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13th October 2012

EDP/SG

E-92/28/2012

The Deputy Controller of Patents & Designs,
Intellectual Property Office,
Intellectual Property Office Building,
G.S.T. Road, Guindy, Chennai-600032
Phone : 044-22502080

Pre grant
opposition


19/10/12

Ref: Patent Application No. 1576/CHENP/2011

Sub: Filing of Written Submission u/s 25(1) of the Act for Pre-Grant Opposition.

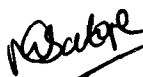
Sir,

With regards to the captioned subject and on behalf of our clients, M/s. Indian
Pharmaceutical Alliance, a society registered under the Societies Registration Act,
Written Submission (In duplicate) u/s 25(1) of the Patent Act for opposing the Pre -
Grant Opposition of Patent Application No. 1576/CHENP/2011

Kindly take them on record and oblige.

Thanking You,

Yours truly,



for Nayan Rawal
Constituted Attorney for
the Opponents



General Stamp Office, Mumbai.

L. S. No. 222
महाराष्ट्र MAHARASHTRA

-7 APR 2010

PROPER OFFICER

SHRI. L. S. BAMBLE

सी. कांचन हर्षद बोगाळे

वाटिका कॉम्प्लेक्स, अ. व. मार्ग
बंद (पूर्व), मुंबई - ५२.

CX 510202

15 APR 2010
Indian Pharmaceutical Alliance
यांना स. कांचन हर्षद बोगाळे

परवाना धारक मुद्रांक विक्रेता

GENERAL POWER OF ATTORNEY

We, Indian Pharmaceutical Alliance, a Society registered under the Societies Registration Act having its mailing address as follows C/o VISION CONSULTING GROUP, 201 Darvesh Chambers, 743 P D Hinduja Road, Khar (W), Mumbai 400 052 hereby authorize Mr. Nayan J. Rawal, Patent Agent (Agent No.654), having its Office at L-303, Panchsheel Gardens, Mahavir Nagar, Kandivli West, Mumbai 400 067, to act as our Agents and Attorneys for various Pre-Grant and Post-Grant Opposition, rectification and any other proceedings under the Patents (Amendment) Act, 1970 in respect of various Patents filed in India.

We request that all notices, requisitions and communications may be sent to the said Agents at the following address:-

Vision Consulting Group, 201 Darvesh Chambers, 743 P D Hinduja Road, Khar (W), Mumbai 400 052.

We hereby revoke all previous authorizations, if any; we hereby ratify all acts done by the said Agents

Dated this 19th day of April 2010

For Indian Pharmaceutical Alliance



D G Shah
Secretary General

IN THE MATTER OF THE PATENTS ACT, 1970

and

**IN THE MATTER OF THE PATENT RULES, 2003
(as amended by the Patents (Amendment) Rules 2006)**

and

**IN THE MATTER OF INDIAN PATENT APPLICATION
NO.1576/CHENP/2011 FILED BY F. HOFFMANN-LA ROCHE AG.**

.....the Applicants

and

**IN THE MATTER OF A REPRESENTATION BY WAY OF AN OPPOSITION
UNDER SECTION 25(1) AND RULE 55 THERETO BY INDIAN
PHARMACEUTICALS ALLIANCE**

.....the Opponents

REPRESENTATION BY WAY OF OPPOSITION U/S 25(1)

1.0 It is respectfully submitted on behalf of Indian Pharmaceutical Alliance, that a pre-grant Opposition under Section 25(1) of the Patents Act, 1970 and rule 55(1) of the Patents Rules, 2003 (as amended by the Patents (Amendment) Rules 2006), is hereby presented by the "Opponents" against Indian Patent Application No. **1576/CHENP/2011** (hereinafter also referred to as the "Opposed Application") in the name of **F. HOFFMANN-LA ROCHE AG.** (hereinafter referred to as the "Applicants").

It is respectfully submitted:

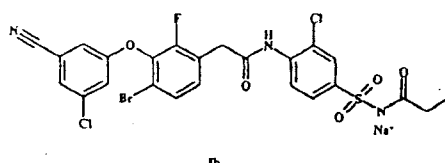
2.0 The Opponents are an associations of person registered under the SOCIETIES REGISTRATION ACT, XXI OF 1860 in the name and style of "INDIAN PHARMACEUTICAL ALLIANCE" having its registered office 115/11, GROUND FLOOR, WORLD TRADE CENTRE, BABAR ROAD, CONNAUGHT PLACE, NEW DELHI - 110001, the main object of as follows:-

- (a) To support the development of international and regional policies, which seek to ensure, access to medical care for all customers.
- (b) To promote balanced and generic friendly intellectual property rights in the pharmaceuticals sector to ensure that timely access to markets is guaranteed for new and generic pharmaceutical products.
- (c) To promote the global harmonization relating to generic products.
- (d) To support the right of all governments to regulate their own pricing, substitution, prescribing and reimbursement policies.
- (e) To suggest measures for enhancing pharmaceutical research in India, both in the areas of basic as well as applied research.
- (f) To interact with the environmental protection agencies to evolve uniform standards of environmental protection measures across the country and ensure implementation of the same.
- (g) To suggest measures to strengthen the pharmaceutical pricing framework that ensures an equitable pricing system for industry and consumers.

- (h) One of the further object of the society is to promote cause of generic pharmaceutical industry and to provide support for the development of competition on the off Patent pharmaceutical sector and to prepare position papers for representing India at international for a to highlight the problems face by generic pharmaceutical companies in international market. It also aims at strengthening regulatory agencies for patenting registration and quality assurance of drugs and pharmaceuticals by providing gaudiness to government and international organization in improving the regulatory and legal expertise relating to registration and marketing of drugs and pharmaceutical. It also further aims at interacting with the regulatory authorities to streamline the guidelines for clinical trials and bio-equivalence studies, to ensure expeditious registration of new as well as existing drugs.

2.1 The opposed patent application is for "Polymorphs of acyl sulfonamides"

The application discloses novel polymorphic crystalline forms of 2-[4-bromo-3-(3-chloro-5cyano-phenoxy)-2-fluoro-phenyl]-N-(2-chloro-4-propionyl sulfamoyl-phenyl)-acetamide, sodium salt (Ib)



with improved stability and physical properties which facilitate manufacturing, handling and formulating for treatment or prophylaxis of HIV mediated diseases, AIDS or ARC, in monotherapy or in combination therapy.

2.2 Although a representation of Opposition can be made by "any person", "in writing" under Section 25(1) of The Patents Act, 1970; however, the Opponents interest in opposing this application is substantial and real. The Opponents, therefore, have *locus standi* in opposing this application.

2.3 It is respectfully submitted that the Opposed Application entitled **"POLYMORPHS OF ACYL SULFONAMIDES"** has been filed on March 7, 2011 and published in the Official Journal of the Indian Patent office on June 8,

2012. The specification of Opposed Application is attached herewith as **Document 6 (D6)**.

2.4 The Opponents are filing this Representation by way of Opposition against Indian Patent Application No. **1576/CHENP/2011** (the Opposed Application), along with documentary evidence and facts in support thereof.

2.5 In this representation by way of opposition, the following grounds enumerated in Section 25 (1) of The Patents Act, 1970 are relied upon (hereinafter referred to as the "Act"):

(a) that the applicant for the patent or the person under or through whom he claims, wrongfully obtained the invention or any part thereof from him or from a person under or through whom he claims;

(b) that the invention so far as claimed in any claim of complete specification has been published before the priority date of the claim –

i) in any specification filed in pursuance of an application for a patent made in India on or after the 1st day of January, 1912; or

ii) in India or elsewhere, in any other document

Provided that the ground specified in sub-clause (ii) shall not be available where such publication does not constitute an anticipation of the invention by virtue of sub-section (2) or sub-section (3) of section 29;

(c) that the invention so far as claimed in any claim of the complete specification is claimed in a claim of a complete specification published on or after the priority date of the applicant's claim and filed in pursuance of an application for a patent in India, being a claim of which the priority date is earlier than that of the applicant's claim;

(d) that the invention so far as claimed in any claim of the complete specification was publicly known or publicly used in India before the priority date of the claim.

Explanation:- For the purpose of this clause, an invention relating to a process for which a patent is claimed shall be deemed to have been publicly known or publicly used in India before the priority date of the claim if a product made by that process had already been imported into India before that date except where such importation has been for the purpose of reasonable trial or experiment only;

(e) That the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step having regard to the matter published as mentioned in clause (b) or having regard what was used in India before the priority date of the applicant's claim;

(f) that the subject matter of any claim of the complete specification is not invention within the meaning of this Act, or is not patentable under this Act

(g) that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed;

(h) that the applicant has failed to disclose to the Controller the information required by section 8 or has furnished the information which in any material particular was false to his knowledge.

(i) that in the case of convention application, the application was not made within twelve months from the date of the first application for protection for the invention made in a convention country by the applicant or a person from whom he derives title;

(j) that the complete specification does not disclose or wrongly mentions the source or geographical origin of biological material used for the invention;

(k) That the invention so far as claimed in any claim of the complete specification is anticipated having regard to the knowledge, oral or otherwise, available within any local or indigenous community in India or elsewhere.

The present Representation By Way Of Opposition U/S 25(1) takes into consideration the following documents:

Document 1 [D1]-US20050239881 published on 27th October 2005

Document 2 [D2] - Drugs and Pharmaceutical Sciences, Volume-95, Polymorphism in pharmaceutical solids, Chapter-5, "Generation of Polymorphs, Hydrates, Solvates and Amorphous Solids", Page no. 183-186, 1999; By J. Keith Gniloroy, Edited by Harvey G. Britain.

Document 3 [D3] - Crystalline Polymorphism of Organic Compounds by Mino R. Cairra, pages 163-203 in Topic in Current Chemistry, vol. 198 published by Springer Verlag.

Document 4 [D4] - Knapman, U; Modern Drug Discovery, 2000, 57.

Document 5 [D5] - European Journal of Pharmaceutics & Biopharmaceutics, 55 (2003), 345-349.

Document 6 [D6] – Specification of Opposed Application.

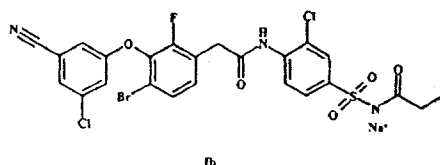
3.0 LACK OF NOVELTY

In connection with the above mentioned ground of Opposition, we would rely upon the following documents, which were available to public prior to the priority date of the Opposed Application:

Document 1 [D1]–US20050239881 published on 27th October 2005

3.1 Claim 1 of the opposed application lacks novelty over D1

Claim 1 of opposed patent application "*A crystalline form of a compound according to formula Ib*"



lacks novelty from following reason.

