaussian
Area: 2.415892

	Center X	Height	Width
Value:	205.34381	.384129731	5.92526329
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.558751

	Center X	Height	Width
Value:	203.430133	.438054419	3.34305046
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Peak #3: Gaussian
Area: -.00009953689

	Center X	Height	Width
Value:	198.008	-.00199071841	.032
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	198.008	None	.032
Standard Error:	.0120330443	.0000572157583	0

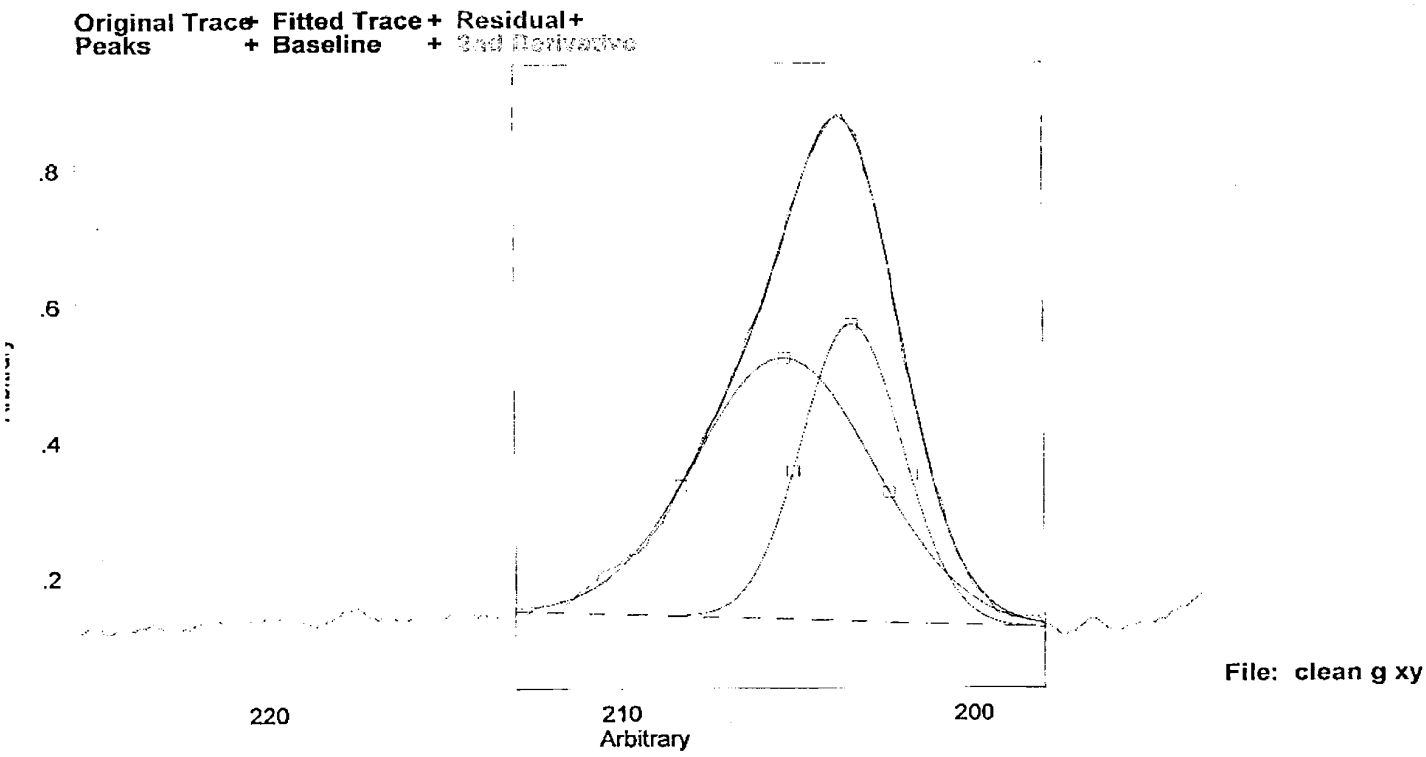
Baseline: Linear

	Offset	Linear
Value:	-.211883986	.00166774226
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	9
Fixed Parameters:	2
Reduced Chi ^{2}:	5.532769
RMS Noise:	.00242
Correlation (R^2):	.9994641
Standard Error:	.005637521

ATWOOD 0000400



Munson Exhibit G

FWHH = 0.6

Requested Peaks = 2

Peak Report

Original File: clean g xy datsa.spc

Date: 10/29/2007 14:13

Peak #1: Gaussian
Area: 2.419913

	Center X	Height	Width
Value:	205.340461	.384561627	5.92856462
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.0221763895	.00298931301	.0205450574

Peak #2: Gaussian
Area: 1.555984

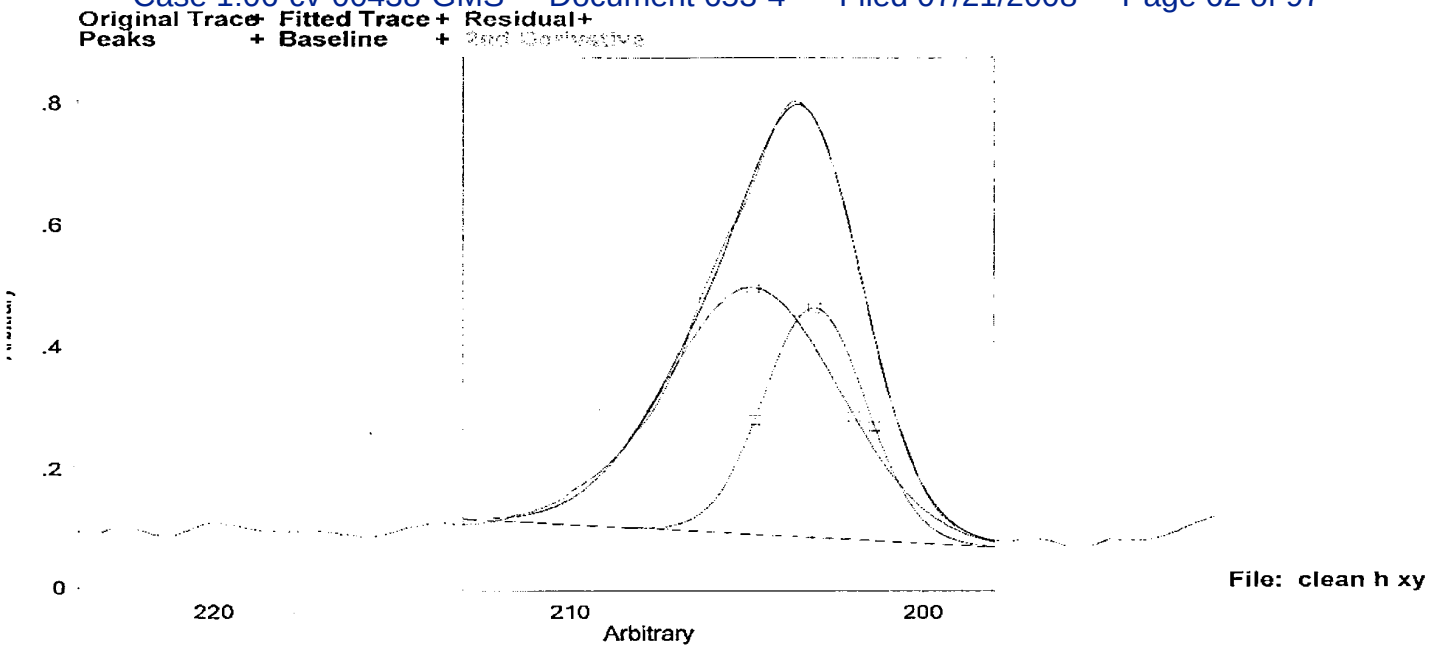
	Center X	Height	Width
Value:	203.430297	.437470065	3.34157085
Fixed:	NO	NO	NO
Low Limit:	198.008	None	.032
High Limit:	213.016	None	None
Standard Error:	.00297024171	.00406108421	.0130250234

Baseline: Linear

	Offset	Linear
Value:	-.214270672	.00167892028
Fixed:	NO	NO
Low Limit:	-10.5455141	-.050079074
High Limit:	11.5413607	.050079074
Standard Error:	.0120330443	.0000572157583

Statistics Summary:
Solution AT A MINIMUM

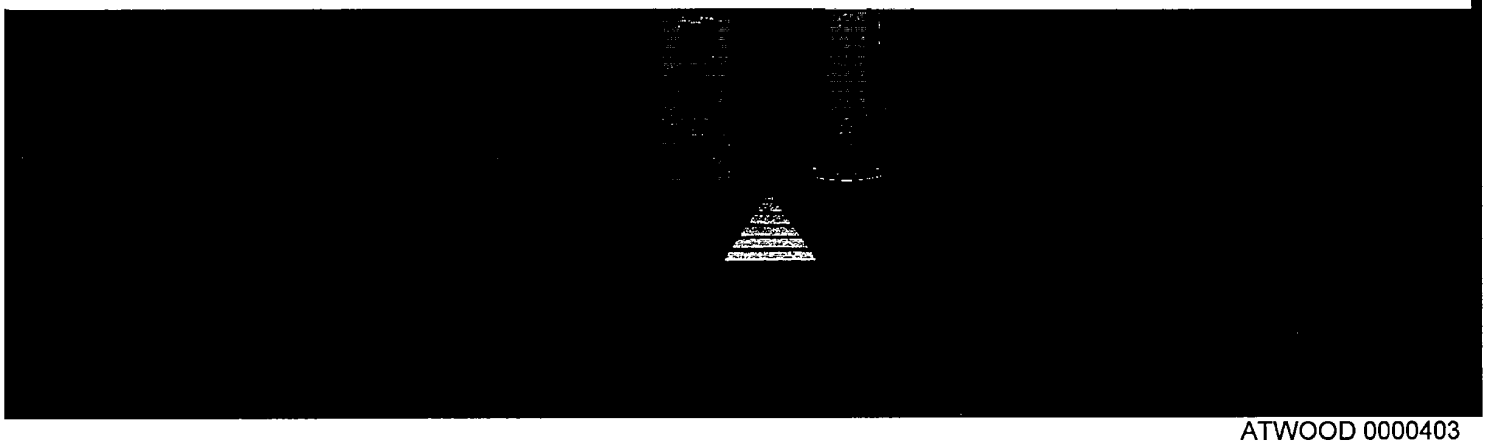
Total Data Points:	470
Fitted Region:	213.016 to 198.008
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ^{2}:	5.52221
RMS Noise:	.00242
Correlation (R^2):	.9994788
Standard Error:	.005638244



Munson Exhibit H

FWHH = 1.6

Requested Peaks = 2



ATWOOD 0000403

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:25

Peak #1: Gaussian
Area: 2.463458

	Center X	Height	Width
Value:	204.840221	.404341384	5.73967662
Fixed:	NO	NO	NO
Low Limit:	198.001961	None	.032
High Limit:	213.009961	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: 1.358395

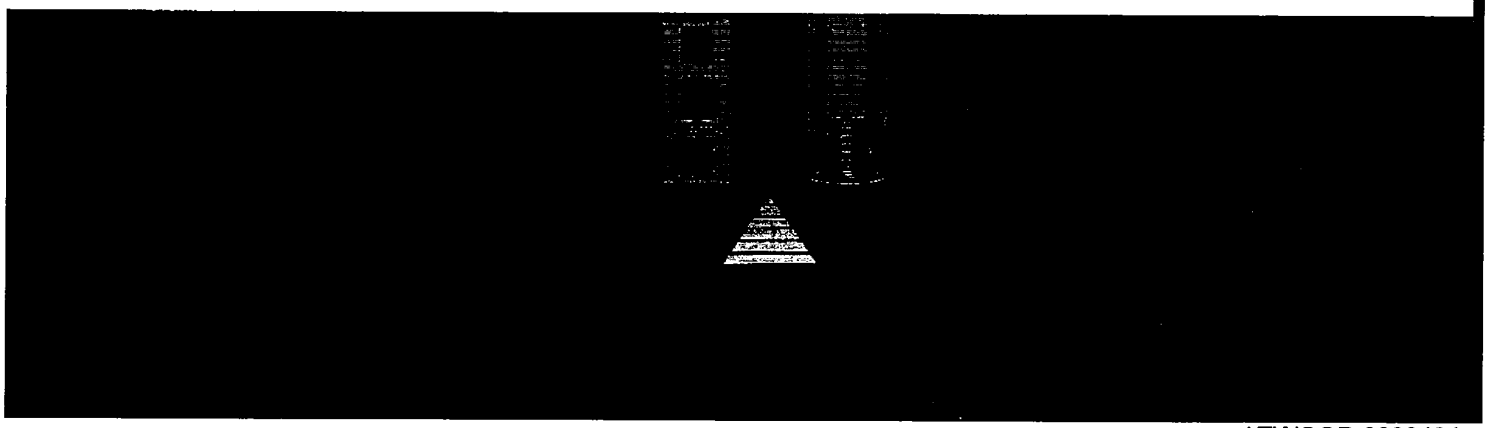
	Center X	Height	Width
Value:	203.092193	.376654639	3.38868253
Fixed:	NO	NO	NO
Low Limit:	198.001961	None	.032
High Limit:	213.009961	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Baseline: Linear

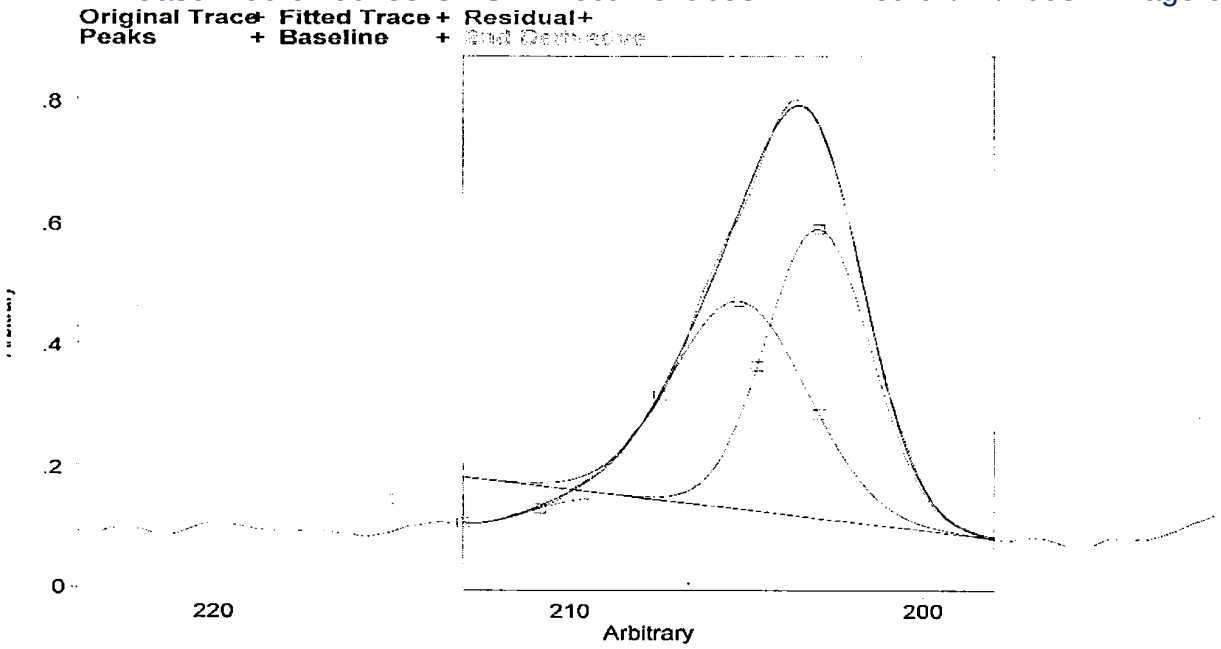
	Offset	Linear
Value:	-.531468362	.00302365451
Fixed:	NO	NO
Low Limit:	-10.1399296	-.0479685437
High Limit:	11.0155375	.0479685437
Standard Error:	.00945502897	.0000447488271

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	22.42578
RMS Noise:	.00154
Correlation (R^2):	.9990779
Standard Error:	.00723047



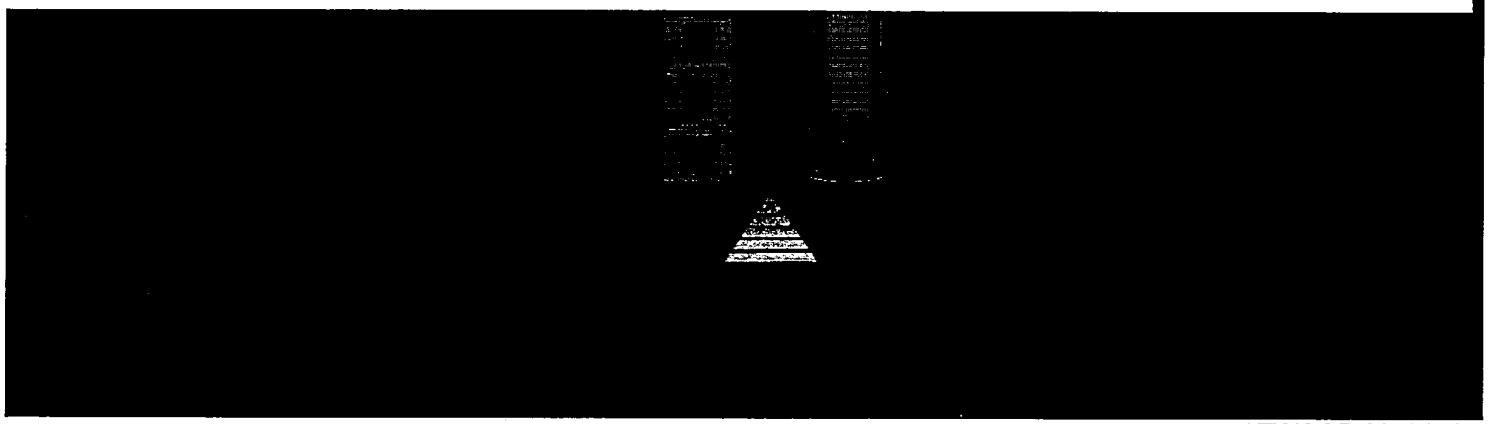
ATWOOD 0000404



Munson Exhibit H

FWHH = 1.6

Requested Peaks = 3



ATWOOD 0000405

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:27

Peak #1: .Gaussian
Area: R -.1684027

	Center X	Height	Width
Value:	213.01	-.0739618445	4.27798341
Fixed:	NO	NO	NO
Low Limit:	213.01	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: .Gaussian
Area: 1.63793

	Center X	Height	Width
Value:	205.222266	.341407961	4.50746826
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: .Gaussian
Area: 1.754239

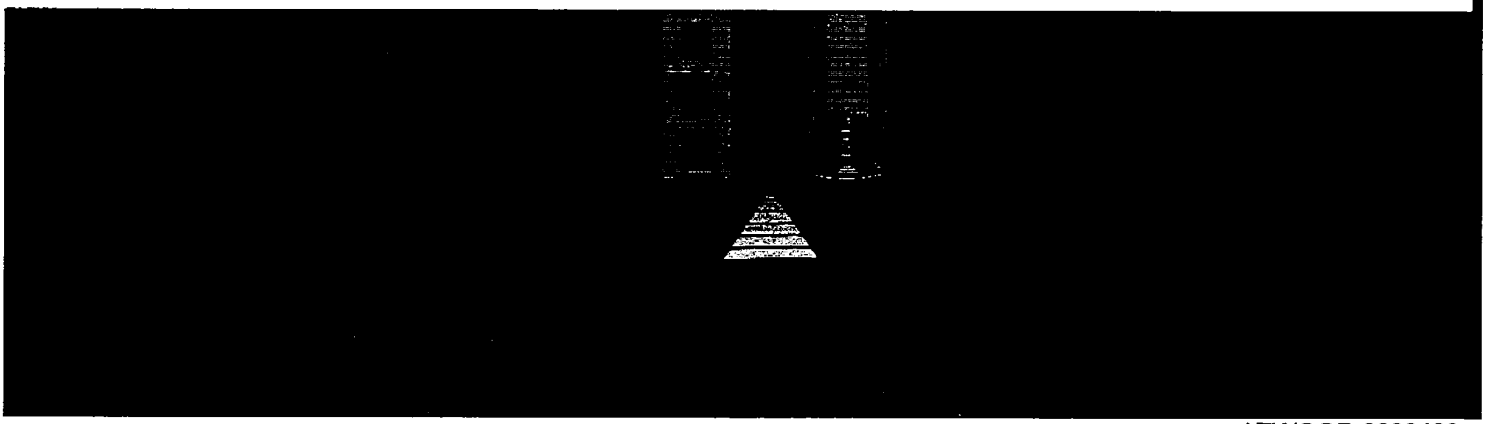
	Center X	Height	Width
Value:	202.977695	.475167012	3.46941939
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Baseline: .Linear

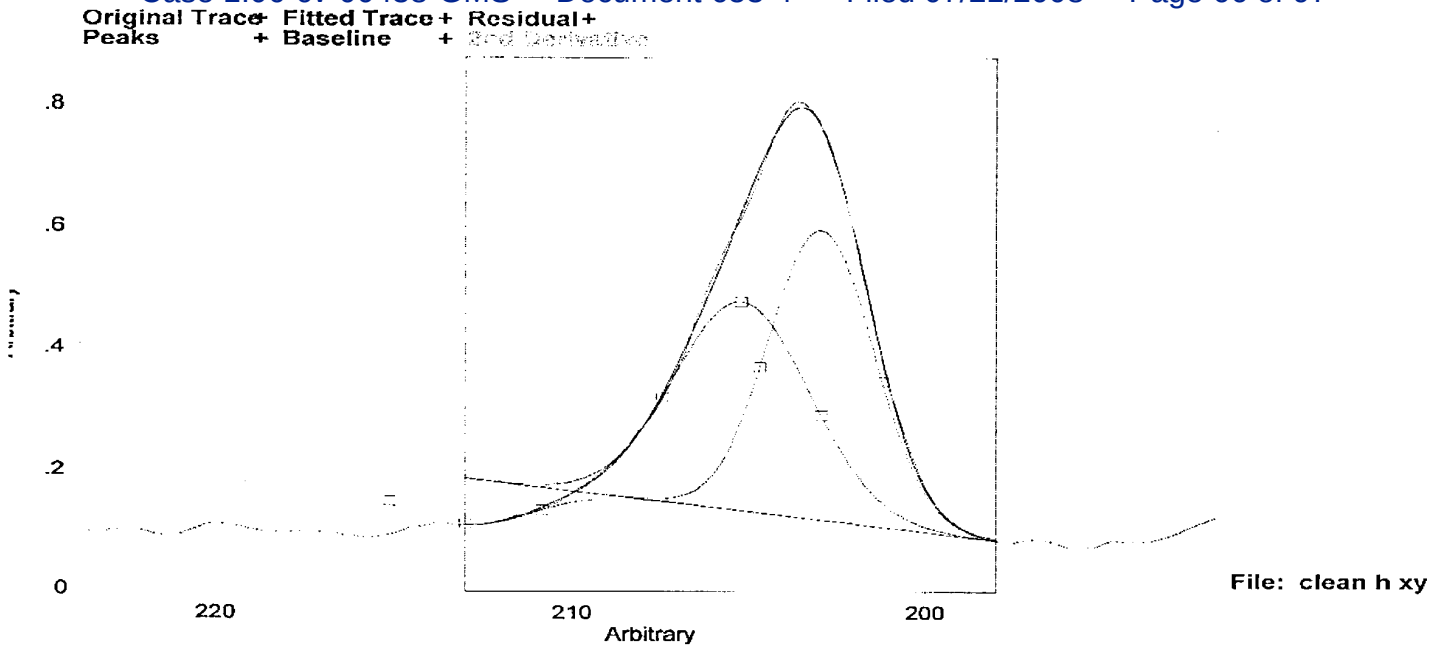
	Offset	Linear
Value:	-1.25164633	.00672189706
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	10
Fixed Parameters:	1
Reduced Chi ²:	13.44056
RMS Noise:	.00154
Correlation (R^2):	.9980408
Standard Error:	.005585466



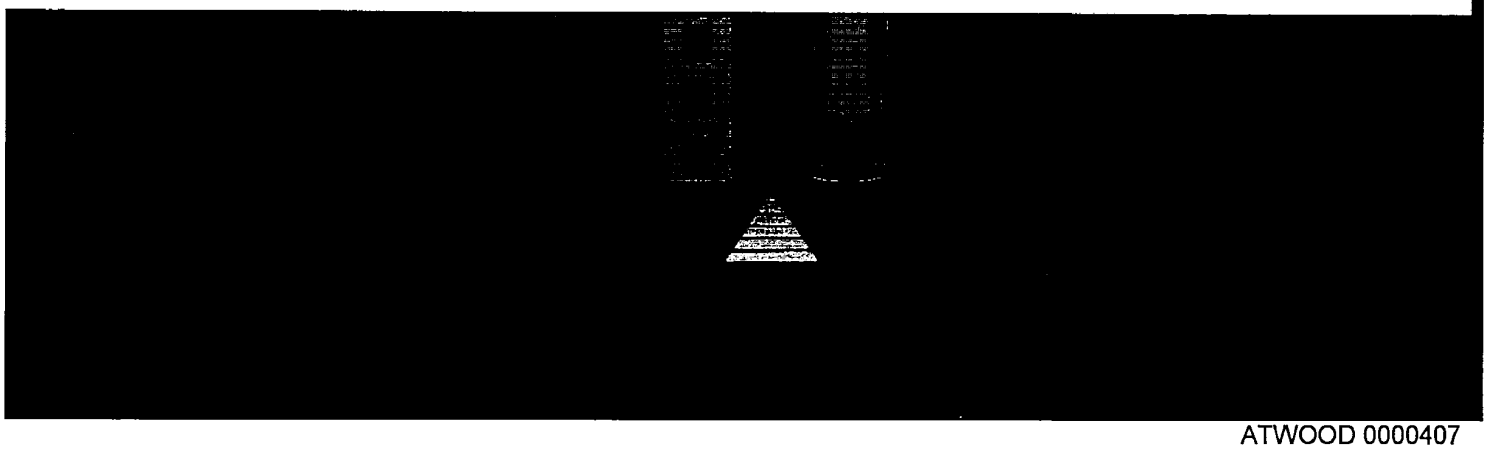
ATWOOD 0000406



Munson Exhibit H

FWHH = 1.6

Requested Peaks = Max



ATWOOD 0000407

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:28

Peak #1: Gaussian
Area: -.1705313

	Center X	Height	Width
Value:	213.01	-.0750128737	4.27135832
Fixed:	NO	NO	NO
Low Limit:	213.01	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: 1.641141

	Center X	Height	Width
Value:	205.227597	.341902538	4.50977212
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: Gaussian
Area: 1.753901

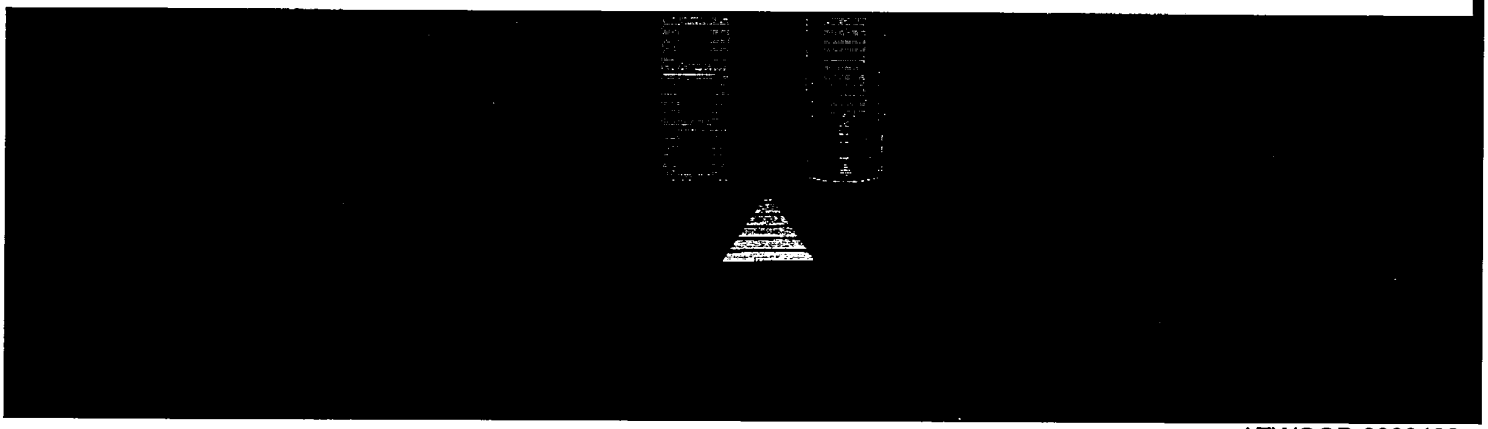
	Center X	Height	Width
Value:	202.976225	.475451436	3.46666918
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Baseline: Linear

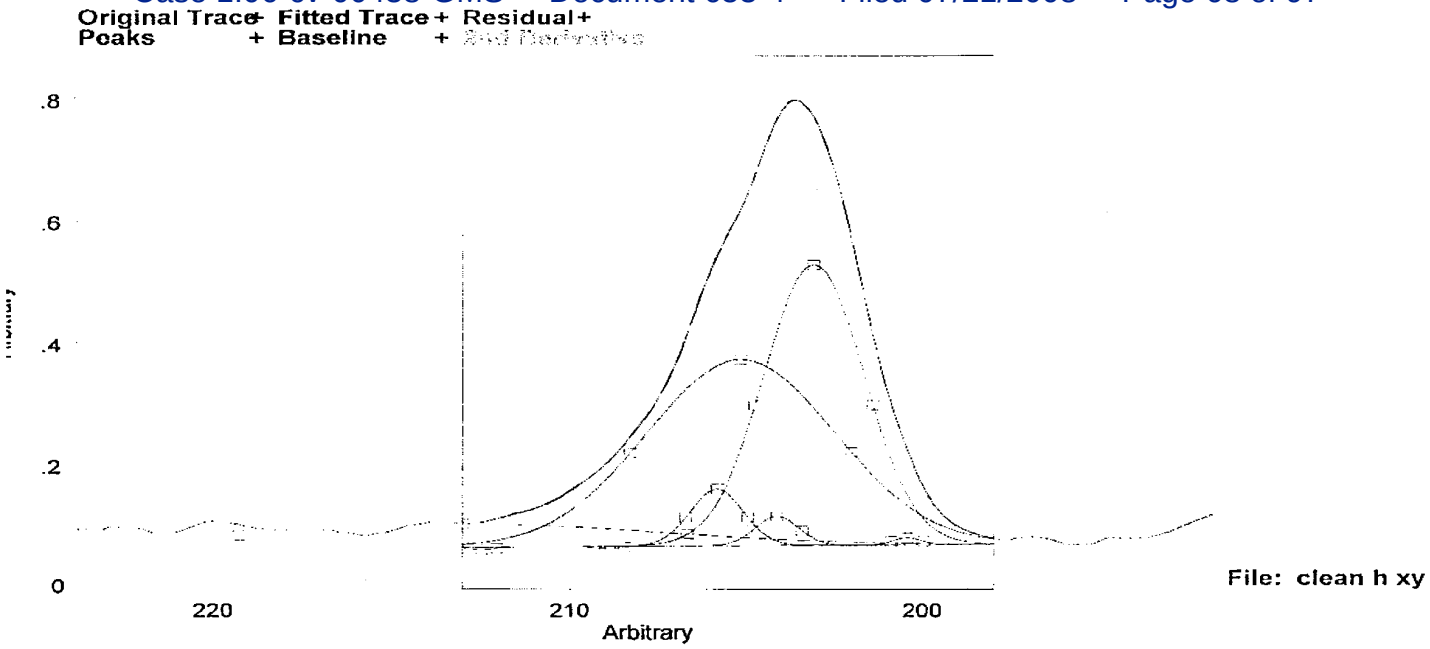
	Offset	Linear
Value:	-1.25331789	.00673003776
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	10
Fixed Parameters:	1
Reduced Chi ²:	13.32228
RMS Noise:	.00154
Correlation (R^2):	.9989624
Standard Error:	.005560835



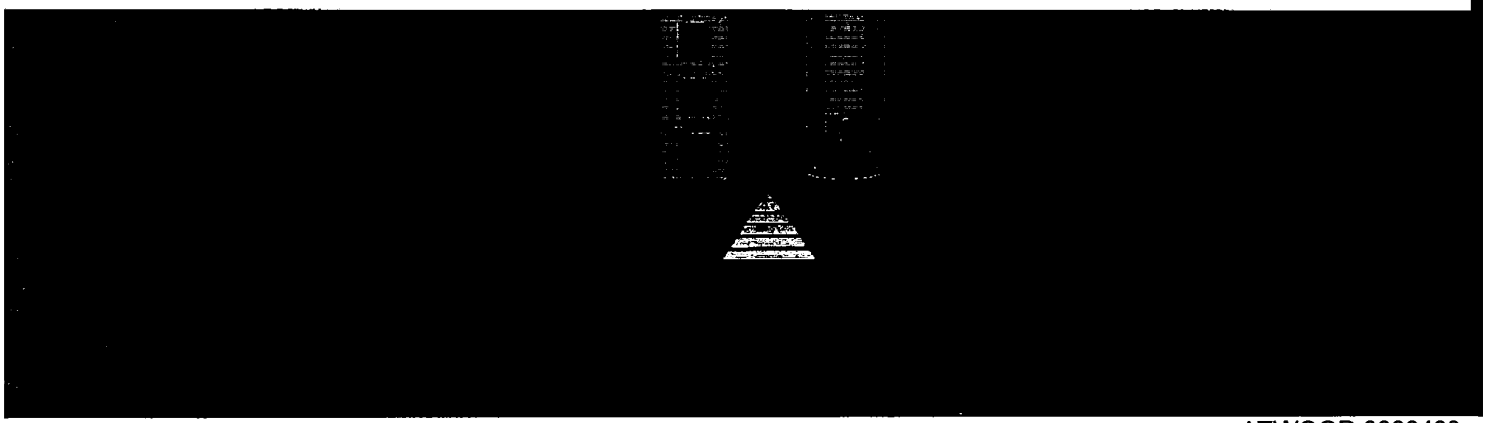
ATWOOD 0000408



Munson Exhibit H

FWHH = 0.8

Requested Peaks = Max



ATWOOD 0000409

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:31

Peak #1: Gaussian
Area: .253051

	Center X	Height	Width
Value:	212.998418	.0378316749	12.6088849
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: 0 -.003179534

	Center X	Height	Width
Value:	212.477158	-.00381231571	.840566546
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: Gaussian
Area: 0 -.002990352

	Center X	Height	Width
Value:	208.882361	-.00329704896	.852049145
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Peak #4: Gaussian
Area: .1741342