D1 discloses the compound I-63 (Paragraph [0068], Table 1) on claim 4 and claim 14 thereof, and describes a process for its preparation is the same as the compound Ib indicated in claim 1 of opposed patent application. Further, discloses compound in D1 is in solid form. This must have been either crystalline or amorphous form hence, is not novel. Therefore, claim 1 of opposed patent application not novel over D1.

3.2 Claims 1 to 18 of the opposed application lacks novelty over D1

D1 discloses the same compound as discloses in opposed patent application. The compound Ib was obtained in D1 in solid form. Discloses compound must have been either crystalline or amorphous form. Therefore, opposed patent application not novel in that the subject-matter of claims 1 to 17, which relates to crystalline forms, and claim 18 which relates to the amorphous form of Ib. Therefore, claims 1 to 18 of opposed patent application not novel over D1.

4.0 LACK OF INVENTIVE STEP

It is respectfully submitted that the alleged invention as described and claimed in the opposed specification lacks inventiveness and is obvious to a person skilled in the art. It is still obvious and lacks inventive step in view of the teachings contained in the following documents:

Document 1 [D1] – US20050239881 published on 27th October 2005

Document 2 [D2] – Drugs and Pharmaceutical Sciences, Volume-95, Polymorphism in pharmaceutical solids, Chapter-5, “Generation of Polymorphs, Hydrates, Solvates and Amorphous Solids”, Page no. 183-186, 1999; By J. Keith Gnillory, Edited by Harvy G. Brittain.

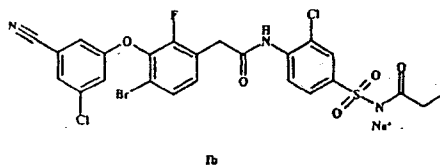
Document 3 [D3] – Crystalline Polymorphism of Organic Compounds by Mino R. Cairá, pages 163-203 in Topic in Current Chemistry, vol. 198 published by Springer Verlag.

Document 4 [D4] – Knapman, U; Modern Drug Discovery, 2000, 57.

Document 5 [D5] – European Journal of Pharmaceutics & Biopharmaceutics, 55 (2003), 345-349.

4.1 Claim 1 of the opposed patent application is obvious over D1 and general art

Claim 1 of the opposed patent application “*A crystalline form of a compound according to formula Ib*”



is obvious over following reason.

D1 discloses the compound I-63 (Paragraph [0068], Table 1) on claim 4 and claim 14 thereof, and describes a process for its preparation is the same as the compound Ib indicated in claim 1 of opposed patent application. Further, discloses compound in D1 is in solid form. This must have been either crystalline or amorphous form. A person skilled in the art who is working in the field of organic chemistry is very well aware about the importance and advantages of crystalline form. Further, It is also well known that organic

compounds exhibit polymorphism and most of the compounds can exist in more than one polymorphic form including their different salts forms (D2, Page-184-186). According to D3, a systematic investigation of a compound to determine whether it is prone to polymorphism or not is a matter of routine practice in pharmaceutical pre-formulation studies. Further, as per (D4) commercial software package are available to predict the possible polymorphs of an organic compound from its molecular structure. So, it is very easy to predict for possible polymorphic form of known compound. D1 disclosed the preparation of Compound Ib. The skilled person, having the commonly available knowledge that temperature and other process parameters may be altered for obtaining alternate polymorphic forms, would try to alter these conditions and thereby reach the polymorphic forms by routine experimentation.

The patent document does not provide any specific surprising effects for the new polymorphic form than the prior art crystalline form. In absence of any data, one cannot conclude that the new form will have the same effect of the earlier known forms.

This is because it is known that different polymorphic forms of the same substance differ in their therapeutic activity to the extent that one of the polymorphic form may be toxic. Thus, it is reported that one polymorph of chloramphenical-3-palmitate can have 8 fold higher bioactivity than the other, creating the danger of fatal doses when the unwanted polymorph is unwittingly administered (D4). Another such example of different polymorphic forms of the same substance having different therapeutic profile as well as toxicity profile is reported for Mebendazole in D5. Therefore, one cannot extrapolate the properties, specifically pharmaceutical properties, of one polymorphic form to the other, and opposed patent application has prepared a new polymorphic form of unapproved compound. Thus, the present invention is not patentable because it fails to provide any utility for the new polymorphic form.

Further, the new polymorphic form does not provide any data showing the new form to be in any way superior or showing any superior beneficial properties compared to the known form. Therefore, since making alternate polymorphic forms of a substance is a routine exercise well within the skills of a person skilled in the art, in the absence of any superior beneficial/surprising effect, the

present invention as claimed in claim 1 of the opposed patent is obvious and no patent can be granted for this invention.

Further, claim 1 of opposed patent application not novel. Therefore, claim 1 of opposed patent application does not fulfill the criteria of non-obviousness, and obvious over D1.

4.2 Claim 2 of the opposed patent application is obvious over general art

Claim 2 of the opposed patent application "*A process for preparing a polymorph of the crystalline form of claim 1, comprising crystallizing the compound (Ib) from THF, water, and nBuAc*" is obvious over following reason.

Claim 2 of opposed patent application stated process of crystallizing compound comprising THF, Water, nBuAc. Selection of solvent it is depended on the solubility of the compound. A person skilled in the art working in the field of organic chemistry, he would able to chose suitable solvent for crystallization. Hence with the teachings of general art any person skilled in the relevant art who is working in the field of organic chemistry is capable enough to prepare crystallization comprising THF, Water and nBuAc as a solvent. Further, claim 2 of opposed patent application depend on claim 1 of opposed patent application, and claim 1 of opposed patent application not novel as well as obvious. Therefore, claim 2 of opposed patent application does not fulfill the criteria of non-obviousness and not comprising any inventive steps

4.3 Claims 3 to 11 of the opposed patent application is obvious over D1 and general art

Claim 3 of the opposed patent application "*A polymorphic crystalline form (Form I) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:*

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
15.1	100.0	6.0	39.5
10.9	21.9	3.2	30.0

Claim 4 of the opposed patent application "*A polymorphic crystalline form (Form II) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:*

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
13.2	100.0	7.6	17.3
9.8	44.0	5.3	30.5
7.9	20.9		

Claim 5 of the opposed patent application "*A polymorphic crystalline form (Form III) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:*

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
6.8	100.0	3.7	36.9
4.6	44.0	3.6	42.7
4.4	31.7	3.4	32.3
4.1	31.5		

Claim 6 of the opposed patent application "*A polymorphic crystalline form (Form IV) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:*

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
12.9	43.0	4.6	25.6
11.3	100.0	4.0	23.7

Claim 7 of the opposed patent application "*A polymorphic crystalline form (Form V) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:*

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
13.9	56.2	5.5	45.4
10.8	58.2	3.4	54.9
10.1	100	3.2	27.5
5.7	87.7		

Claim 8 of the opposed patent application "*A polymorphic crystalline form (Form VI) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:*

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
13.4	100.0	5.4	37.6
10.9	38.4	3.6	41.8
9.8	48.4	3.4	38.8

5.7	40.1	3.2	35.4
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Claim 9 of the opposed patent application "A polymorphic crystalline form (Form VII) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
13.9	100.0	3.7	26.2
10.2	33.4	3.4	36.7
5.6	33.0	3.3	27.9

Claim 10 of the opposed patent application "A polymorphic crystalline form (Form VIII) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
7.2	65.6	4.1	45.2
6.7	34.3	3.9	100.0
6.1	45.3	3.4	43.4
4.7	53.9		

Claim 11 of the opposed patent application "A polymorphic crystalline form (Form IX) of the compound of claim 1 with an x-ray powder diffraction trace having a D-spacing essentially as shown:

D-space	I/I ₀ X 100	D-space	I/I ₀ X 100
7.2	65.6	4.1	45.2
6.7	34.3	3.9	100.0
6.1	45.3	3.4	43.4
4.7	53.9		

are obvious over D1 and general art following reason.

It is also well known that organic compounds exhibit polymorphism and most of the compounds can exist in more than one polymorphic form including their different salts forms (D3, Page-184-186). According to D2, a systematic investigation of a compound to determine whether it is prone to polymorphism or not is a matter of routine practice in pharmaceutical pre-formulation studies. Claims 3 to 11 of opposed patent application describe the X-ray powder diffraction data of form I to IX. It is well known in general art to characterize polymorphic form of a compound by X-ray powder diffraction method (D2). A

skilled person from knowledge of D3 and reading of D2 can acquire data from X-ray. Finding the XRD or other physical characterization data of a known compound is not inventive. Further, a claim 3 to 11 of opposed patent application is depends on claim 1 of opposed patent application and claim 1 of opposed patent application does not fulfill the criteria of novelty over D1.

Therefore, claims 3 to 11 of opposed patent application is obvious over D2, D3 and general art.

4.4 Claim 17 of the opposed patent application is obvious over D1 and general art

Claim 17 of the opposed patent application "*A pharmaceutical composition comprising any one of the polymorphic crystalline form of claims 3-11 in admixture with at least one pharmaceutically acceptable carrier, diluents or excipient*" is obvious over D1 and general art following reason.

Claim 17 of opposed patent application directed towards a pharmaceutical composition comprising polymorphic crystalline form of **Ib**. D1 is directed towards compounds having a pharmaceutical activity. It is considered, therefore, that it would be obvious for the skilled person to provide a pharmaceutical composition comprising **Ib**. Further, Several books and general art [e.g. (1) Remington: The Science and Practice of Pharmacy, Volume I & II. 19th edition, (2) Pharmaceutics: The Science of Dosage Form Design edited by M. E. Aulton (1998 edition), (3) Pharmaceutical Dosage Forms and Drug Delivery Systems by Anael, Popovich and Allen, 6th edition, (4) The Theory and Practice of Industrial Pharmacy, by Lachman, Lieberman and Kanig. 3rd edition, (5) Pharmaceutical Dosage form: Tablets, Volume - II edited by Lieberman Lachman and Schwartz 2nd edition] are available which teach preparation of pharmaceutical compositions and other pharmaceutical compositions along with use of pharmaceutically acceptable carriers, excipients and diluents to a person skilled in the relevant art. Hence with the teachings of aforementioned prior art any person skilled in the relevant art who is working in the field of formulation and development is capable enough to prepare pharmaceutical composition comprising polymorphic crystalline form of compound **Ib** with the suitable pharmaceutically acceptable carrier.