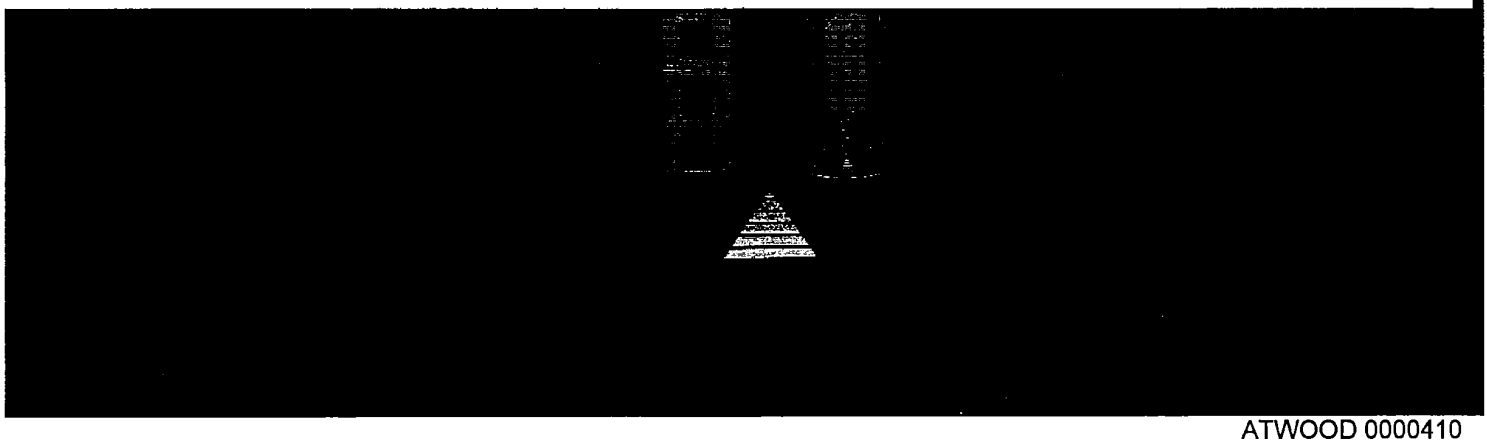
	Center X	Height	Width
Value:	205.84591	.0925963235	1.76668128
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: 2.02872

	Center X	Height	Width
Value:	205.163114	.305611074	6.26779252
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: .07203122

	Center X	Height	Width
Value:	204.133495	.0474546032	1.4259694
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0



ATWOOD 0000410

Peak #7: Gaussian
Area: 1.641824

	Center X	Height	Width
Value:	203.085273	.460885351	3.34711629
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #8: Gaussian
Area: .01095139

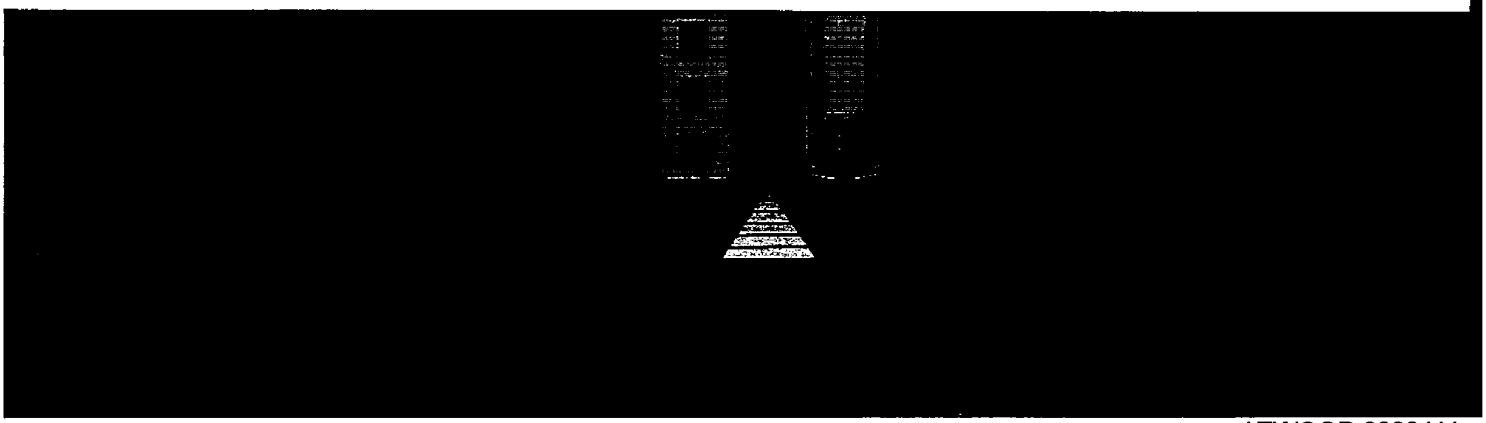
	Center X	Height	Width
Value:	200.445612	.0111669956	.921299009
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Baseline: Linear

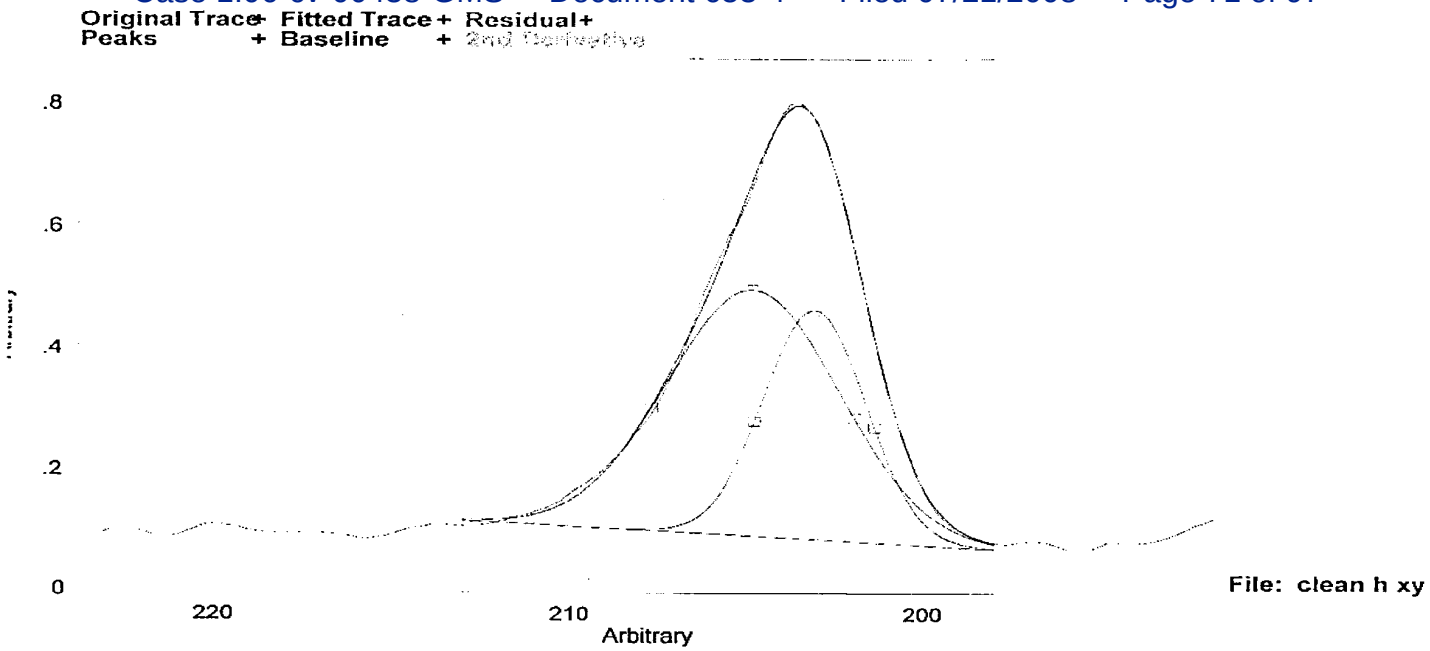
	Offset	Linear
Value:	.134227013	-.000329949198
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution has CONVERGED

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	9
Total Parameters:	26
Fitted Parameters:	26
Fixed Parameters:	0
Reduced Chi ²:	.4828785
RMS Noise:	.00154
Correlation (R^2):	.9999831
Standard Error:	.001040117



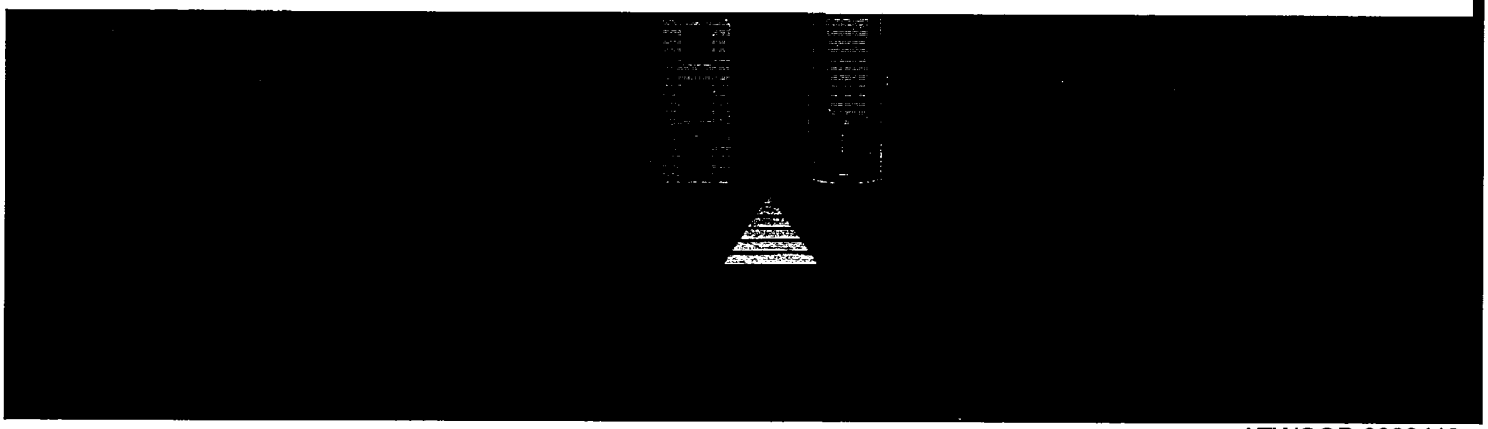
ATWOOD 0000411



Munson Exhibit H

FWHH = 0.8

Requested Peaks = 2



ATWOOD 0000412

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:32

Peak #1: Gaussian
Area: 2.463451

	Center X	Height	Width
Value:	204.840227	.404340484	5.73967325
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: 7 1.3584

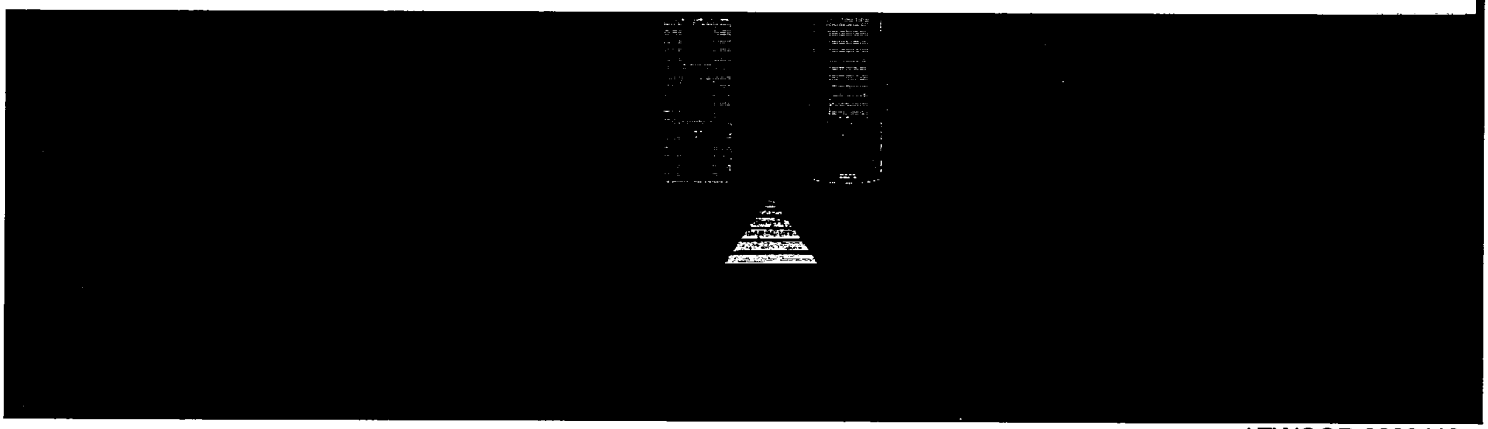
	Center X	Height	Width
Value:	203.092193	.376655804	3.38868642
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Baseline: Linear

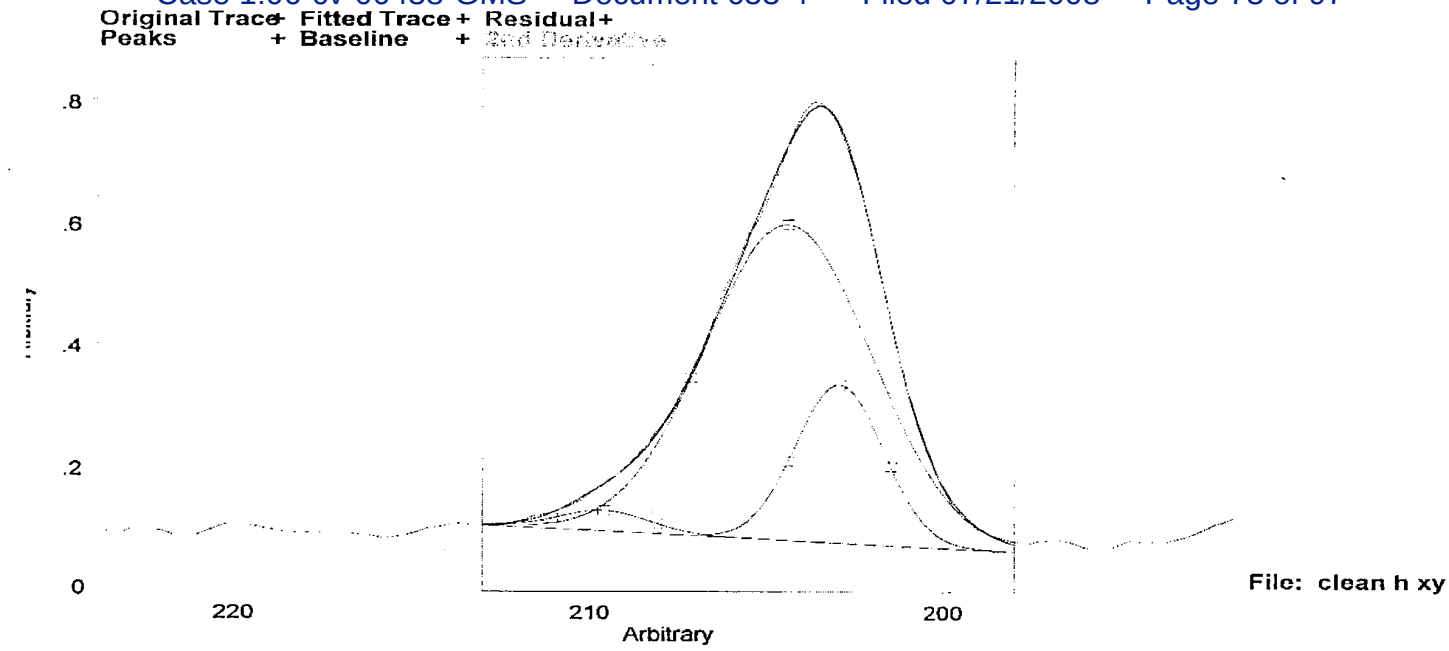
	Offset	Linear
Value:	-.531465671	.00302364178
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	.00945502897	.0000447488271

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	22.42578
RMS Noise:	.00154
Correlation (R^2):	.9990779
Standard Error:	.00723047



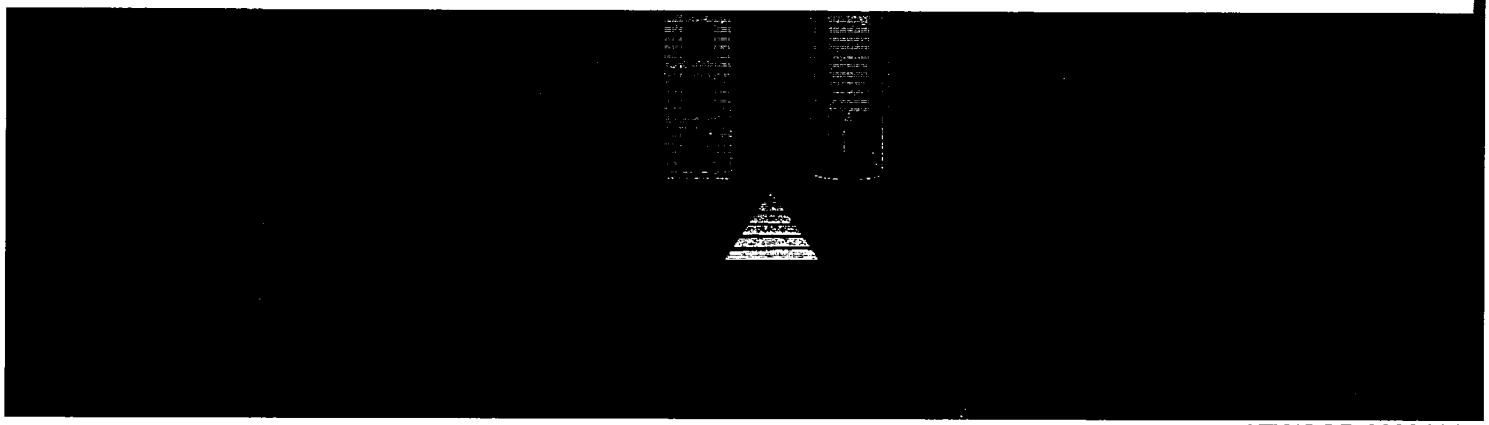
ATWOOD 0000413



Munson Exhibit H

FWHH = 0.8

Requested Peaks = 3



ATWOOD 0000414

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:33

Peak #1: Gaussian
Area: .1067302

	Center X	Height	Width
Value:	209.614671	.0339716251	2.96175951
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: 3.021425