The objection against obviousness could have been overcome by showing any surprising/superior or unexpected effect of the allegedly claimed pharmaceutical composition but no evidences from the opposed specification are revealed that the pharmaceutical composition of the present invention has any unexpected effect.

Therefore, one has to reach the conclusion that the pharmaceutical composition allegedly claimed in Claim 17 of the opposed application is well within the capabilities of those skilled in the art and therefore Claim 17 is deemed to be obvious in the light of above submissions.

Therefore, claim 17 of the opposed patent application is obvious over general art.

4.5 Claim 18 of the opposed patent application is obvious over D1 and general art

Claim 18 of the opposed patent application "*A pharmaceutical composition comprising of the amorphous state of Ib in admixture with at least one pharmaceutically acceptable carrier, diluents or excipient*" is obvious over D1 and general art following reason.

Claim 18 of opposed patent application directed towards a pharmaceutical composition comprising amorphous Ib. D1 is directed towards compounds having a pharmaceutical activity. It is considered, therefore, that it would be obvious for the skilled person to provide a pharmaceutical composition comprising Ib. Further, Several books and general art [e.g. (1) Remington: The Science and Practice of Pharmacy, Volume I & II. 19th edition, (2) Pharmaceutics: The Science of Dosage Form Design edited by M. E. Aulton (1998 edition), (3) Pharmaceutical Dosage Forms and Drug Delivery Systems by Anacleto, Popovich and Allen, 6th edition, (4) The Theory and Practice of Industrial Pharmacy, by Lachman, Lieberman and Kanig. 3rd edition, (5) Pharmaceutical Dosage form: Tablets, Volume - II edited by Lieberman Lachman and Schwartz 2nd edition] are available which teach preparation of pharmaceutical compositions and other pharmaceutical compositions along with use of pharmaceutically acceptable carriers, excipients and diluents to a person skilled in the relevant art. Hence with the teachings of aforementioned prior art

any person skilled in the relevant art who is working in the field of formulation and development is capable enough to prepare pharmaceutical composition comprising amorphous form of compound Ib with the suitable pharmaceutically acceptable carrier.

The objection against obviousness could have been overcome by showing any surprising/superior or unexpected effect of the allegedly claimed pharmaceutical composition but no evidences from the opposed specification are revealed that the pharmaceutical composition of the present invention has any unexpected effect.

Therefore, one has to reach the conclusion that the pharmaceutical composition allegedly claimed in Claim 18 of the opposed application is well within the capabilities of those skilled in the art and therefore Claim 18 is deemed to be obvious in the light of above submissions.

Therefore, claim 18 of the opposed patent application is obvious over general art.

4.6 Claim 16 of the opposed patent application is obvious over D1 and general art

Claim 16 of the opposed patent application "*Use of any of the polymorphic crystalline form of claims 3-11 and the a,prujpris state pf Ib for the preparation of medicament for the therapeutic and/or prophylactic treatment of diseases which are associated with HIV*" is obvious over D1 and general art following reason.

D1 teaches in the example 51 and 52 that the compound I-63 is the use for the preparation of medicament for the therapeutic and/or prophylactic treatment of diseases which are associated with HIV. Claim 16 also describe the same compound for the preparation of medicament for the therapeutic and/or prophylactic treatment of diseases which are associated with HIV. Therefore it is respectfully submitted that Claim 16 of the present application is well within the capabilities of those skilled in the art and is obvious in the light of above submissions.

Therefore, claim 16 of the opposed patent application is obvious over D1 and general art.

5.0 NON-PATENTABLE SUBJECT MATTER

5.1 Section 3(d)

According to Section 3(d) of Indian Patent Act, "the mere discovery of a new form of a substance which does not result in the enhancement of a known efficacy of that substance or the mere discovery of a new property or new use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant.

Explanation: For the purpose of this clause, salts, esters, ethers, polymorphs, metabolites, pure form, particle size, isomers mixtures of isomers, complexes, combinations and other derivatives of known substance shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy."

5.1.1 Claims 1 to 15 are not patentable under section 3(d) of the Act

It is respectfully submitted that the polymorphic form of 2-[4-Bromo-3-(3-chloro-5-cyano-phenoxy)-2-fluoro-phenyl]-N-(2-chloro-4-propionylsulfamoyl-phenyl)-acetamide sodium of the present invention does not fulfill the requirement to be patentable under Section 3(d) and hence is not patentable under Section 3(d) of the Indian Patent Act for following reason.

It is respectfully submitted that claims 1-15 of the opposed patent application are not an invention within the meaning of this Act or are not patentable under Section 3(d) of this Act. According to Section 3(d) of Indian Patent Act, mere discovery of new form in way of salt of known substance is not patentable unless there is increase in therapeutic efficacy. Applicant of patent has not showing any surprising effect to prove better efficacy or comparison data of the claimed polymorphic form of 2-[4-Bromo-3-(3-chloro-5-cyano-phenoxy)-2-fluoro-phenyl]-N-(2-chloro-4-propionylsulfamoyl-phenyl)-acetamide sodium compared to prior art compound. Further, applicant has not provided any comparative data of the new crystalline form that shows the new form to be in any way superior or showing any superior beneficial properties compared to the known compound.

According to Intellectual Property Appellate Board (IPAB) decision dated 26th June 2009, a new form of a known substance can be patentable provided they show substantial improvement in the therapeutic efficacy as compared to prior art. This means there has to be an improvement in the therapeutic content or capacity in the same amount of drug compound of the present invention vis-à-vis prior art compound or marketed drug. Thus according to the decision of Intellectual Property Appellate Board (IPAB) pharmacokinetics or bioavailability increase is not related to therapeutic efficacy.

In such circumstances of failure to prove efficacy of the claimed new crystalline form of compound 2-[4-Bromo-3-(3-chloro-5-cyano-phenoxy)-2-fluoro-phenyl]-N-(2-chloro-4-propionylsulfamoyl-phenyl)-acetamide sodium is merely a new form of known substance which is not patentable u/s 3(d) of the act. Therefore, the present invention as claimed in claims 1-15 of the present application constitute non-patentable subject matter as per the provisions of section 3(d) of the Indian Patent Act.

5.2 Section 3(e)

According to Section 3(e) of the Indian Patent Act, "*a substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance;*"

5.2.1 Claim 17 are not patentable under Section 3(e) of the Act

Claim 17 of the opposed patent application are not patentable under Section 3(e) for the following reason.

Claim 17 of the opposed patent application describe the pharmaceutical composition comprising any one of the polymorphic crystalline form of claims 3-11 in admixture with at least one pharmaceutically acceptable carrier, diluents or excipient. Further, the opposed application does not state anything surprising or superior regarding the pharmaceutical composition of the present invention; hence in absence of such essential information, the pharmaceutical composition of the present invention is deemed to have no surprising effect. In absence of any data to the contrary such pharmaceutical composition will be consider a

substance obtained by a mere admixture and therefore is not patentable under Section 3(e) of the Indian Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005.

Therefore, in absence of any synergistic effect of the pharmaceutical composition comprising any one of the polymorphic crystalline form of claims 3-11 in admixture with at least one pharmaceutically acceptable carrier, diluents or excipient is not patentable under Section 3(e) of the Indian Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005.

5.2.2 Claim 18 are not patentable under Section 3(e) of the Act

Claim 18 of the opposed patent application are not patentable under Section 3(e) for the following reason.

Claim 18 of the opposed patent application describe the pharmaceutical composition comprising amorphous state of **Ib** in admixture with at least one pharmaceutically acceptable carrier, diluents or excipient. Further, the opposed application does not state anything surprising or superior regarding the pharmaceutical composition of the present invention; hence in absence of such essential information, the pharmaceutical composition of the present invention is deemed to have no surprising effect. In absence of any data to the contrary such pharmaceutical composition will be consider a substance obtained by a mere admixture and therefore is not patentable under Section 3(e) of the Indian Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005.

Therefore, in absence of any synergistic effect of the pharmaceutical composition comprising amorphous state of **Ib** in admixture with at least one pharmaceutically acceptable carrier, diluents or excipient is not patentable under Section 3(e) of the Indian Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005.

6.0 Lack of clarity and insufficiency according to section 25(1)(g)

Without prejudice to what is submitted above, it is submitted that the Opposed Application does not contain sufficient information to enable the person skilled in the art to carry out the alleged invention as claimed.

According to section 12(1) (a) read with section 10(4) of the Indian Patent Act during the examination the learned Controller considers whether the complete specification fully and particularly describes the invention:

According to section 25(1) (g), whereas in opposition proceedings the Learned controller decides whether the complete specification sufficiently and clearly describes the invention or the method by which it is to be performed.

It is frequently stressed that the monopoly of a patent may only be awarded for subject matter which is re-workable for the skilled person within the entire claimed range. Subject matter which is not accessible based on the provided teaching and by using ordinary skill is to be excluded from the patent protection.

Claim 2 is directed towards the preparation of a polymorph of the crystalline form of claim 1. Claim 1 is, however, just directed to a crystalline form and not a polymorphic form. As such this claim is unclear since there is a contradiction between claims 1 and 2.

Claim 15 of opposed patent application is directed towards a polymorphic crystalline form of compound Ib prepared by the process of claim 2, that is the product is defined by means of the process. It is considered that this claim does not clearly identify the polymorph using unique identifying features.

In the description it is stated that the form II can be prepared from heating/drying form I (without indicating conditions such as temperature and pressure), but also by suspending form II in methanol (page 28, without indicating conditions such as temperature/time). Form II, however, is anhydrous, and it is, therefore, impossible to see how the anhydrous form III can be converted to the hydrate by suspending in methanol.

The application contains an example, example 3, in which it is stated that the polymorph II is prepared. It appears that the compound Ib is crystallized from a

mixture of at least nBuOH and nBuOAc (considering the conditions used THF is expected to be distilled out of the solution) to obtain the polymorph II. These conditions are not indicated on page 28.

Example 7 describes the preparation of polymorph VII from polymorph III. It is considered that nowhere in the application is there a specific example for the preparation of form III (the only preparation being the general description on page 28, where no crystallization conditions are shown). It is therefore, unclear how the form VIII can be prepared from this if it is not clear how to obtain the form III. Also on page 28, line 17-22 conditions for obtaining form VIII are describe, the first of these being crystallization from THF/water/butyl acetate (that is from a ternary solvent mixture). In example 7, however, it is not clear that the conversion of the dry free acid into the salt is performed using water, it only states that the Na-2-ethyl hexanoate is soluble but not in what (it could just mean soluble in THF), then further on in the example it states that the "THF is replaced by atmospheric distillation by butyl acetate". The normal interpretation of replaced is that the first component is removed and substituted by the second component completely (otherwise the example should have stated partially replaced, which is not the case). That is the example is worded such that the skilled man would consider that the final solution from which the crystals are separated there is no more THF present. The example 7 teaches then that the crystallization is performed from butyl acetate and not from a ternary mixture of THF/water/butyl acetate as stated on page 28. There appears to be a contradiction within the application which creates unclarity as to how the form III can actually be prepared.