	Center X	Height	Width
Value:	204.415081	.517718128	5.49912304
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: Gaussian
Area: .8021106

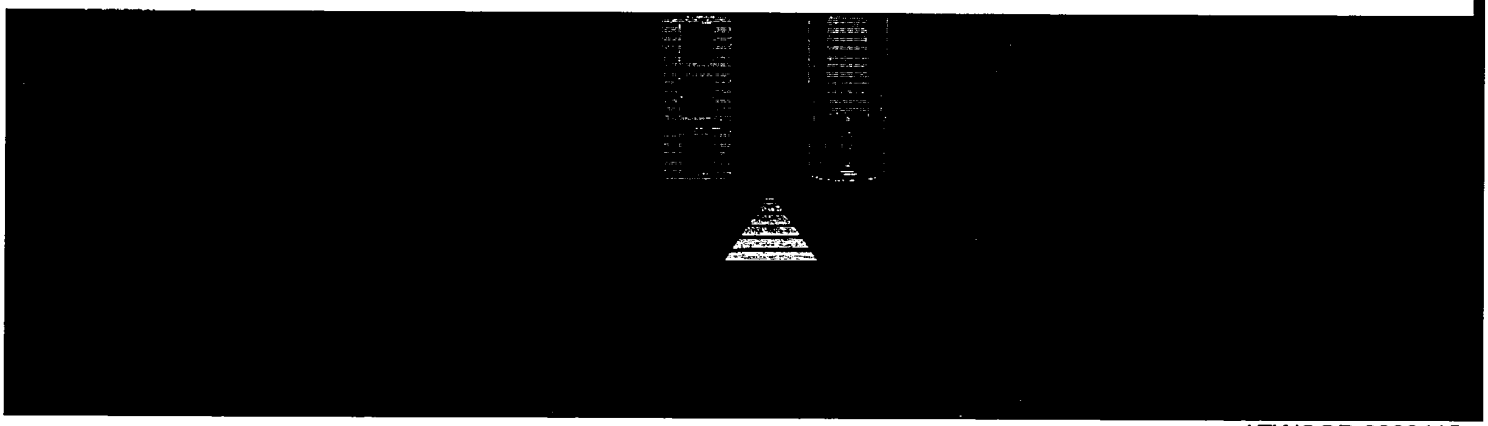
	Center X	Height	Width
Value:	202.962111	.259638292	2.90231367
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Baseline: Linear

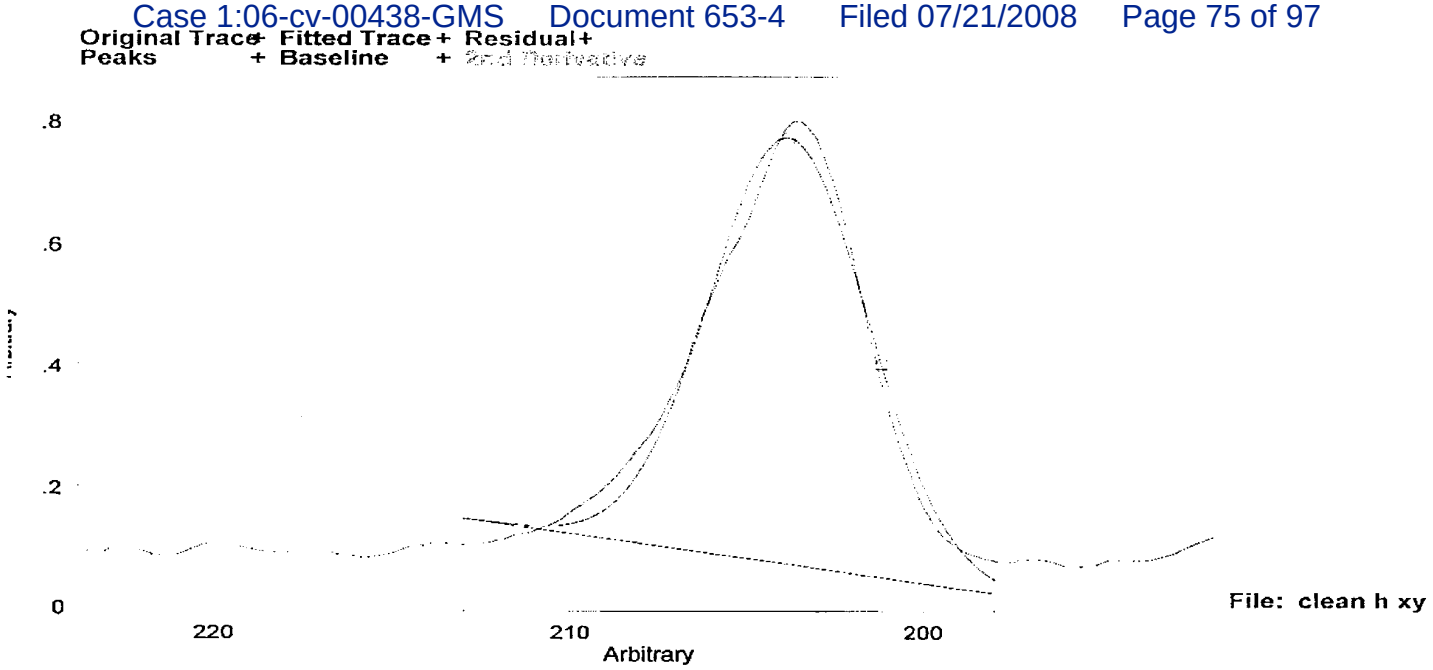
	Offset	Linear
Value:	-.508557716	.00287711202
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	11
Fixed Parameters:	0
Reduced Chi ²:	10.1337
RMS Noise:	.00154
Correlation (R^2):	.9995861
Standard Error:	.004844648



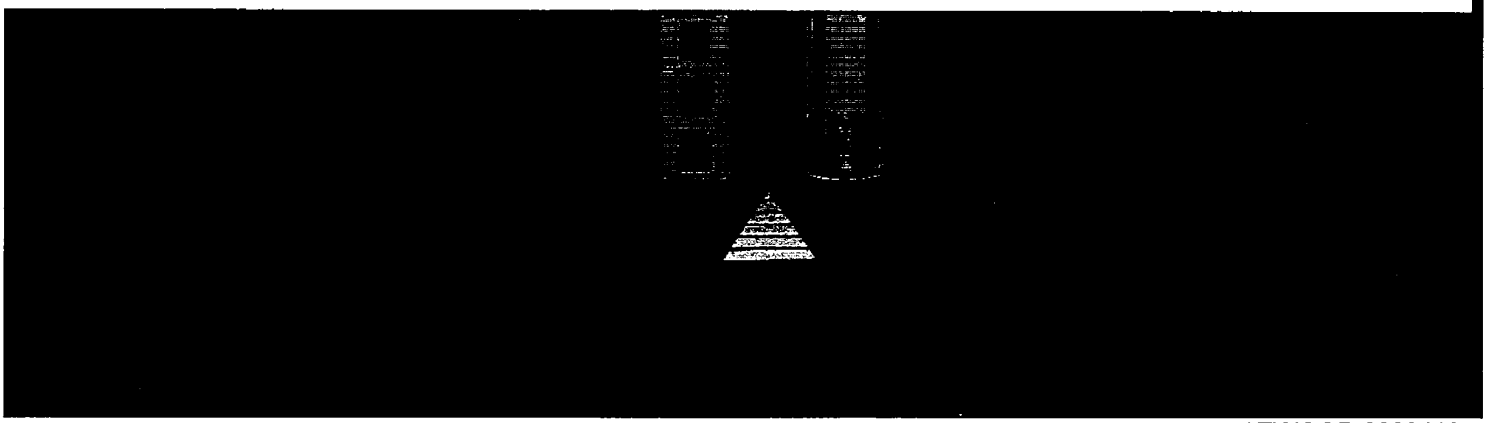
ATWOOD 0000415



Munson Exhibit H

FWIIII = 3.0

Requested Peaks = Max (1 found)



ATWOOD 0000416

Peak Report
Original File: clean h xy data.spc

Date: 10/29/2007 16:36

Peak #1: Gaussian
Area: 3.887423

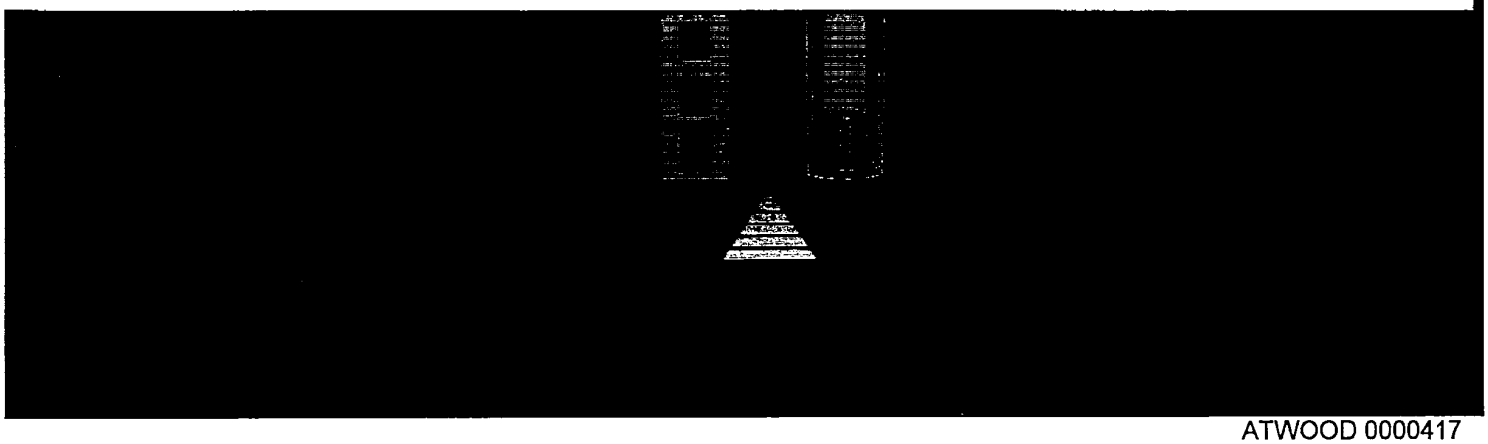
	Center X	Height	Width
Value:	203.847829	.698847444	5.24773179
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Baseline: Linear

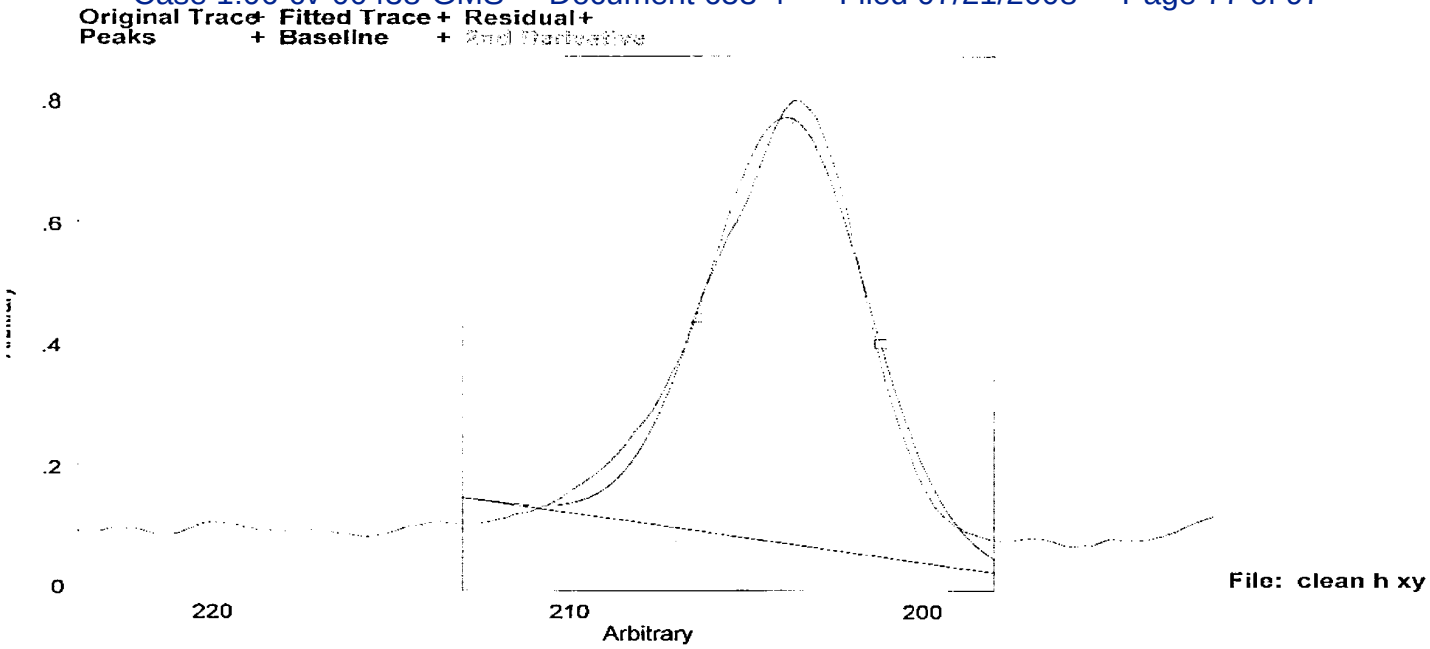
	Offset	Linear
Value:	-1.59199631	.00816308727
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	.00254332255	.00338793906

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	2
Total Parameters:	5
Fitted Parameters:	5
Fixed Parameters:	0
Reduced Chi ²:	369.1531
RMS Noise:	.00154
Correlation (R^2):	.9847234
Standard Error:	.02943076



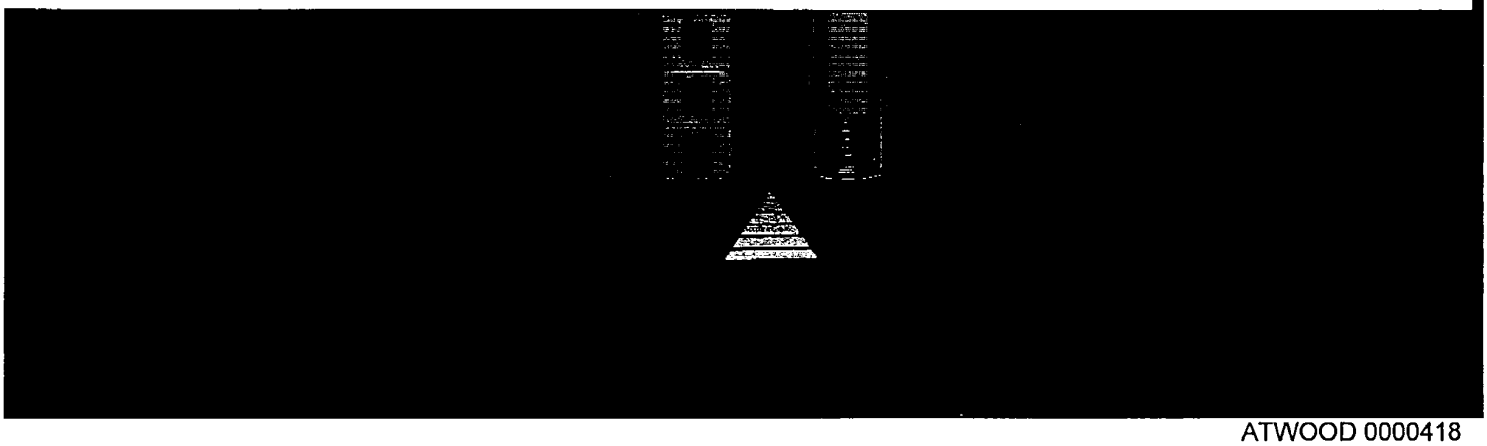
ATWOOD 0000417



Munson Exhibit H

FWHH = 3.0

Requested Peaks = 3 (1 found)



ATWOOD 0000418

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:36

Peak #1: Gaussian
Area: 3.887423

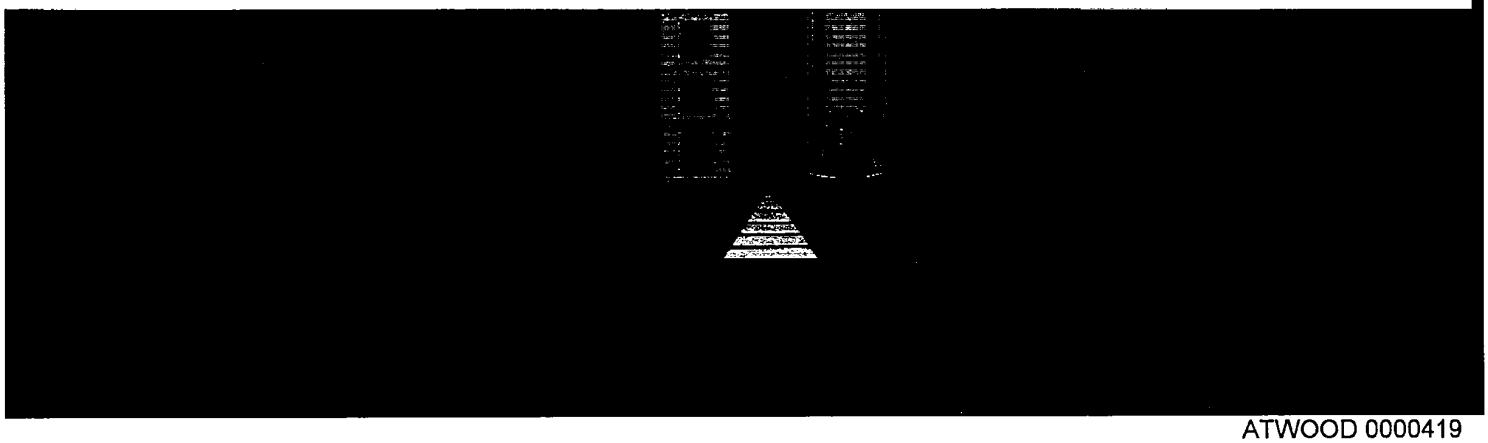
	Center X	Height	Width
Value:	203.847829	.698847444	5.24773179
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Baseline: Linear