The application states that the form I can be obtained by crystallization from THF/water/n-butanol/n-butyl acetate (page-27, line 28-29). Then on page 28 it is indicated that form III can be obtained by crystallization from THF/water/n-butanol/n-butyl acetate (page 28, line 4-5). That is from the same solvent mixture two separate forms can be obtained. As such it is considered that the preparation of forms II and III is not defined in such a manner that it is exactly clear how they are obtained since apparently essential technical features are missing.

For the forms I, IV, V, VI, VII and IX there are no concrete examples as to how they can be prepared apart from the very general indications given on page 27-28. It appears that it would require extensive experimentation on the part of the skilled person to determine the conditions under which the desired product can be obtained, since the application gives no guidance whatsoever as to the crystallization conditions.

Claim 16 defines the "a,prujpris state pf lb". the meaning of this term is not clear which leads to a lack of clarity of this claim.

7.0 **Section 25(1)(h)**

The controller should verify that the information of corresponding application in other application in other countries have been correctly provided and if not the patent application should be rejected under section 8.

It is also respectfully prayed that the Controller should check whether the Applicant of the opposed application has dutifully informed the status of every other application relating to the same or substantially the same invention. If any, filed in any country outside India subsequently to the filing of the statement referred to in the Section 8(1)(a) as required under Section 8(1)(b) of the Patents Act, 1970 as amended by the Patents (Amendment) Act, 2005. If such information is not provided, it is respectfully submitted that the opposed patent under opposition is liable to be rejected on this ground alone. Therefore, it is our contention that the present application is obvious and constitutes subject matter which is not patentable under the Indian Patent Act. Therefore, this application should not be granted.

8.0 The Opponents' hereby submit that Claims 1-18 contained in the opposed Indian Patent Application No. 1576/CHENP/2011 is not patentable under the Act.

9.0 The Opponents further submits that Claims 1-18 contained in the opposed Indian Patent Application No. 1576/CHENP/2011 are not inventive and not patentable under the Act. The Opposed Application concerns "*Polymorphs of acyl sulfonamides*". The Opposed Application does not fulfill the patentability

criteria under the Act. The subject matter of the claims lack of inventive step over the prior art. The Opposed Application also does not sufficiently and clearly describe the alleged invention for it to be carried out by a person skilled in the art.

10.0 Accordingly, it is respectfully submitted that the Opposed Application does not contain sufficient information to enable the person skilled in the art to perform the invention disclosed and claimed in opposed Indian Patent Application No. 1576/CHENP/2011. Therefore, this ground of opposition has been established and the entire Opposed Application ought to be rejected on this ground alone.

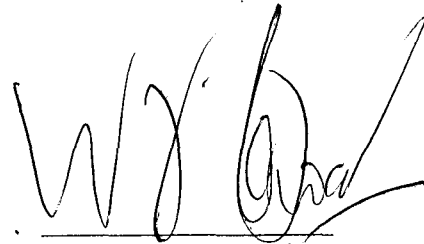
CONCLUSION

11.0 In view of the submissions presented above, we humbly pray that:

- i) the Indian Patent Application No. **1576/CHENP/2011** be dismissed *into to*;
- ii) any other relief as the Learned Controller may deem fit be awarded in favor of the Opponents.

As a matter of precaution we request the Learned Controller to grant us an oral hearing before disposing of this application.

Dated this the ¹³ day of ^{Jan} ~~Oct~~ 2012



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Constituted Attorney
for the Opponent
IPA NO 654



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23, 2004.****Publication Classification**(51) **Int. Cl.⁷** A61K 31/277; C07C 311/18(52) **U.S. Cl.** 514/522; 558/410(57) **ABSTRACT**

The present invention provides compounds for treating or preventing an HIV infection, or treating AIDS or ARC comprising administering a compound according to formula I where Ar, R¹-R⁵, R^{7a}, R^{7b}, R^{7c}, R⁸-R¹⁰, X¹, X² m, n, o and p are as defined herein.

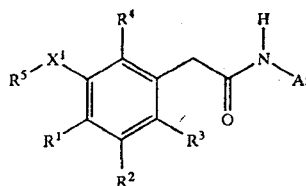
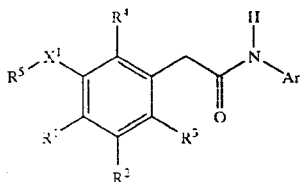


TABLE 1-continued

Cpd. No.	NAME	MS	MP
I-63	2-[4-Bromo-3-(3-chloro-5-cyano-phenoxy)-2-fluoro-phenyl]-N-(2-chloro-4-propionylsulfamoyl-phenyl)-acetamide; sodium salt	(M + I) ⁺ 630	164.8–166.2
I-64	2-[3-(3-Chloro-5-cyano-phenoxy)-4-methoxy-phenyl]-N-(2-chloro-4-sulfamoyl-phenyl)-acetamide	(M) + 505	186.3–189.7
I-65	2-[3-(2-Chloro-5-cyano-phenoxy)-4-methoxy-phenyl]-N-(4-sulfamoyl-phenyl)-acetamide	(M) + .471	252.3–254.3
I-66	2-[3-(2-Chloro-5-cyano-phenoxy)-4-methoxy-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M) + .485	232.9–236.9
I-67	2-[3-(2-Chloro-5-cyano-phenoxy)-4-methoxy-phenyl]-N-(2-chloro-4-sulfamoyl-phenyl)-acetamide	(M) + .505	214.6–216.4
I-68	2-[4-Chloro-3-(4-cyano-2,6-dimethyl-phenoxy)-phenyl]-N-(2-chloro-phenyl)-acetamide	(M + H) ⁺ 425	183.9–185.1
I-69	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-(4-methylsulfamoyl-phenyl)-acetamide		
I-70	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-(4-dimethylsulfamoyl-phenyl)-acetamide		
I-71	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-[4-(piperidine-1-sulfonyl)-phenyl]-acetamide		
I-72	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-[4-(morpholine-4-sulfonyl)-phenyl]-acetamide		
I-73	2-[3-(3-Chloro-5-cyano-phenoxy)-4-methoxy-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 484	222.3–224.3
I-74	2-[3-(3-Chloro-5-cyano-phenoxy)-2-fluoro-4-methyl-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 486	230.0–232.2
I-75	2-[4-Chloro-3-(3-cyano-5-difluoromethyl-phenoxy)-2-fluoro-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M + H) ⁺ 524	
I-76	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-(2-chloro-4-methanesulfonyl-phenyl)-acetamide		
I-77	N-{4-[(S)-2-Amino-3-methyl-butylsulfamoyl]-2-methyl-phenyl}-2-[4-chloro-3-(3-chloro-5-cyano-phenoxy)-2-fluoro-phenyl]-acetamide; compound with hydrochloric acid	(M + H) ⁺ 607	169.0–180.3
I-78	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-(4-cyanomethoxy-phenyl)-acetamide		
I-79	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-(4-methanesulfonyl-phenyl)-acetamide		
I-80	3-Chloro-4-{2-[4-chloro-3-(3,5-dicyano-phenoxy)-phenyl]-acetyl amino}-benzoic acid methyl ester		
I-81	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-[4-{(2-hydroxy-ethyl)-methyl-sulfamoyl}-phenyl]-acetamide		
I-82	2-[4-Chloro-3-(3,5-dicyano-phenoxy)-phenyl]-N-[4-(4-hydroxy-piperidine-1-sulfonyl)-phenyl]-acetamide		
I-83	2-[3-(3-Chloro-5-cyano-phenoxy)-2-fluoro-4-methyl-phenyl]-N-(2-chloro-4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 506	228.9–230.6
I-84	N-(2-Chloro-4-sulfamoyl-phenyl)-2-[3-(3-cyano-5-difluoromethyl-phenoxy)-2-fluoro-4-methyl-phenyl]-acetamide	(M – H) [–] 522	218.0–218.7
I-85	2-[3-(3-Cyano-5-difluoromethyl-phenoxy)-2-fluoro-4-methyl-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M + H) ⁺ 504	218.0–220.4
I-86	N-(2-Chloro-phenyl)-2-[3-(3-cyano-5-difluoromethyl-phenoxy)-2-fluoro-4-methyl-phenyl]-acetamide	(M + H) ⁺ 445	163.3–164.4
I-87	2-[4-Chloro-3-(3-chloro-5-cyano-phenoxy)-2-fluoro-phenyl]-N-[2-methyl-4-{(pyridine-3-carbonyl)-sulfamoyl}-phenyl]-acetamide; compound with hydrochloric acid	(M + H) ⁺ 613	261.8–263.6
I-88	2-[4-Chloro-3-(3-cyano-5-trifluoromethyl-phenoxy)-phenyl]-N-(4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 508	219–221.4
I-89	2-[4-Chloro-3-(3-cyano-5-trifluoromethyl-phenoxy)-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 522	211.6–214.6
I-90	2-[4-Chloro-3-(3-cyano-5-trifluoromethyl-phenoxy)-phenyl]-N-(2-chloro-4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 542	198.0–202.0
I-91	2-[3-(3-Cyano-5-difluoromethyl-phenoxy)-4-ethyl-phenyl]-N-(4-sulfamoyl-phenyl)-acetamide	(M + H) ⁺ 486	188.0–198.0
I-92	2-[3-(3-Cyano-5-difluoromethyl-phenoxy)-4-methoxy-phenyl]-N-(4-sulfamoyl-phenyl)-acetamide	(M + H) ⁺ 488	213.9–214.2
I-93	2-[3-(3-Cyano-5-difluoromethyl-phenoxy)-4-methoxy-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M + I) ⁺ 502	175.0–177.2
I-94	N-(2-Chloro-4-sulfamoyl-phenyl)-2-[3-(3-cyano-5-difluoromethyl-phenoxy)-4-methoxy-phenyl]-acetamide	(M – H) [–] 520	185.0–187.9
I-95	2-[4-Chloro-3-(3-cyano-5-(1,1-difluoro-ethyl)-phenoxy)-2-fluoro-phenyl]-N-(4-sulfamoyl-phenyl)-acetamide	(M – H) [–] 522	207.0–209.8
I-96	2-[4-Chloro-3-(3-cyano-5-(1,1-difluoro-ethyl)-phenoxy)-2-fluoro-phenyl]-N-(2-methyl-4-sulfamoyl-phenyl)-acetamide	(M + H) ⁺ 538	198.5–201.0

We claim:

1. A compound according to formula I



wherein

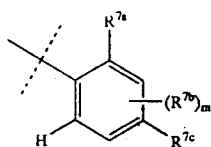
X^1 is $-O-$;

R^1 and R^2 are (i) each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} sulfonyl, C_{1-6} haloalkoxy, C_{1-6} haloalkylthio, halogen, amino, alkylamino, dialkylamino, aminoacyl, nitro and cyano; or, (ii) together R^1 and R^2 are $-O-CH=CH-$ or $-O-CH_2CH_2-$ provided that R^1 is not hydrogen;

R^3 and R^4 are independently selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{1-6} alkylthio, C_{1-6} haloalkylthio, halogen, amino, nitro and cyano;

R^5 is aryl substituted with one to three substituents independently selected from the group consisting of C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, $-C\equiv CCH_2OH$, $-C\equiv CCH_2NMe_2$, C_{1-6} haloalkyl, C_{3-8} cycloalkyl, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} sulfonyl, C_{1-6} haloalkoxy, C_{1-6} haloalkylthio, hydroxy, halogen, nitro and cyano, said alkyl and said cycloalkyl are optionally substituted with one or two substituents independently selected from the group consisting of alkyl, hydroxy, alkoxy, thiol, alkylthio, halogen, amino, alkylamino, dialkylamino, amino alkyl, alkylaminoalkyl, and dialkylamino;

Ar is a substituted phenyl ring according to formula IIa with the proviso that R^{7a} and R^{7c} are not both hydrogen or if R^{7c} is hydrogen, then R^{7a} is chlorine:



R^{7a} is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-3} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, halogen and cyano;

R^{7b} in each incidence is independently selected from the group consisting of C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} sulfonyl, amino, C_{1-6} alkylsulfonyl, $SO_2NR^{11a}R^{11b}$, C_{1-6} haloalkoxy, C_{1-6} haloalkylthio, hydroxy, amino, C_{1-6} alkylamino,

C_{1-6} dialkylamino, aminoacyl, acyl, $CONR^8R^9$, nitro, cyano, C_{1-6} heteroalkoxy, $-X^2(CH_2)_pS(O)_2NR^8R^9$, $-X^2(CH_2)_pNHC(O)NHR^8R^9$, $-X^2(CH_2)_pS(O)_2NR^8R^9$, and $-X^2(CH_2)_pNHCOOR^{10}$;

R^{7c} is selected from the group consisting of C_{1-6} heteroalkoxy, $-S(O)_2NR^8R^9$, $-X^2CH_2(CH_2)_pS(O)_2NR^8R^9$, $-X^2(CH_2)_pNHC(O)NHR^8R^9$, $X^6(CH_2)_vCoNR^8R^9$, $-SO_2R^{13}$, $-NR^8R^9$, $X^2(CH_2)_pNR^{11}S(O)_2NR^8R^9$, $-X^2(CH_2)_pNHCOOR^{10}$, $-X^6(CH_2)_vCOOR^{10}$, $-X^6(CH_2)_vCN$, $-OR^{15}$ and $C(=O)CH_2N[(CH_2)_2]_2X^4$;

R^8 and R^9 (i) taken independently, one of R^8 and R^9 is hydrogen or C_{1-6} alkyl and the other of R^8 and R^9 is selected from the group consisting of hydrogen, $-C(=O)R^{14}$, $-C(=O)CR^{12}NH_2$, $-(CH_2)_2N[(CH_2)_2]_2O$, $COCO_2Me$, C_{3-8} cycloalkyl said cycloalkyl optionally substituted with one or two hydroxyl substituents, pyranyl, C_{1-6} alkyl and aryl said alkyl and said aryl groups optionally substituted with one or two substituents independently selected from the group consisting of hydroxy, C_{1-6} alkoxy, thiol, C_{1-6} alkylthio, C_{1-6} alkylsulfinyl, C_{1-6} sulfonyl, and halogen; or, (ii) R^8 and R^9 taken together are $(CH_2)_2-X^5-(CH_2)_2$, $-(CH_2)_6-$ or $(CH_2)_2S(O)_n-$ optionally substituted with one or two substituents selected from the group consisting of halogen, hydroxyl and $NR^{11a}R^{11b}$;

R^{10} is C_{1-6} alkyl;

R^{11} is hydrogen or C_{1-6} alkyl;

R^{11a} and R^{11b} are independently R^{11} ;

R^{12} is the sidechain of a naturally occurring α -amino acid;

R^{13} is C_{1-6} alkyl; $-(CH_2)_uCO_2R^{11}$, $-(CH_2)_2CN$, $-(CH_2)_2NH_2$, $-(CH_2)_vOH$;

R^{14} is C_{1-10} alkyl, $-(CH_2)_nNHR^{11a}R^{11b}$, $(CH_2)_nOR^{11}$, $-CH_2CH(OH)CH_3$, $CH_2N[(CH_2)_2]_2O$, $-(CH_2)_2CO_2R^{11}$, optionally substituted phenyl or pyridinyl;

R^{15} is C_{1-6} alkyl substituted with one to three hydroxyl groups;

X^2 is $-O-$ or a bond;

X^4 is $-O-$ or $-NMe-$;

X^5 is $-O-$, $-S(O)_n-$ or NR^{11} ;

X^6 is $O-$ or $-S(O)_n-$;

m is an integer from 0 to 2;

n is an integer from 0 to 2;

o is an integer from 4 to 6;

p is an integer from 0 to 6;

r is an integer from 3 to 4

s is an integer from 1 to 2;

u is an integer from 2 to 3;

v is an integer from 2 to 6; and,

17. A compound according to claim 16 wherein R^5 is 2,4-disubstituted phenyl, 2,5-disubstituted phenyl, 3,5-disubstituted phenyl or 2,3,5-trisubstituted phenyl.

18. A compound according to claim 17 wherein R^5 is substituted with 2 to 3 groups independently selected from the group consisting of halogen, cyano, C_{1-6} alkyl, C_{3-8} cycloalkyl and C_{1-6} haloalkyl.

19. A compound according to claim 18 wherein R^{7c} is $-S(O)NR^8R^9$ or $-X^2CH_2(CH_2)_pS(O)_2NR^8R^9$, R^8 is hydrogen and R^9 is hydrogen, $C(=O)R^{14}$ or $-C(=O)R^{12}NH_2$.

* * * * *

D₂

Polymorphism in Pharmaceutical Solids

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5

Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids

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I. METHODS EMPLOYED TO OBTAIN UNIQUE POLYMORPHIC FORMS

Organic medicinal agents that can exist in two or more solid phases often can provide some distinct advantages in particular applications. The metastable solid may be preferred in those instances where absorption of the drug is dissolution rate dependent. The stable phase may be less susceptible to chemical decomposition and may be the only form that can be used in suspension formulations. Often a metastable polymorph can be used in capsules or for tableting, and the thermodynamically stable form for suspensions. Factors related to processing, such as powder flow characteristics, compressibility, filterability, or hygroscopicity, may dictate the use of one polymorph in preference to another. In other cases, a particular form may be selected because of the high reproducibility associated with its isolation in the synthetic procedure.

It is essential to ascertain whether the crystalline material that results from a synthetic procedure is thermodynamically stable before conducting pivotal trials, since a more stable form may be obtained subsequently, and it may be impossible to produce the metastable form in future syntheses. Conversion from one polymorph to another can occur during processing or upon storage. An additional incentive for

isolating and identifying polymorphs that provides certain advantages is the availability of subsidiary patents for desirable polymorphic forms, or for retaining a competitive edge through unpublished knowledge. In 1990 Byrn and Pfeiffer found more than 350 patents on crystal forms granted on the basis of an advantage in terms of stability, formulation, solubility, bioavailability, ease of purification, preparation or synthesis, hygroscopicity, recovery, or prevention of precipitation [1].

One question that is likely to arise during the registration process is "What assurance can be provided that no other crystalline forms of this compound exist?" It is incumbent on the manufacturer of a new drug substance to show that due diligence has been employed to isolate and characterize the various solid-state forms of a new chemical entity. This may seem to be a daunting task, particularly in light of the widely quoted statement by Walter C. McCrone [2] that "Those who study polymorphism are rapidly reaching the conclusion that all compounds, organic and inorganic, can crystallize in different crystal forms or polymorphs. In fact, the more diligently any system is studied the larger the number of polymorphs discovered." On the other hand, one can take comfort from the fact that some important pharmaceuticals have been in use for many years and have, at least until now, exhibited only one stable form. Indeed, it seems to this author that there must be particular bonding arrangements of some molecules that are so favorable energetically as to make alternate arrangements unstable or nonisolatable.

In the future, computer programs using force-field optimization should be perfected to the point where it will be possible to predict, with confidence, that a particular crystalline packing arrangement is the most stable that is likely to be found. These programs also may make it possible to predict how many alternate arrangements having somewhat higher energy can potentially be isolated [3,4]. Until that time, the developmental scientist is handicapped in attempting to predict how many solid forms of a drug are likely to be found. The situation is further complicated by the phenomenon of "disappearing polymorphs" [5], or metastable crystal forms that seem to disappear in favor of more stable ones.

Some polymorphs can be detected, but not isolated. Hot stage microscopy has been used extensively to study polymorphic transfor-

mations. The microscopist can detect numerous polymorphic transformations, but the individual polymorphs often prove to be so unstable that they cannot be isolated by the usual methods. An excellent example of this is the work of Grieser and Burger on etofylline [6]. These authors identified five polymorphic forms by thermomicroscopy, but only stable Modification I could be obtained by recrystallization, even when seed crystals from the hot stage were used. Similarly, Kuhnert-Brandstätter, Burger, and Völlenkle [7] described six polymorphic forms of piracetam, only three of which could be obtained by solvent crystallization. All the others were found only by crystallization from the melt. What, then, is a careful investigator to do?

In this chapter, the various methods used to isolate polymorphs, hydrates, and solvates will be described. As Bernstein [8] has observed, "The conditions under which different polymorphs are obtained exclusively or together also can provide very useful information about the relative stability of different phases and the methods and techniques that might be necessary to obtain similar structures of different chemical systems." In this context, it is hoped that the following information will prove useful in devising a "screening" protocol for the preparation of the various solid state forms of pharmaceuticals. While one cannot be absolutely certain that no additional forms will be identified in the future, this approach should provide some assurance that "due diligence" has been exercised to isolate and identify crystalline forms that are likely to arise during the normal course of drug development and storage.