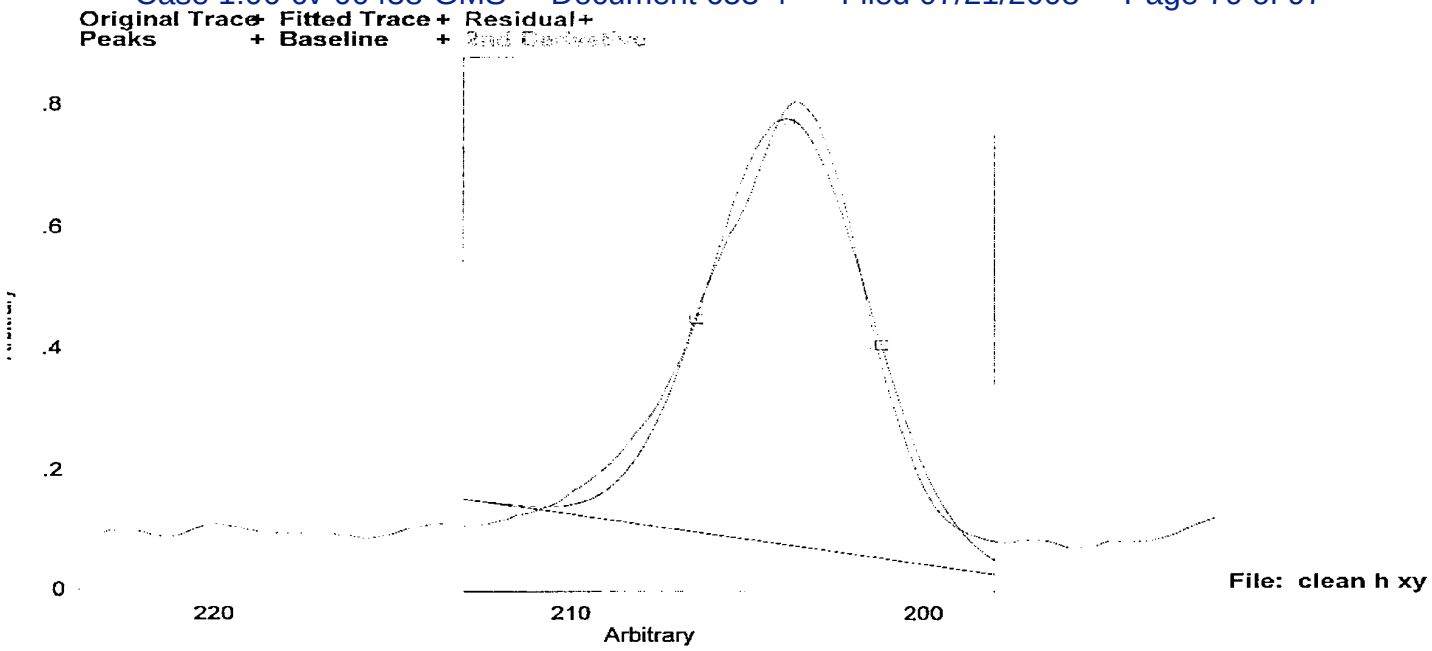
	Offset	Linear
Value:	-1.59199631	.00816308727
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	.00254332255	.00338793906

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	2
Total Parameters:	5
Fitted Parameters:	5
Fixed Parameters:	0
Reduced Chi ²:	369.1531
RMS Noise:	.00154
Correlation (R^2):	.9847234
Standard Error:	.02943076



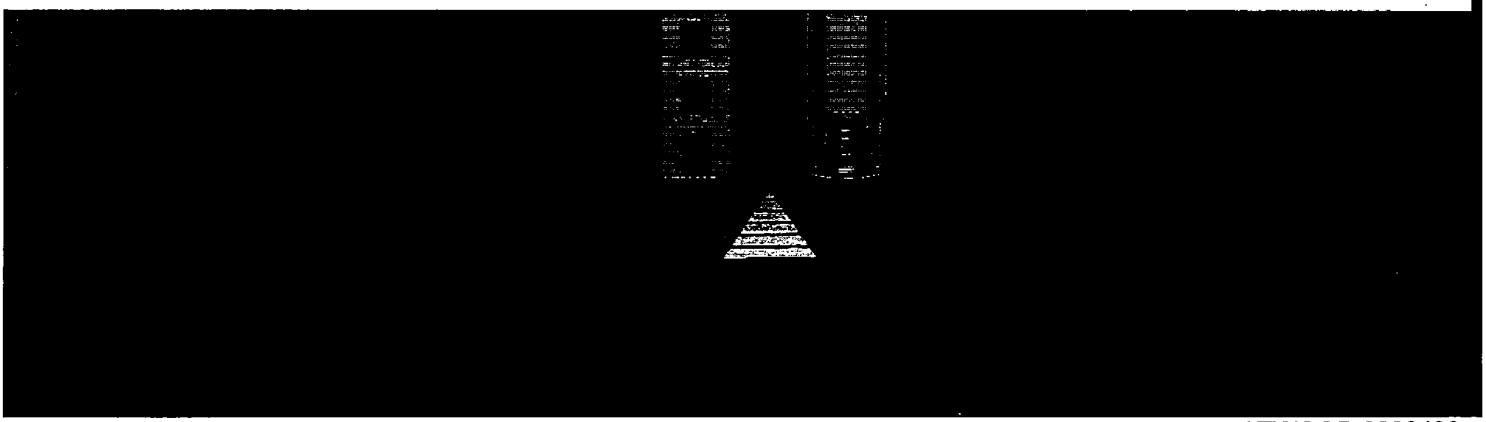
ATWOOD 0000419



Munson Exhibit H

FWHH = 5.0

Requested Peaks = Max (1 found)



ATWOOD 0000420

Peak Report
Original File: clean h xy data.spc

Date: 10/29/2007 16:37

Peak #1: Gaussian
Area: 3.887422

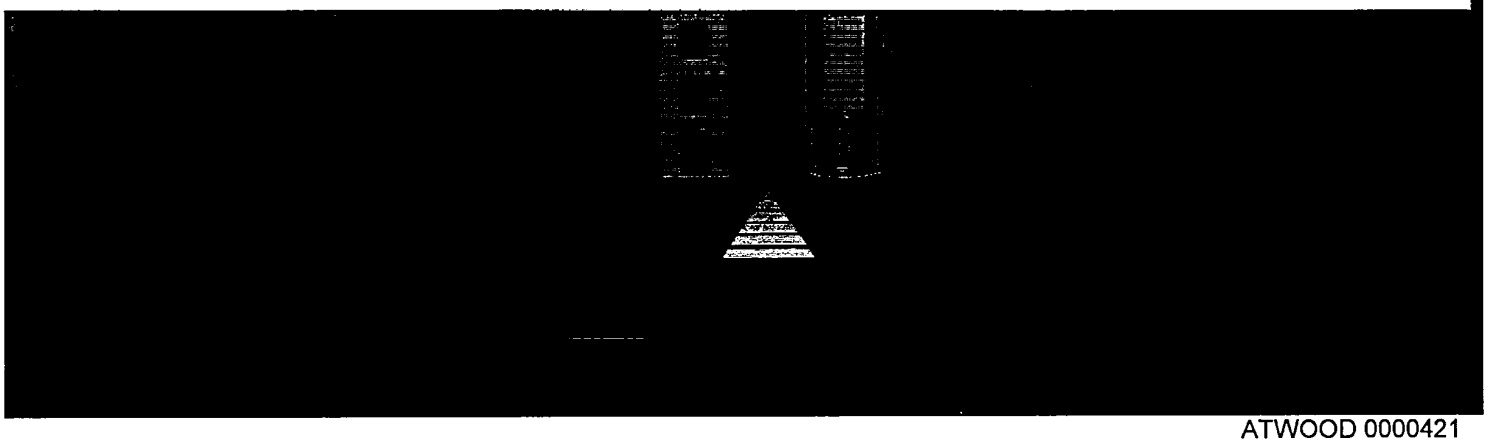
	Center X	Height	Width
Value:	203.847829	.698847433	5.24773077
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Baseline: Linear

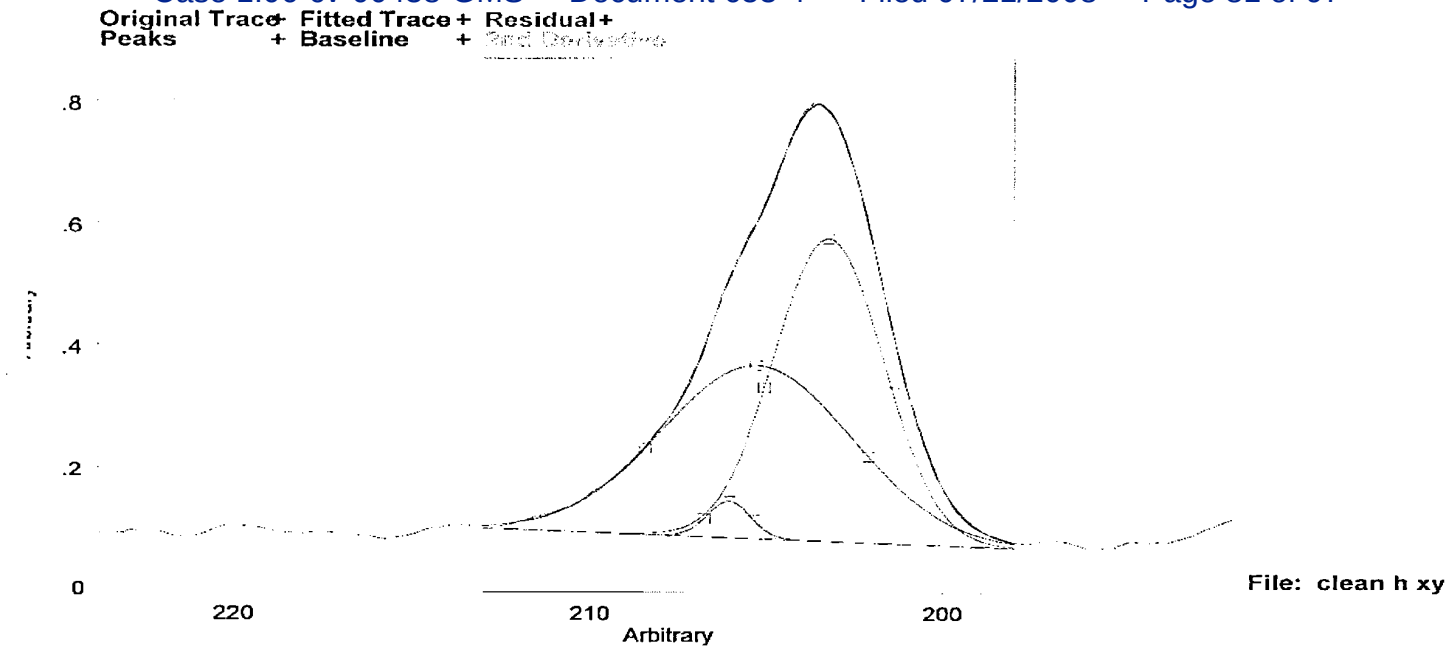
	Offset	Linear
Value:	-1.59199586	.00816308537
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	.00254332255	.00338793906

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	2
Total Parameters:	5
Fitted Parameters:	5
Fixed Parameters:	0
Reduced Chi ²:	369.1531
RMS Noise:	.00154
Correlation (R^2):	.9847234
Standard Error:	.02943076



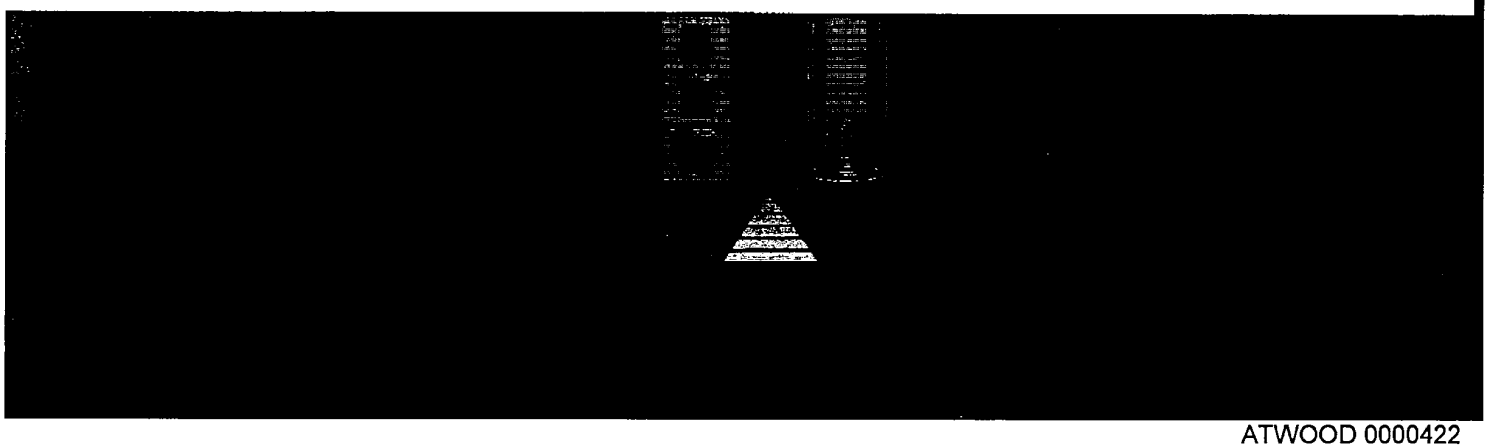
ATWOOD 0000421



Munson Exhibit H

FWHH = 0.6

Requested Peaks = 3



ATWOOD 0000422

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:39

Peak #1: Gaussian
Area: .09076191

	Center X	Height	Width
Value:	206.078075	.0596765236	1.42878834
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: 1.897129

	Center X	Height	Width
Value:	205.297074	.283461658	6.32021505
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: Gaussian
Area: 1.918522

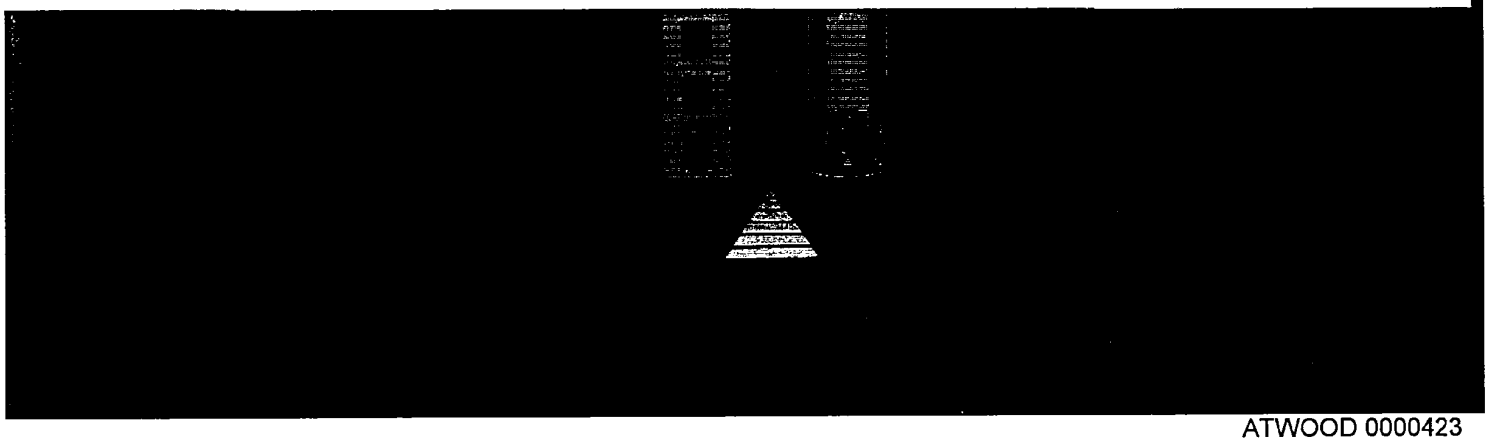
	Center X	Height	Width
Value:	203.25672	.496000908	3.63483944
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Baseline: Linear