A. Sublimation

On heating, approximately two-thirds of all organic compounds are converted partially from the solid to the gaseous state and back to solid, i.e., they sublime [9]. While strictly speaking the term sublimation refers only to the phase change from solid to vapor without the intervention of the liquid phase, it is often found that crystals are formed on cooler surfaces in close proximity to the melt of organic compounds when no crystals were formed at temperatures below the melting point. The most comprehensive information concerning sublimation temperatures of compounds of pharmaceutical interest can be found in tables

in the textbook of Kuhnert-Brandstätter [9]. While the information in these tables is designed primarily for the microscopic examination of compounds, it is also possible to utilize it to determine which compounds might be susceptible to the application of techniques (such as vacuum sublimation) that can be carried out on larger scales and at lower temperatures.

The sublimation temperature and the distance of the collecting surface from the material undergoing sublimation have a great influence on the form and size of the crystals produced. The occurrence of polymorphic modifications depends on the temperature of sublimation. In general, it may be assumed that unstable crystals form preferentially at lower temperatures, while at higher temperatures stable forms are to be expected. Nevertheless, mixtures consisting of several modifications are frequently found together. This is the case for barbital and for estradiol benzoate. It should be obvious that the sublimation technique is applicable only to those compounds that are thermally stable.

A simple test can be used to determine if a material sublimates. A small quantity (10–20 mg) of the solid is placed in a petri dish that is covered with an inverted watch glass. The petri dish is heated gently on a hot plate and the watch glass is observed to determine if crystals are growing on it. According to McCrone [2], one of the best methods for obtaining a good sublimate is to spread the material thinly over a portion of a half-slide, cover with a large cover glass, and heat slowly using a Kofler block. When the sublimate is well formed, the cover glass is removed to a clean slide for examination. It is also possible to form good crystals by sublimation from one microscope slide to a second held above it, with the upper slide also being heated so that its temperature is only slightly below that of the lower slide. Cooling of the cover slip by placing drops of various low-boiling solvents on the top surface will cause condensation of the more unstable forms, the lower temperatures leading to the most unstable forms. On a larger scale, a glass cold finger or a commercial sublimator can be employed. Once crystals of various modifications have been obtained, they can be used as seeds for the solution phase crystallization of larger quantities.

Form I of 9,10-anthraquinone-2-carboxylic acid was obtained as needle-like crystals upon sublimation at temperatures exceeding 250°C [10]. Fokkens et al. have used sublimation to purify theophylline for

from the literature that for some solutes it is the choice of solvent rather than the effects of supersaturation that determines the form that crystallizes [18].

Crystallization of mannitol as a single solute was found to be influenced by both the initial mannitol concentration and by the rate of freezing [19]. In the range of 2.5% to 15%, the δ -polymorph is favored by higher concentrations, whereas the β -polymorph is favored at lower concentrations. At constant mannitol concentration (10%), the α -polymorph is favored by a slow freezing rate, whereas the δ -polymorph is favored by a fast freezing rate.

Kaneko et al. [20] observed that both the cooling rate and the initial concentration of stearic acid in *n*-hexane solutions influenced the proportion of polymorphs A, B, C, and E that could be isolated. Garti et al. [21] reported that for stearic acid polymorphs crystallized from various organic solvents, a correlation was observed between the polymorph isolated and the extent of solvent-solute interaction.

The reason for using crystallization solvents having varying polarities is that molecules in solution often tend to form different types of hydrogen-bonded aggregates, and that these aggregate precursors are related to the crystal structures that develop in the supersaturated solution [22]. Crystal structure analysis of acetanilide shows that a hydrogen-bonded chain of molecules is aligned along the needle axis of the crystals. This pattern is characteristic of secondary amides that crystallize in a *trans* conformation so that the carbonyl acceptor group and the -NH hydrogen bond donor are anti to one another. The morphology of acetanilide crystals can be controlled by choosing solvents that promote or inhibit the formation of this hydrogen-bond chain. Hydrophobic solvents such as benzene and carbon tetrachloride will not participate in hydrogen-bond formation, so they will induce the formation of rapidly growing chains of hydrogen-bonded anides. Crystals grown by evaporation methods from benzene or carbon tetrachloride are long needles. Solvents that are proton donors or proton acceptors inhibit chain formation by competing with amide molecules for hydrogen-bonding sites. Thus acetone inhibits chain growth at the -NH end, and methanol inhibits chain growth at the carbonyl end of the chain. Both solvents encourage the formation of rod-like acetanilide crystals, while

mixtures of benzene and acetone give hybrid crystals that are rod-shaped, with fine needles growing on the ends [23].

Some solvents favor the crystallization of a particular form or forms because they selectively adsorb to certain faces of some polymorphs, thereby either inhibiting their nucleation or retarding their growth to the advantage of others. Among the factors affecting the types of crystal formed are (a) the solvent composition or polarity, (b) the concentration or degree of supersaturation, (c) the temperature, including cooling rate and the cooling profile, (d) additives, (e) the presence of seeds, (f) pH, especially for salt crystallization, and (g) agitation [22].

Martínez-Ohárriz et al. [24] found that Form III of diflunisal is obtained from polar solvents, whereas Forms I and IV are obtained from nonpolar solvents. Likewise, Wu et al. [25] observed that when moricizine hydrochloride is recrystallized from relatively polar solvents (ethanol, acetone, and acetonitrile), Form I is obtained, whereas nonpolar solvents (methylene chloride or methylene chloride/ethyl acetate) yield Form II.

In determining what solvents to use for crystallization, one should be careful to select those likely to be encountered during formulation and processing. Typically these are water, methanol, ethanol, propanol, isopropanol, acetone, acetonitrile, ethyl acetate, and hexane. Matsuda employed 27 organic solvents to prepare two polymorphs and six solvates of piretanide [26].

According to McCrone [27], in a poor solvent the rate of transformation of a metastable to a more stable polymorph is slower. Hence a metastable form once crystallized can be isolated and dried before it is converted to a more stable phase by solution phase mediated transformation. In some systems the metastable form is extremely unstable and may be prepared only with more extreme supercooling. This is usually performed on a very small scale with high boiling liquids so that a saturated solution at a high temperature that is suddenly cooled to room temperature will achieve a high degree of supersaturation [28].

There are many examples in the literature of the use of single solvents as crystallization screens. Slow crystallization from acetone, acetonitrile, alcohols, or mixtures of solvents yields the Form A of

fosinopril sodium, but rapid drying of a solution of this compound yields Form B, sometimes contaminated with a small amount of Form A [29]. A rotary evaporator can be used to maintain a solution at the appropriate temperature as solvent is being removed.

Form I of dehydroepiandrosterone was obtained by recrystallization from warm ethyl acetate, acetone, acetonitrile, or 2-propanol. Form II was obtained by rapid evaporation, using a vacuum from solutions in dioxane, tetrahydrofuran, or chloroform (which are higher boiling, less polar solvents) [30].

C. Evaporation from a Binary Mixture of Solvents

If single-solvent solutions do not yield the desired phase, mixtures of solvents can be tried. Multicomponent solvent evaporation methods depend on the difference in the solubility of the solute in various solvents. In this approach, a second solvent in which the solute is sparingly soluble is added to a saturated solution of the compound in a good solvent. Often a solvent system is selected in which the solute is more soluble in the component with the higher vapor pressure. As the solution evaporates, the volume of the solution is reduced and, because the solvents evaporate at different rates, the composition of the solvent mixture changes.

Occasionally, crystals are obtained by heating the solid in one solvent and then pouring the solution into another solvent or over cracked ice. Otsuka et al. [31] obtained phenobarbital Form B by adding dropwise a saturated solution of the compound in methanol to water at room temperature. Form E was obtained by the same technique, but by using a saturated solution of phenobarbital in dioxane.

Kitamura et al. have shown that the fraction of Form A of *L*-histidine decreases quickly when the volume fraction of ethanol in an ethanol-water solvent system increases above 0.2, and that pure Form B is obtained at a 0.4 volume fraction of ethanol [32]. The transformation rate for conversion of Form B to Form A decreases with ethanol concentration. The authors postulated that the concentration of the conformer that corresponds to Form A decreases more with ethanol concentration than that of Form B, and so the growth rate of Form A will also decrease.

An example of precipitation in the presence of a second solvent is seen in the case of indomethacin. The γ -crystal form of indomethacin can be obtained by recrystallization from ethyl ether at room temperature, but the α -form is prepared by dissolution in methanol and precipitation with water at room temperature [33]. Precipitation can also result from the addition of a less polar solvent. Form II of midodrine hydrochloride, metastable with respect to Form I, can be prepared by precipitation from a methanolic solution by means of a less polar solvent such as ethyl acetate or dichloromethane [34].

In Fig. 2, three crystalline modifications of thalidomide are illustrated. These were obtained by solvent recrystallization techniques and differ both in crystal habit and in crystal structure. Two of the forms were obtained from a single solvent, and one from a binary mixture.

D. Vapor Diffusion

In the vapor diffusion method, a solution of the solute in a good solvent is placed in a small, open container that is then stored in a larger vessel containing a small amount of a miscible, volatile nonsolvent. The larger vessel (often a desiccator) is then tightly closed. As solvent equilibrium is approached, the nonsolvent diffuses through the vapor phase into the solution, and saturation or supersaturation is achieved. The solubility of the compound in a precipitant used in a two-solvent crystallization method such as vapor diffusion should be as low as possible (much less than 1 mg/mL), and the precipitant (the solvent in which the compound is poorly soluble) should be miscible with the solvent and the saturated solution. The most frequent application of this technique is in the preparation of single crystals for crystallographic analysis. An illustration of the technique is provided in Fig. 3 [35].