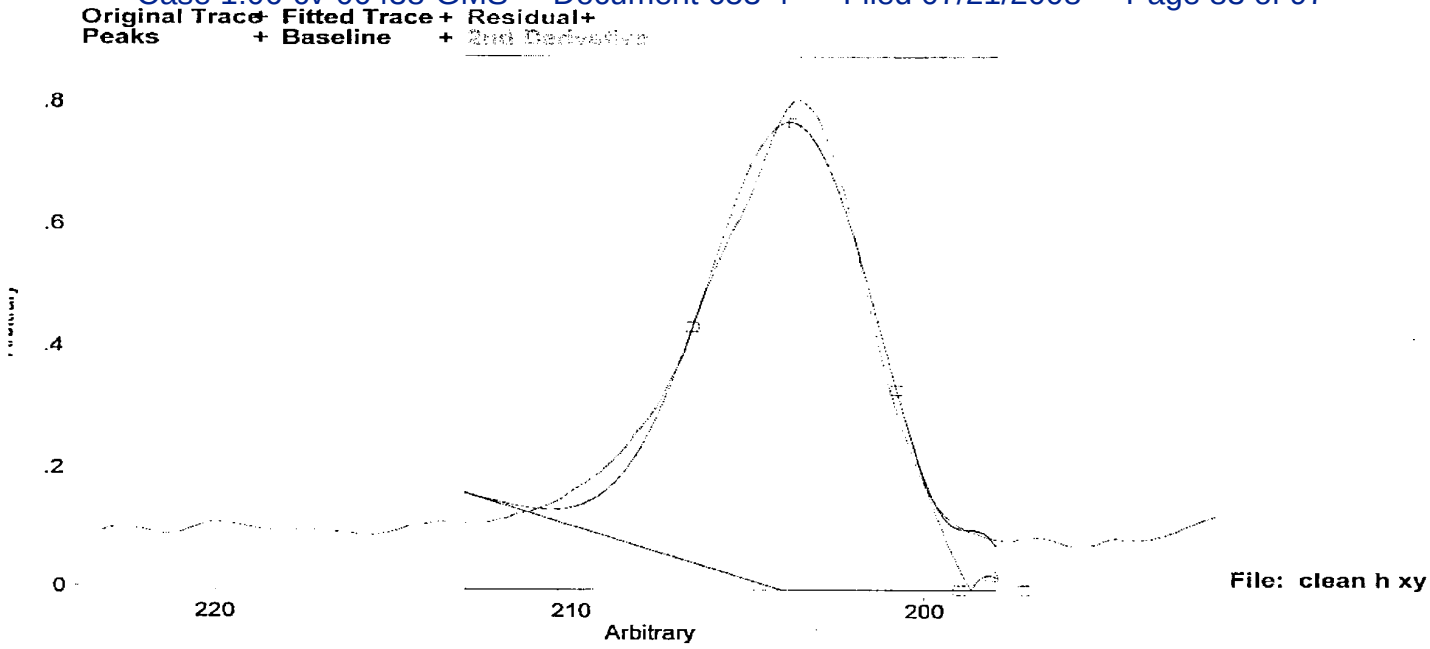
	Offset	Linear
Value:	-.362089299	.00217207494
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:

Solution has CONVERGED	
Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	4
Total Parameters:	11
Fitted Parameters:	11
Fixed Parameters:	0
Reduced Chi ²:	2.053915
RMS Noise:	.00154
Correlation (R^2):	.9999161
Standard Error:	.002181069



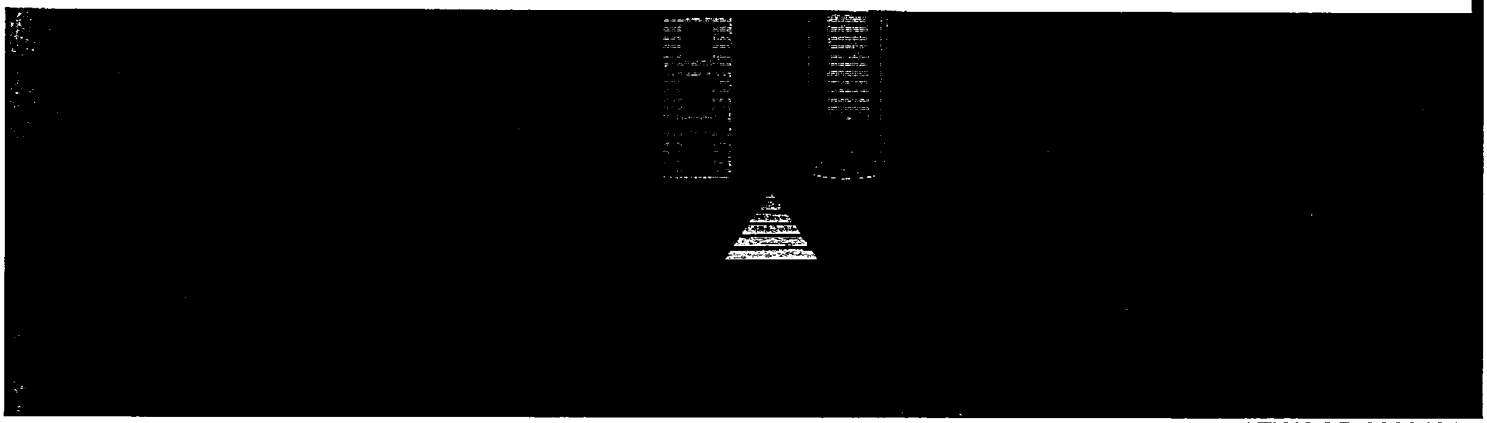
ATWOOD 0000423



Munson Exhibit H

FWHH = 0.6

Requested Peaks = 2



ATWOOD 0000424

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:39

Peak #1: Gaussian
Area: 4.728738

	Center X	Height	Width
Value:	203.727383	.773388229	5.80081892
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: .1445126

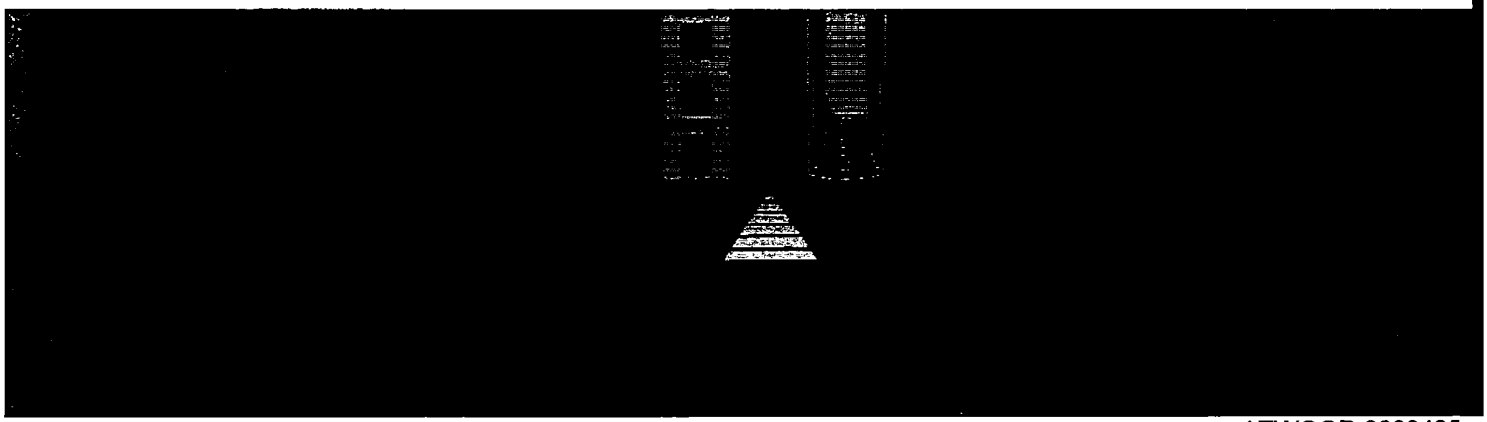
	Center X	Height	Width
Value:	198.121909	.129687634	1.80990645
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Baseline: Linear

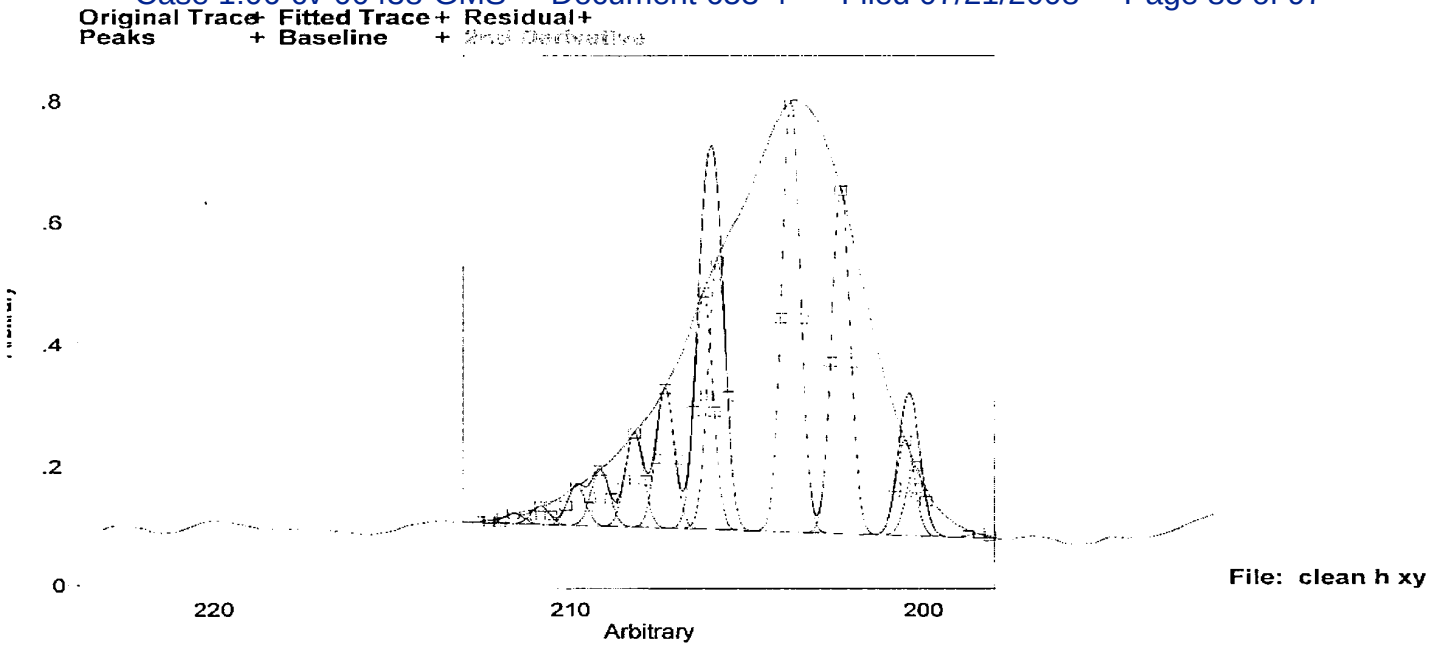
	Offset	Linear
Value:	-3.64474099	.0178336802
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	.00945502897	.0000447488271

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	3
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	326.9733
RMS Noise:	.00154
Correlation (R^2):	.9865352
Standard Error:	.02760889



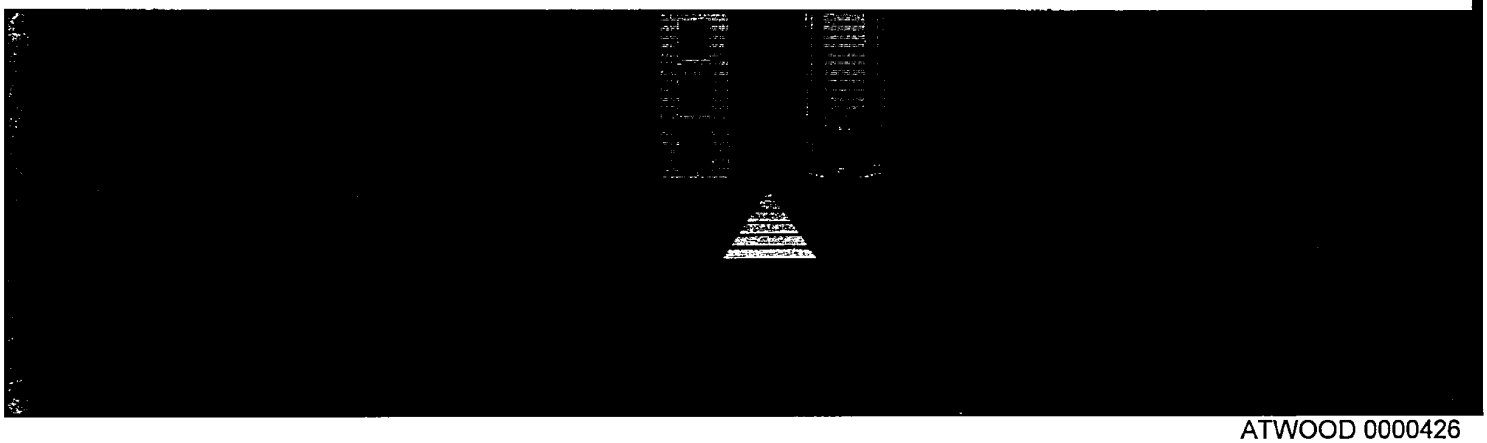
ATWOOD 0000425



Munson Exhibit H

FWHH = 0.6

Requested Peaks = Max (non-refined)



ATWOOD 0000426

Peak Report
Original File: clean h xy data.spc

Date: 10/29/2007 16:43

Peak #1: Gaussian
Area: .002303416

	Center X	Height	Width
Value:	212.305961	.00361699251	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: .01073488

	Center X	Height	Width
Value:	211.601961	.0168079042	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: Gaussian
Area: .01906856

	Center X	Height	Width
Value:	210.865961	.0298561935	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Peak #4: Gaussian
Area: .04159195

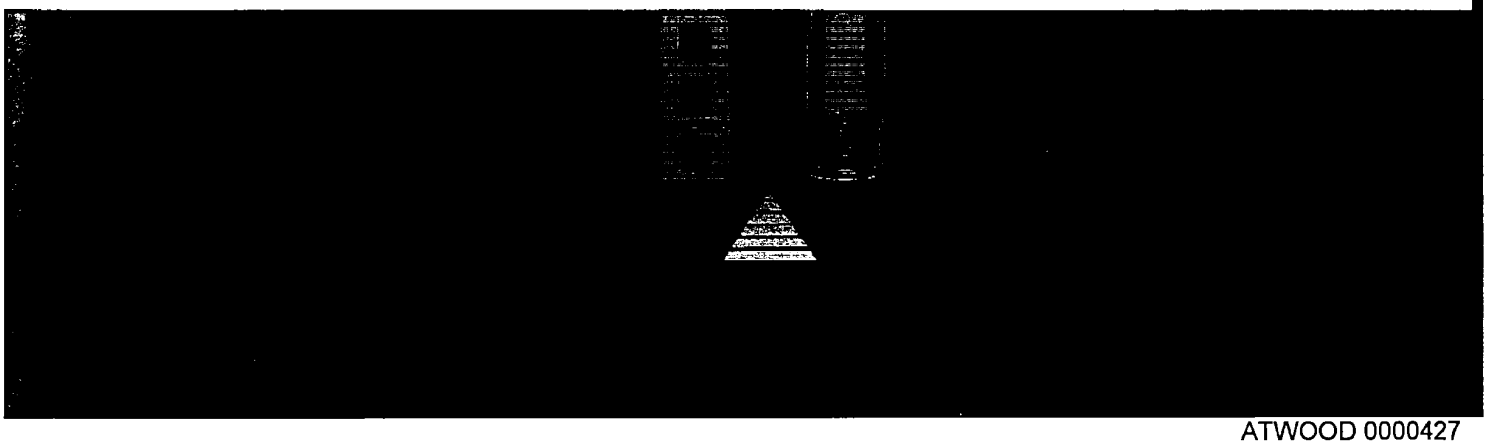
	Center X	Height	Width
Value:	209.841961	.0651217118	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: .05922819

	Center X	Height	Width
Value:	209.169961	.0927352894	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: .09952622

	Center X	Height	Width
Value:	208.209961	.155831072	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0



ATWOOD 0000427

Peak #7:	Gaussian		
Area:	.1472527		
	Center X	Height	Width
Value:	207.345961	.230557765	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #8:	Gaussian		
Area:	.2501595		
	Center X	Height	Width
Value:	206.257961	.391681986	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

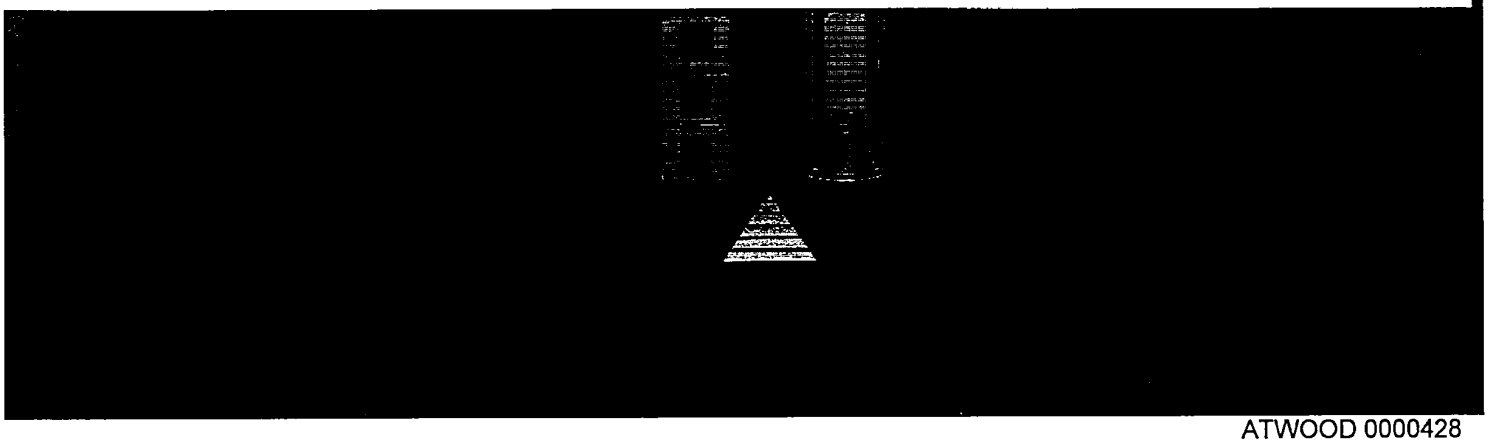
Peak #9:	Gaussian		
Area:	.2834924		
	Center X	Height	Width
Value:	205.873961	.44387225	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #10:	Gaussian		
Area:	.4515431		
	Center X	Height	Width
Value:	203.761961	.706994068	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #11:	Gaussian		
Area:	.3628828		
	Center X	Height	Width
Value:	202.353961	.568175986	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #12:	Gaussian		
Area:	.1008377		
	Center X	Height	Width
Value:	200.529961	.15788455	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #13:	Gaussian		
Area: :	.07413597		
	Center X	Height	Width
Value:	200.241961	.116076831	.6



ATWOOD 0000428

Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #14: Gaussian
Area: .003929696

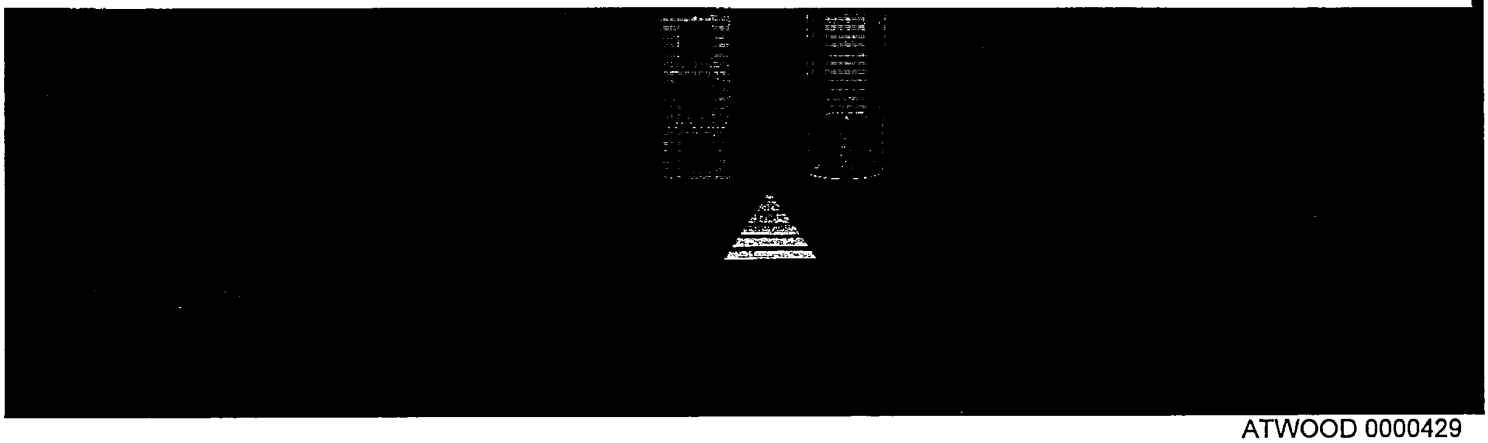
	Center X	Height	Width
Value:	198.449961	.00633580654	.6
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Baseline: Linear

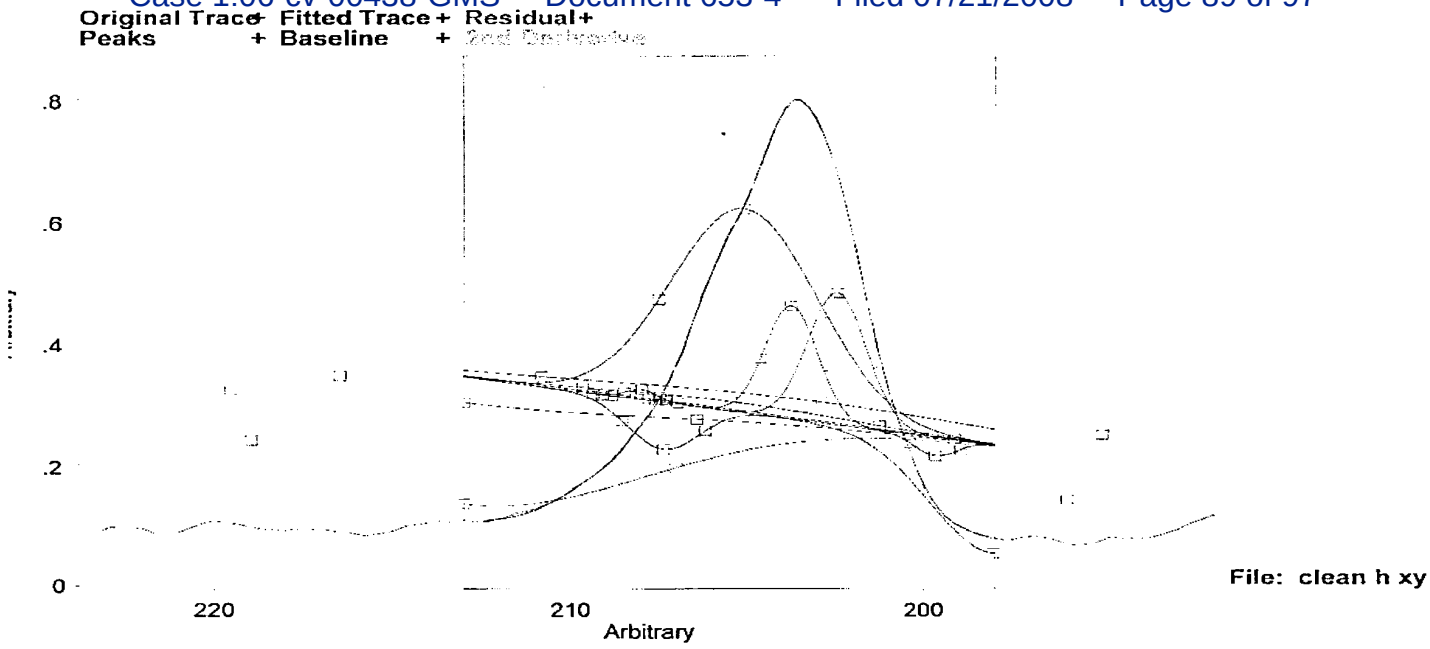
	Offset	Linear
Value:	-.270885028	.00176126006
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	15
Total Parameters:	8
Fitted Parameters:	8
Fixed Parameters:	0
Reduced Chi ²:	27995.92
RMS Noise:	.00154
Correlation (R^2):	.806036
Standard Error:	.240664



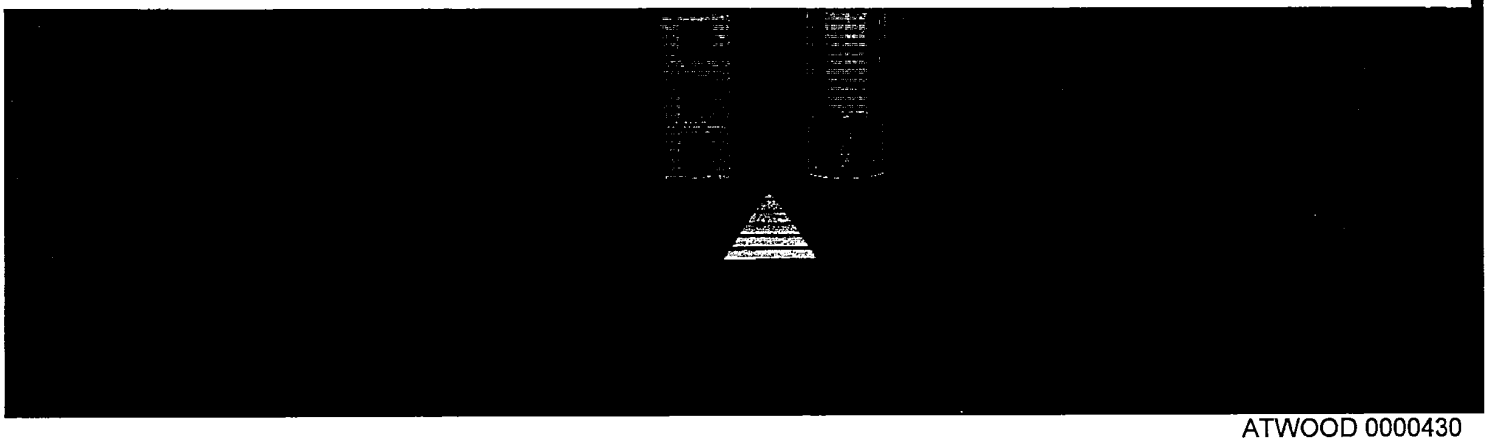
ATWOOD 0000429



Munson Exhibit H

FWHH = 0.6

Requested Peaks = Max



ATWOOD 0000430

Peak Report
Original File: clean h xy data.spc

Date: 10/29/2007 16:44

Peak #1: Gaussian
Area: -.2911809

	Center X	Height	Width
Value:	213.01	-.042047031	13.101387
Fixed:	NO	NO	NO
Low Limit:	213.01	None	.032
High Limit:	213.01	None	None
Standard Error:	.0163188637	.0026004938	.0139093745

Peak #2: Gaussian
Area: -1.327795

	Center X	Height	Width
Value:	213.01	-.209469265	11.946097
Fixed:	NO	NO	NO
Low Limit:	213.01	None	.032
High Limit:	213.01	None	None
Standard Error:	.00254332255	.00338793906	.011370806

Peak #3: Gaussian
Area: 0 -.0005384655

	Center X	Height	Width
Value:	209.115293	-.000979697636	.516337441
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	.00945502897	.0000447488271	0

Peak #4: Gaussian
Area: .01752457

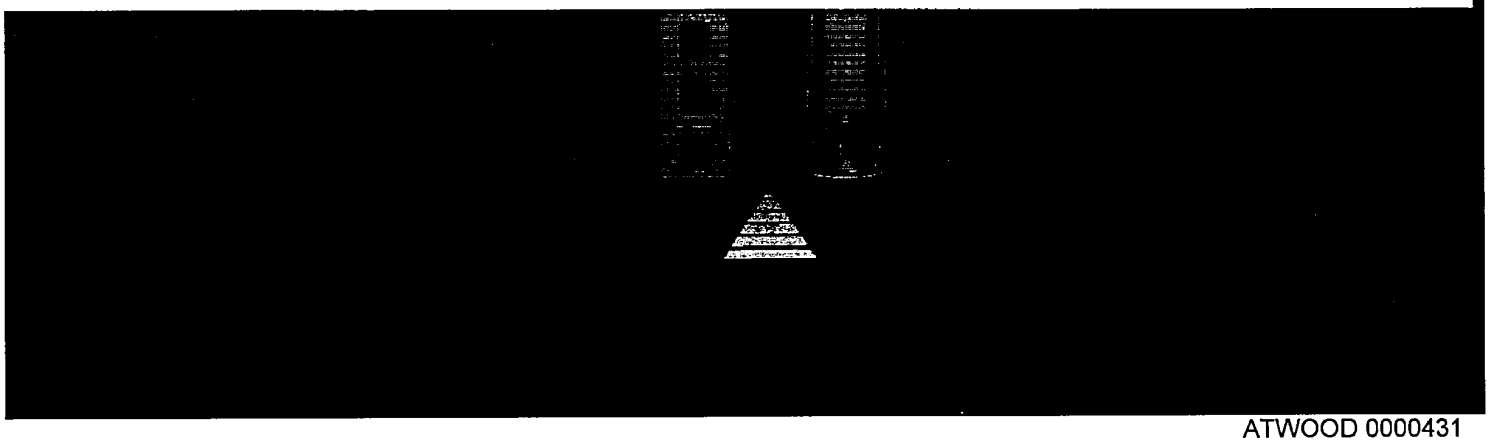
	Center X	Height	Width
Value:	207.999534	.0164143892	1.0029757
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #5: Gaussian
Area: .03459869

	Center X	Height	Width
Value:	207.812295	.00280082285	17.3450133
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #6: Gaussian
Area: -.1936706

	Center X	Height	Width
Value:	207.401193	-.0776416812	2.34334685
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0



ATWOOD 0000431

Peak #7: Gaussian
Area: .002545293

	Center X	Height	Width
Value:	207.310519	.00396286059	.603388063
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #8: Gaussian
Area: .1307885

	Center X	Height	Width
Value:	205.468147	.0151556991	8.40153276
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #9: Gaussian
Area: 1.73017

	Center X	Height	Width
Value:	205.09834	.334738052	4.85733208
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #10: Gaussian
Area: .3430198

	Center X	Height	Width
Value:	203.768678	.18516104	1.74035318
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #11: Gaussian
Area: .398656

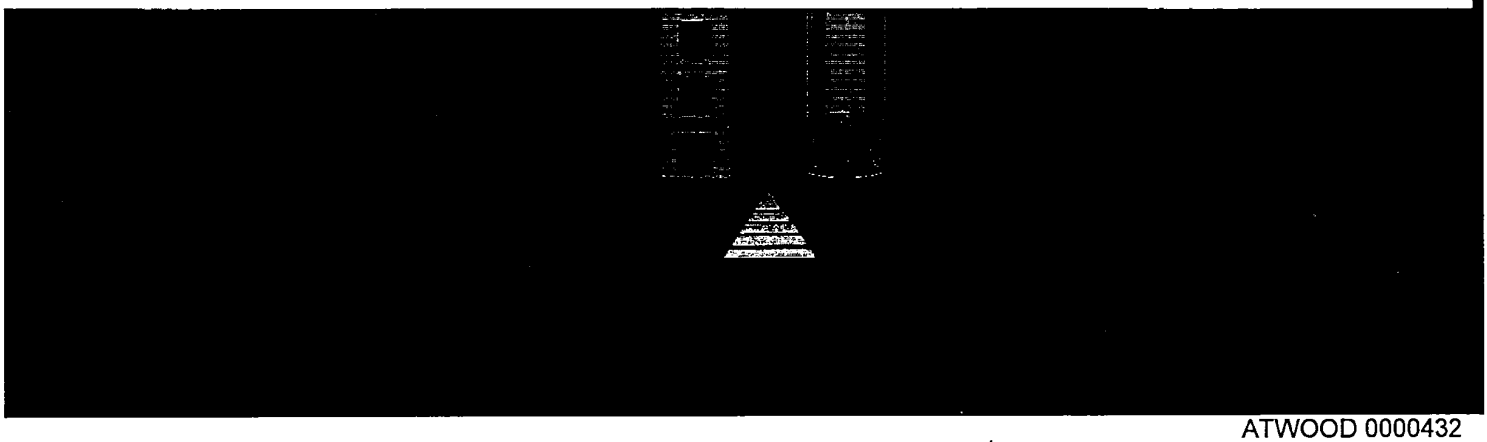
	Center X	Height	Width
Value:	202.907039	.0336686697	15.8557546
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #12: Gaussian
Area: .4516587

	Center X	Height	Width
Value:	202.474327	.216082107	1.96362893
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #13: Gaussian
Area: -.04566066

	Center X	Height	Width
Value:	199.689922	-.0298896471	1.43859011



ATWOOD 0000432

Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Peak #14: Gaussian
Area: : -.4157481

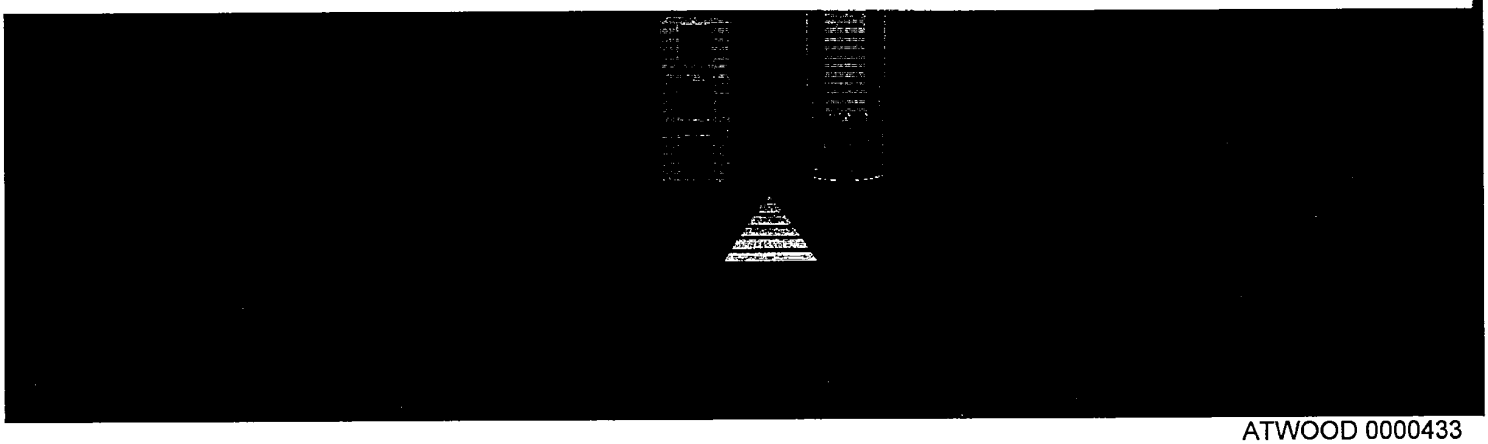
	Center X	Height	Width
Value:	198.068002	-.179003823	4.17974979
Fixed:	NO	NO	NO
Low Limit:	198.002	None	.032
High Limit:	213.01	None	None
Standard Error:	0	0	0