E. Thermal Treatment

Frequently when using differential scanning calorimetry as an analysis technique, one can observe an endothermic peak corresponding to a phase transition, followed by a second endothermic peak corresponding to melting. Sometimes there is an exothermic peak between the two endotherms, representing a crystallization step. In these cases it is often

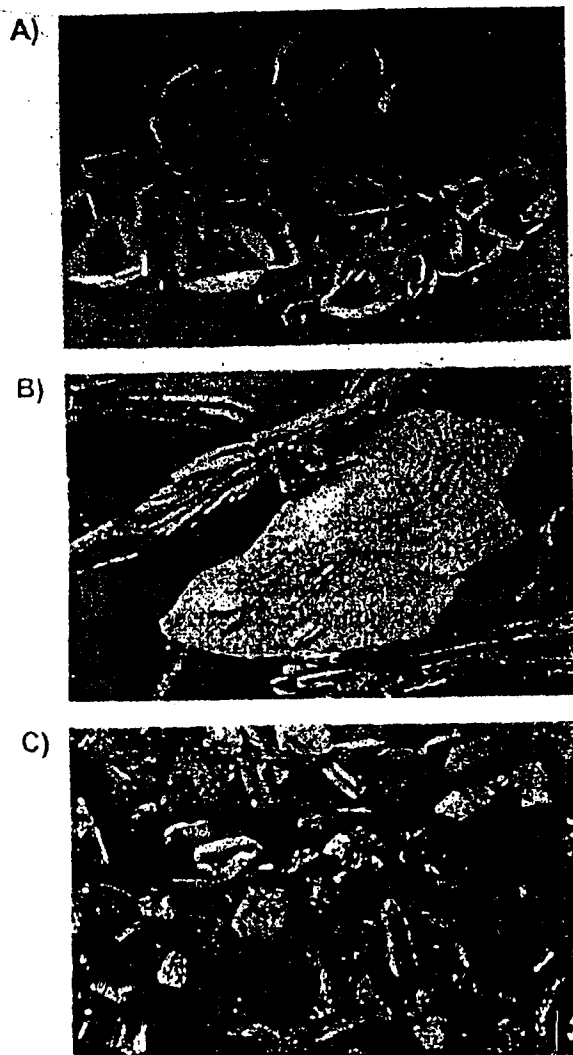


Fig. 2 Three crystalline modifications of thalidomide obtained by solvent recrystallization. (A) Form I obtained as bipyramids by slow crystallization of thalidomide in 1:1 dimethylformamide:ethanol at room temperature. (B) Form II obtained by immersing a saturated solution of thalidomide in acetonitrile in an ice bath. (C) Form III prepared as tabular crystals from a solution in boiling 1,4-dioxane, filtered, then allowed to cool to room temperature. (Photomicrographs courtesy of Dr. S. A. Botha, the University of Iowa.)

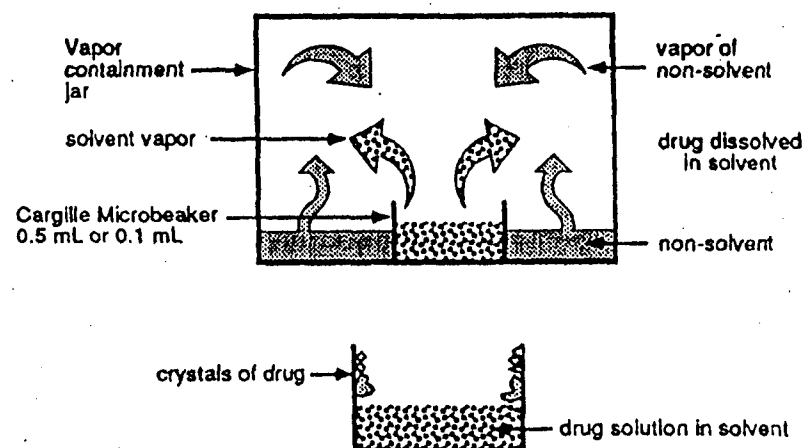


Fig. 3 Crystallization by vapor diffusion. (Reproduced with permission of the author [35] and the copyright holder, Pfizer, Inc.)

possible to prepare the higher melting polymorph by thermal treatment. Thus chlorpropamide Form A is obtained by recrystallization from ethanol solution, but Form C is obtained by heating Form A in an oven maintained at 100°C for 3 hours [36]. While the β -form of tegafur is obtained by the evaporation of a saturated methanol solution, the γ -form is obtained by heating the β -form at 130°C for one hour [37]. Form II of caffeine is prepared by recrystallization from distilled water, but Form I is prepared by heating Form II at 180°C for 10 hours [38].

F. Crystallization from the Melt

In accordance with Ostwald's rule [17], the cooling of melts of polymorphic substances often first yields the least stable modification, which subsequently rearranges into the stable modification in stages. Since the metastable form will have the lower melting point, it follows that supercooling is necessary to crystallize it from the melt. After melting, the system must be supercooled below the melting point of the metastable form, while at the same time the crystallization of the more stable form or forms must be prevented. Quench cooling a melt can

sometimes result in formation of an amorphous solid that on subsequent heating undergoes a glass transition followed by crystallization [39].

On a somewhat larger scale, one can use a vacuum drying pistol and a high boiling liquid such as chlorobenzene to achieve the desired end. Form II of *p*-(1*R*,3*S*)-3-thioanisoyl-1,2,2-trimethylcyclopentane carboxylic acid was obtained by recrystallization from a 50:50 v/v benzene:petroleum ether mixture. Form I then was obtained by melting Form II in the vacuum drying pistol [40]. Caffeine Form I is prepared by heating Form II at 180°C for 10 hours [38]. Yoshioka et al. [41] observed that when the amorphous solidified melt of indomethacin was stored at 40°C, it partly crystallized as the thermodynamically stable γ -form. Yet at 50°C, 60°C, and 70°C, mixtures of the α - and the γ -form were obtained. Sulfathiazole Form I is obtained by heating Form III crystals (grown from a dilute ammonium hydroxide solution at room temperature) at 170°C for 30–40 minutes [42].

G. Rapidly Changing Solution pH to Precipitate Acidic or Basic Substances

Many drug substances fall in the category of slightly soluble weak acids, or slightly soluble weak bases, whose salt forms are much more soluble in water. Upon addition of acid to an aqueous solution of a soluble salt of a weak acid, or upon addition of alkali to an aqueous solution of a soluble salt of a weak base, crystals often result. These crystals may be different from those obtained by solvent crystallization of the weak acid or weak base. Nucleation does not necessarily commence as soon as the reactants are mixed, unless the level of supersaturation is high, and the mixing stage may be followed by an appreciable time lag before the first crystals can be detected. Well-formed crystals are more likely to result in these instances than when rapid precipitation occurs.

Form I of the x-ray contrast agent iopanoic acid was prepared [43] by dissolving the acid in 0.1 N NaOH, adjusting the pH to 12.5, bubbling nitrogen into the solution, and adding 0.1 N hydrochloric acid until the pH reached 2.15. The resulting precipitate was vacuum filtered

and stored *in vacuo* (380 torr) for 12 hours at 35°C. Similarly, Form III of hydrochlorothiazide was precipitated from sodium hydroxide aqueous solution by the addition of hydrochloric acid [44].

When piretanide was dissolved in 0.1 N NaOH at room temperature and acid was added in a 1:1 ratio (to pH 3.3), piretanide Form C precipitated. However, when the base:acid ratio used was 1:0.95, a mixture of amorphous piretanide and Form C precipitated [45].

H. Thermal Desolvation of Crystalline Solvates

The term "desolvated solvates" has been applied to compounds that were originally crystallized as solvates but from which the solvent has been removed (generally by vaporization induced by heat and vacuum). Frequently, these "desolvated solvates" retain the crystal structure of the original solvate form and exhibit relatively small changes in lattice parameters. For this reason, these types have been referred to as pseudopolymorphic solvates. However, in instances where the solvent serves to stabilize the lattice, the process of desolvation may produce a change in lattice parameters, resulting in the formation of either a new crystal form or an amorphous form. These solvates have been referred to as polymorphic solvates. Byrn [46] has characterized the desolvation of polymorphic solvates as occurring in four steps, (a) molecular loosening, (b) breaking of the host-solvent hydrogen bonds (or other associations), (c) solid solution formation, and (d) separation of the product phase.

The process of desolvating pseudopolymorphic solvates is simpler, involving only the two steps of (a) molecular loosening and (b) breaking of host-solvent hydrogen bonds or associations. Byrn [46] has summarized the desolvation studies performed on caffeine hydrate, theophylline hydrate, thymine hydrate, cytosine hydrate, dihydrophenylalanine hydrate, dialuric acid hydrate, cycloserine hydrate, erythromycin hydrate, fenoprofen hydrate, manganous formate dehydrate, bis(salicylaldehyde) ethylenediamine cobalt (II) chloroformate, cephatoglycine hydrates and solvates, and cephalixin solvates and hydrates. Among factors that influence the desolvation reaction are the appearance of defects, the size of tunnels in the crystal packing arrange-

to exist as the anhydrous form, a "hemihydrate," a sesquihydrate, and a trihydrate [62], while the unit cell parameters and the molecular geometry of these are all the same as those of the hemihydrate. This finding suggests that the "hemihydrate" is actually a partially desolvated sesquihydrate.

Ouabaine is another example of a compound that exhibits many different hydration levels, the most hydrated form being stable at the lowest temperature. Thus the nonahydrate phase of ouabaine is obtained from water at 0–15°C, the octahydrate phase at 15–28°C, and the dihydrate phase at 28–90°C. In addition, ouabaine phases corresponding to 4.5 H₂O, 4 H₂O, and 3 H₂O may be obtained from mixtures of water with other solvents. The anhydrous phase of ouabaine anhydrate is crystallized from ethanol at high temperatures [63].

Typically, hydrates are obtained by recrystallization from water. For example, trazodone hydrochloride tetrahydrate was prepared by dissolving the anhydrate in hot distilled water, allowing the solution to remain at room temperature overnight, and storing the collected crystals at 75% relative humidity and 25°C until they reached constant weight [64].

Hydrates can sometimes be obtained by simply suspending the anhydrous material in water, whereupon a form of Ostwald ripening occurs. For instance, aqueous suspensions of anhydrous metronidazole benzoate are metastable, and storage at temperatures lower than 38°C leads to monohydrate formation accompanied by crystal growth [65]. Sorbitol provides another example of this behavior, where slow cooling of a saturated aqueous solution yields long thin needles of sorbitol hydrate [66]. When suspended in water, anhydrous carbamazepine is transformed to carbamazepine dihydrate [67]. In other instances, hydrates can be obtained from mixed solvent systems. Acemetacin monohydrate can be obtained by slow evaporation from a mixture of acetone and water at room temperature [68].

Simply exposing an anhydrous powder to high relative humidity can often lead to formation of a hydrate. On exposure to a relative humidity of 100%, dexmedetomidine hydrochloride is converted to a monohydrate [69]. Droloxifene citrate is an example of a compound that is not very hygroscopic and yet forms a hydrate. Only after storage of the anhydrous form at 85% relative humidity does some sorption of

water occur. The monohydrate phase can be formed by exposing the anhydrous form to 98% relative humidity for ten days at 24°C [70].