Baseline: Linear

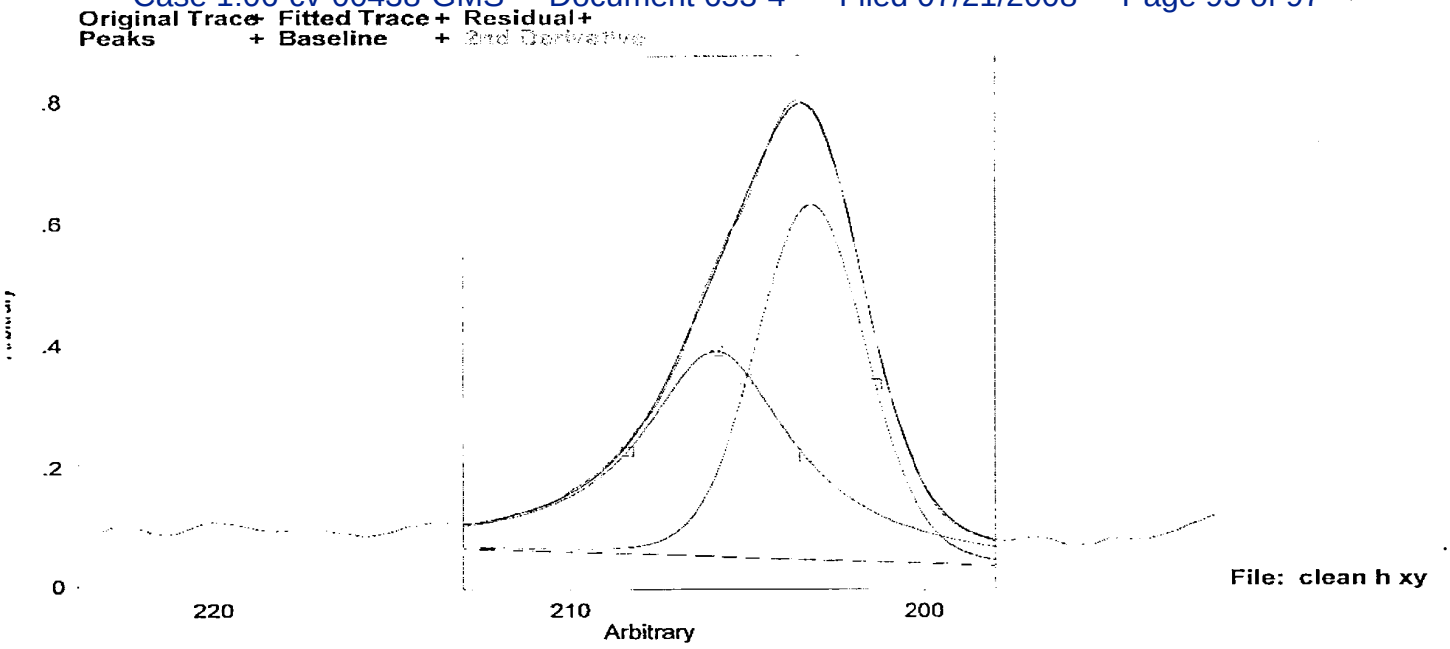
	Offset	Linear
Value:	-1.24506067	.00746061119
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution has CONVERGED

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	15
Total Parameters:	44
Fitted Parameters:	42
Fixed Parameters:	2
Reduced Chi ²:	1.284017
RMS Noise:	.00154
Correlation (R^2):	.9991907
Standard Error:	.001665249



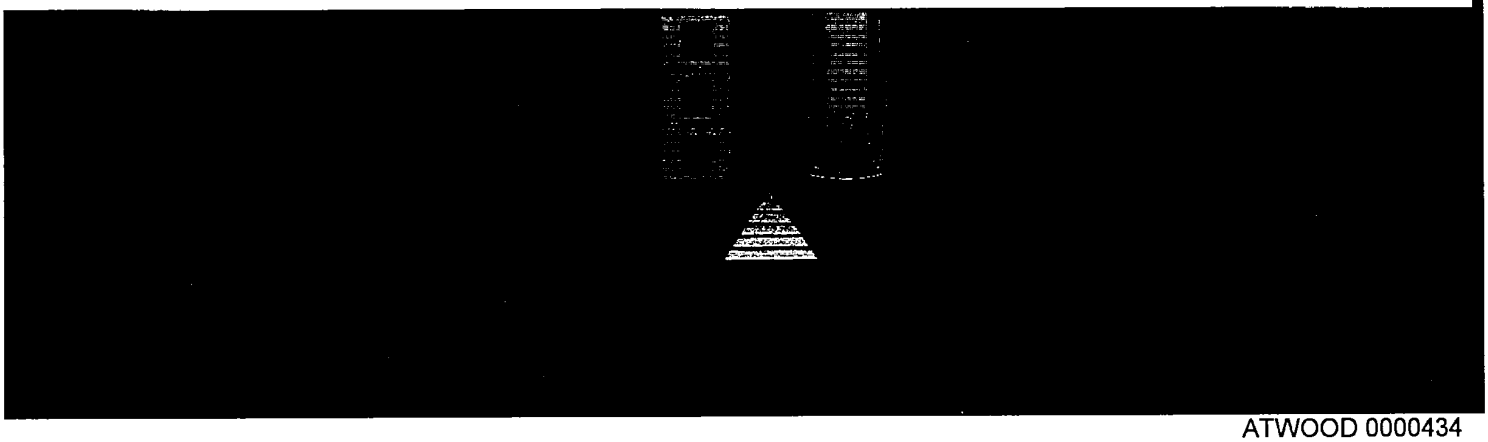
ATWOOD 0000433



Munson Exhibit H

FWHH = 1.6

Requested Peaks = 2 (G&L)



ATWOOD 0000434

Peak Report

Original File: clean h xy data.spc

Date: 10/29/2007 16:46

Peak #1: Mixed G+L
Area: 2.114591

	Center X	Height	Width	Lorentz %
Value:	205.899036	.336936863	5.03239969	1
Fixed:	NO	NO	NO	NO
Low Limit:	198.002	None	.032	1
High Limit:	213.01	None	None	1
Standard Error:	.0163188637	.0026004938	.0139093745	0

Peak #2: wMixed G+L
Area: 2.33344

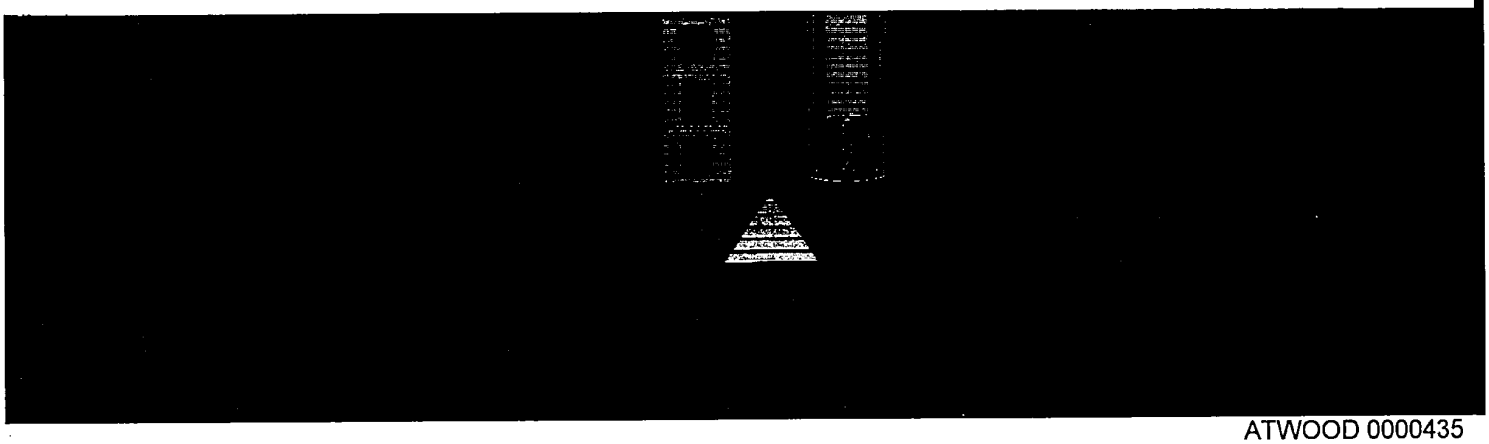
	Center X	Height	Width	Lorentz %
Value:	203.218958	.583769609	3.64561391	.13064937
Fixed:	NO	NO	NO	NO
Low Limit:	198.002	None	.032	0
High Limit:	213.01	None	None	1
Standard Error:	.00254332255	.00338793906	.011370806	0

Baseline: wLinear

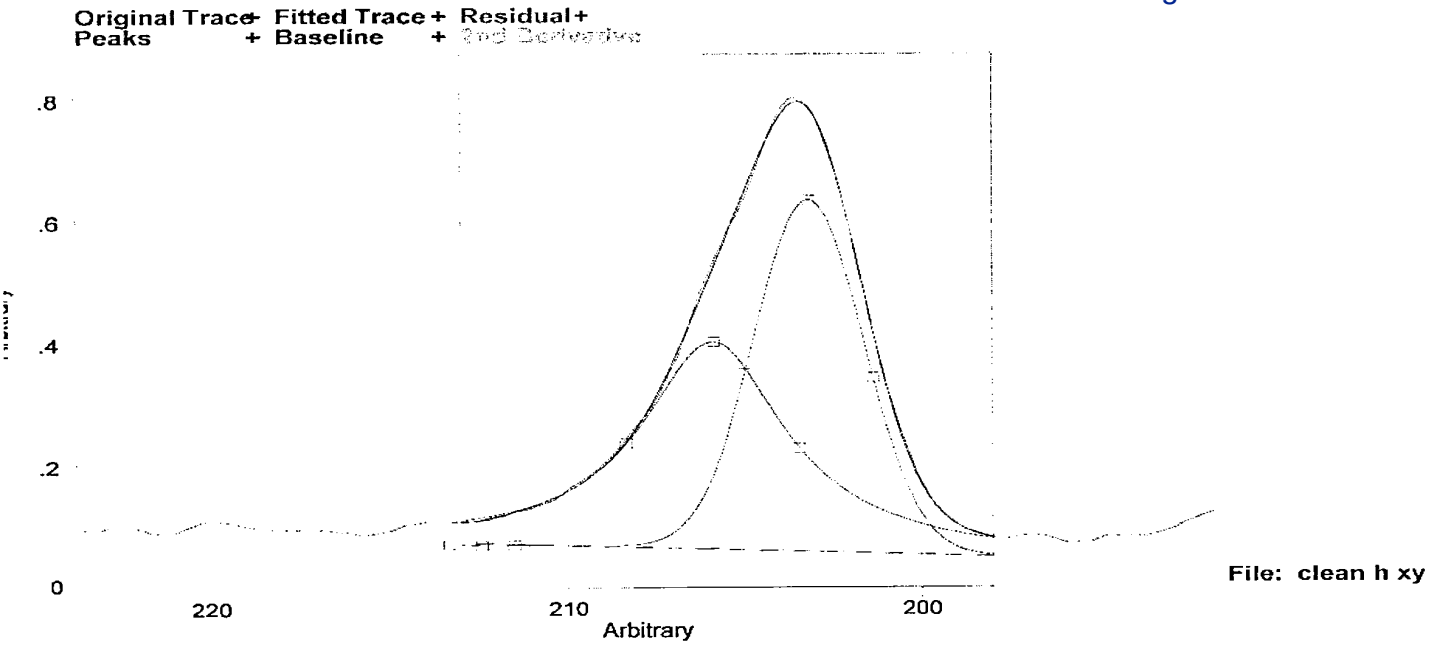
	Offset	Linear
Value:	-.331621631	.00184838705
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	.00945502897	.0000447488271

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	3
Total Parameters:	10
Fitted Parameters:	9
Fixed Parameters:	1
Reduced Chi ²:	8.289484
RMS Noise:	.00154
Correlation (R^2):	.9998108
Standard Error:	.004391228



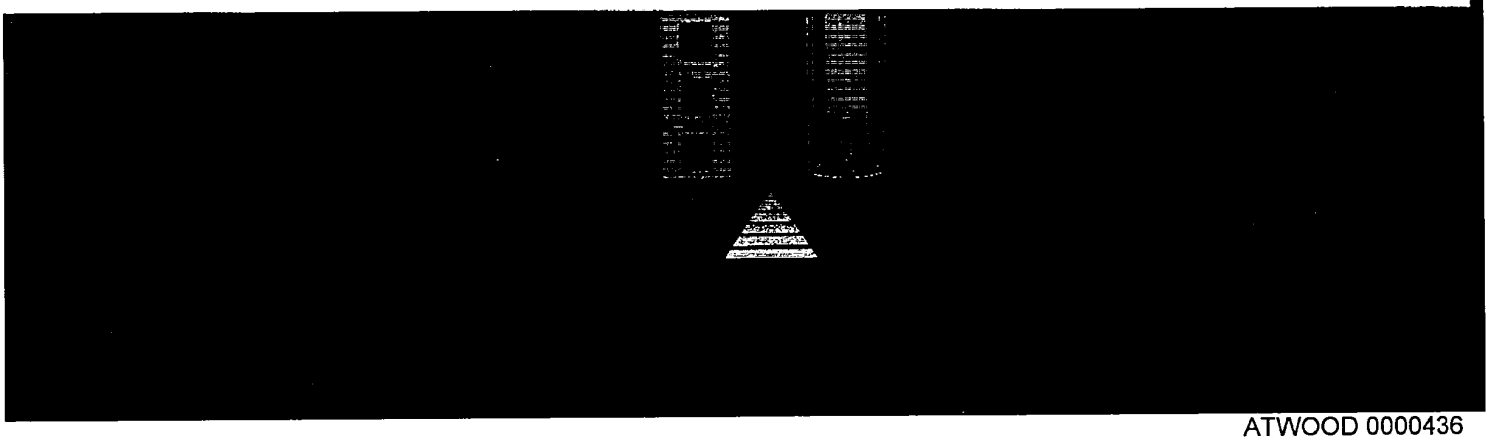
ATWOOD 0000435



Munson Exhibit H

FWHH = 1.6

Requested Peaks = 3 (G&L)



ATWOOD 0000436

Peak Report
Original File: clean h xy data.spc

Date: 10/29/2007 16:47

Peak #1: Mixed G+L
Area: 0 -.005388611

	Center X	Height	Width	Lorentz %
Value:	212.443265	-.00364530201	1.80249385	0
Fixed:	NO	NO	NO	NO
Low Limit:	198.002	None	.032	0
High Limit:	213.01	None	None	0
Standard Error:	.0163188637	.0026004938	.0139093745	0

Peak #2: Mixed G+L
Area: 2.084834

	Center X	Height	Width	Lorentz %
Value:	205.929737	.339191022	4.89721426	1
Fixed:	NO	NO	NO	NO
Low Limit:	198.002	None	.032	1
High Limit:	213.01	None	None	1
Standard Error:	.00254332255	.00338793906	.011370806	0

Peak #3: Mixed G+L
Area: 2.223729

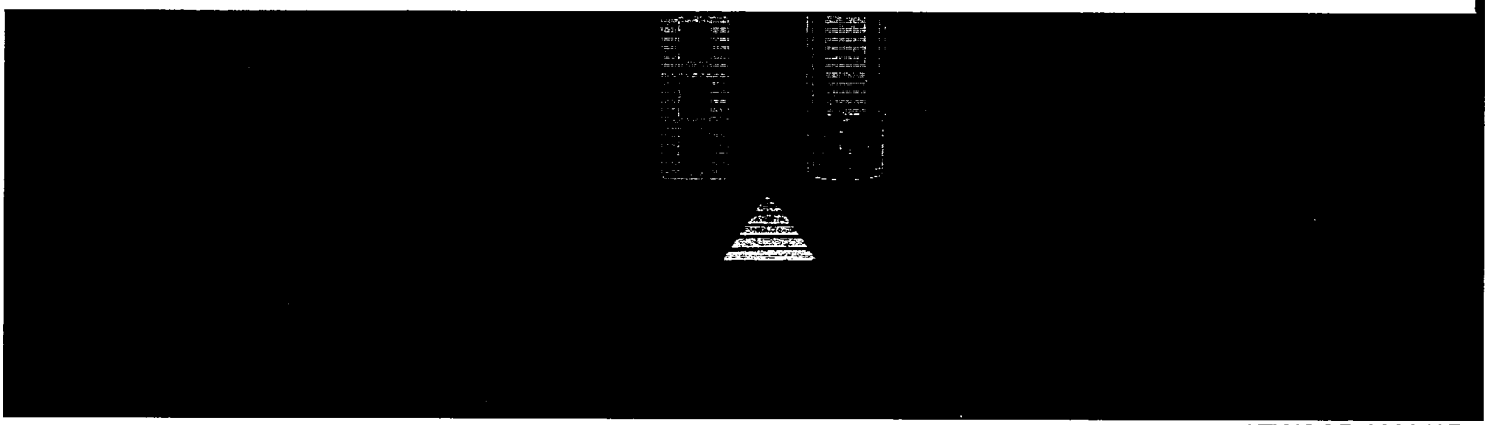
	Center X	Height	Width	Lorentz %
Value:	203.216701	.57693025	3.62215217	0
Fixed:	NO	NO	NO	NO
Low Limit:	198.002	None	.032	0
High Limit:	213.01	None	None	0
Standard Error:	.00945502897	.0000447488271	0	0

Baseline: Linear

	Offset	Linear
Value:	-.266806282	.00157861908
Fixed:	NO	NO
Low Limit:	-10.1399315	-.0479685437
High Limit:	11.0155394	.0479685437
Standard Error:	0	0

Statistics Summary:
Solution AT A MINIMUM

Total Data Points:	470
Fitted Region:	213.01 to 198.002
Fitted Equations:	4
Total Parameters:	14
Fitted Parameters:	11
Fixed Parameters:	3
Reduced Chi ²:	8.971452
RMS Noise:	.00154
Correlation (R^2):	1.001659
Standard Error:	.004558369



ATWOOD 0000437

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REDACTED IN THEIR ENTIRETY