III. METHODS EMPLOYED TO OBTAIN SOLVATE FORMS

Often, when solvents are employed in the purification of new drug substances by recrystallization, it is observed that the isolated crystals include solvent molecules, either entrapped within empty spaces in the lattice or interacting via hydrogen bonding or van der Waals force with molecules constituting the crystal lattice. Solvent molecules also can be found in close association with metal ions, completing the coordination sphere of the metal atom. Coordinated solvent molecules are considered as part of the crystallized molecule. A crystal with large empty channels or cavities is not stable because of packing demands. The size and chemical environment of the cavity or channel determine what kind of solvent molecule can be included in the structure and what kind of interaction occurs between solvent and structure.

Depending on the nature of molecular packing arrangements, it may happen that the inclusion of solvent is necessary to build a stable crystal structure. van Geerestein et al. [71] found during numerous crystallization attempts of 11β-[4-(dimethylamino)phenyl]-17β-hydroxy-17α-(1-propynyl)estra-4,9-diene-3-one that crystals were only obtainable in the presence of *n*-butyl acetate or *n*-propyl acetate. The crystal structure of the compound crystallized from *n*-butyl acetate/methylcyclohexane was solved, and one solvent molecule was found in the crystal structure that showed no strong interactions with the rest of the structure. Apparently, this solvent molecule was necessary to fill empty space resulting after the molecular packing. Solvates in which the solvent fills empty space are generally nonstoichiometric, such as the nonstoichiometric solvates formed by droloxifene citrate with acetonitrile, 2-propanol, ethanol, 1-propanol, and 1-butanol. Typically such solvates exhibit the same x-ray diffraction pattern as does the nonsolvated compound.

When solvent molecules increase the strength of the crystal lattice, they can affect the stability of the compound to solid state decom-

position. It has been observed that the four solvated and one nonsolvated structures of prenisolone *tert*-butyl acetate affect the flexibility of the steroid nucleus and the structure-dependent degradation of the compound when exposed to air and light [72].

van der Sluis and Kroon found 1,247 different compounds with cocrystallized solvents in the Cambridge Crystallographic Database [73]. Out of 46,460 total structures, they found 9,464 solvate structures, and 95% of these contained one of the 15 solvents given in Table 2.

The most commonly encountered solvates among pharmaceuticals are those of 1:1 stoichiometry, but occasionally mixed solvate species are encountered. For structures containing more than one solvent type, one generally finds nonpolar solvents crystallizing together on the one hand and polar solvents on the other. For example, the most common solvents found cocrystallizing with water are (in order of im-

Table 2 Distribution of the 15 Most Abundant Solvents in the Cambridge Crystallographic Database, as the Percentage of Solvate Structures

Solvent	Occurrence (%)
Water	61.4
Methylene dichloride	5.9
Benzene	4.7
Methanol	4.1
Acetone	2.8
Chloroform	2.8
Ethanol	2.6
Tetrahydrofuran	2.3
Toluene	2.2
Acetonitrile	1.9
<i>N,N</i> -dimethylformamide	0.9
Diethyl ether	0.9
Pyridine	0.7
Dimethyl sulfoxide	0.5
Dioxane	0.5

Source: From Ref. 73. Reproduced with permission of the copyright owner.

portance) ethanol, methanol, and acetone. An interesting example of a structure containing a polar and a nonpolar solvent is the sodium salt of the antibiotic K-41, *p*-bromobenzoate monohydrate *n*-hexane solvate [74], which is crystallized from *n*-hexane saturated with water. Perhaps the best known mixed solvate is doxycycline hyclate: (doxycycline · HCl)₂ · C₂H₆O · H₂O. Triamterene also forms a mixed solvate, containing one *N,N*-dimethylformamide molecule and one water molecule within the crystal lattice [75].

The techniques used to obtain solvates are generally similar to the solvent methods used to obtain polymorphs, i.e. crystallization from a single solvent, from mixed solvents, or by vapor diffusion. Sometimes, it is possible to exchange one solvent within the crystal structure for another. When one recrystallizes a hydrate from dry methanol, in most cases one is left with either a methanol solvate or an anhydrous, unsolvated form of the compound.

A large number of solvates have been reported, especially for steroids and antibiotics. It has been observed that cortisone acetate and dexamethasone acetate can be crystallized as 10 different solvates. Dirithromycin, a semisynthetic macrolide antibiotic, crystallizes in two anhydrous polymorphic forms and in at least nine stoichiometric solvate forms. Six of the known solvates are isomorphous, having nearly identical x-ray powder diffraction patterns [76]. In addition to the anhydrate and dihydrate, erythromycin also forms solvates with acetone, chloroform, ethanol, *n*-butanol, and *i*-propanol [77].

It may be instructive to consider some examples of solvate formation. The compound 5-methoxysulphadiazine forms 1:1 host-guest solvates with dioxane, chloroform, and tetrahydrofuran [78]. These were prepared by heating to boiling a solution of the sulfonamide in the appropriate solvent, followed by slow cooling to obtain large crystals. Spironolactone forms 1:1 solvates with methanol, ethanol, ethyl acetate, and benzene. It also forms a 2:1 spironolactone-acetonitrile solvate [79,80]. The spironolactone solvates were prepared by crystallization in a refrigerator from solutions that were nearly saturated at room temperature.

Another steroid that forms solvates is stanozolol [81]. Solvates having 1:1 stoichiometry were prepared by recrystallization from methanol, ethanol, and 2-propanol, by heating the compound in the

Table 5 Amorphous Pharmaceuticals Obtained by Milling

Compound	Method used	Reference
Cimetidine	Milling	[96]
FR76505	Grinding in a ball mill	[97]
Cephalexin	Grinding in an agate centrifugal ball mill for 4 hours	[98]
Indomethacin	Grinding for 4 hours at 4°C in a centrifugal ball mill; grinding the γ -form at 4°C	[57,99]
(E)-6-(3,4-Dimethoxyphenyl)-1-ethyl-4-mesitylimino-3-methyl-3,4-dihydro-2(1H)-pyrimidinone	Grinding in a stainless steel shaker ball mill for 60 minutes	[100]
9,3'-Diacetyl-midecamycin	Mixed grinding with polyvinylpyrrolidone or polyvinylpyrrolidone + hydroxypropylmethylcellulose for 9 hours	[101]
Chloramphenicol stearate	Milling in a Pulverisette 5 grinder (Fritsch) (agate mortar and balls) with colloidal silica or microcrystalline cellulose	[102,103]
Calcium gluceptate	Milling in a Pulverisette 2 grinder (Fritsch) (agate mortar and balls) for 4 hours	[104]
Chloramphenicol palmitate	Milling in a Pulverisette 0 grinder (Fritsch) (agate mortar and balls) for 85 hours	[105]
Aspirin	Grinding with adsorbents under reduced pressure	[106]
	Grinding with β -cyclodextrin	[107]
Ibuprofen	Roll mixing with β -cyclodextrin	[108]
Hydrocortisone acetate	Grinding with crystalline cellulose	[109]

Table 5 Continued

Compound	Method used	Reference
Digoxin	Milling in a Glen Creston Model M270 ball mill for 8 hours	[110]
	Comminution of 1 g at 196°C for 15 minutes in a freezer mill	[111]
Amobarbital	Ball-milling with methylcellulose, microcrystalline cellulose, or dextran 2000	[112,113]
Acetaminophen	Ball milling for 24 hours with α - and β -cyclodextrin	[114]
6-Methyleneandrosta-1, 4-diene-3,17-dione	Co-grinding with β -cyclodextrin for 2 hours	[115]

C. Spray-Drying

In the pharmaceutical industry, spray-drying is used to dry heat-sensitive pharmaceuticals, to change the physical form of materials for use in tablet and capsule manufacture, and to encapsulate solid and liquid particles. This methodology is also used extensively in the processing of foods [116]. In the spray-drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles are then dried in the airstream in seconds owing to the high surface area in contact with the drying gas. Spray-drying can produce spherical particles that have good flow properties, and the process can be optimized to produce particles of a range of sizes required by the particular application. The process can be run using either aqueous or nonaqueous solutions. Examples of pharmaceuticals obtained in the form of amorphous powders by spray-drying are found in Table 6.

D. Lyophilization

Lyophilization (also known as freeze-drying) is a technique that is widely employed for the preparation of dry powders to be reconstituted at the time of administration. It is a particularly useful technique in the

listing of ten pharmaceuticals that form glasses (Table 3). It is often found that the presence of impurities that facilitate glass formation increases the ratio T_g/T_m either by raising T_g or by lowering T_m . Hence one might wonder if some of the high values in the last column of Table 3 are due to partial decomposition of the drug substance upon melting. Of course, this is an important concern when employing the melt solidification procedure for the preparation of amorphous materials.

There are many examples given in the monograph *Thermomicroscopy in the Analysis of Pharmaceuticals* [9] of other compounds that solidify on the microscope hot stage to form glasses. However, Table 4 contains examples from the literature in which solidification from the melt (either by slow cooling to room temperature or by quench cooling with liquid nitrogen) has been employed as the specific method for obtaining amorphous material.

B. Reduction of Particle Size

Reduction of the particle size of crystalline materials to the microcrystalline level can yield a material incapable of exhibiting an x-ray pow-

Table 3 Pharmaceuticals Forming Glasses above Room Temperature

Compound	T_g (K)	T_m (K)	T_g/T_m
Cholecalciferol	296	352	0.84
Sulfisoxazole	306	460	0.67
Stilbestrol	308	439	0.70
Phenobarbital	321	443	0.72
Quinidine	326	445	0.73
Salicin	333	466	0.71
Sulfathiazole	334	471	0.71
Sulfadimethoxine	339	465	0.73
Dehydrocholic acid	348	502	0.69
17- β -Estradiol	354	445	0.80

Source: Ref. 84.

Table 4 Amorphous Pharmaceuticals Obtained by Solidification from the Melt

Compound	Method used	Reference
Phenylbutazone	Solidification from the melt	[85]
Indomethacin	Quench cooling using liquid nitrogen or slow cooling from the melt over 30 min	[86,87]
Felodipine	Cooling of the melt in liquid nitrogen or at ambient temperature	[88,89]
Nifedipine	Melting at 180°C followed by immersion in liquid nitrogen	[90]
Benperidol	Melt in an oven at 277°C then cool to room temperature	[91]
Acetaminophen	Solidification of the melt at -5°C/min	[92]
Sulfapyridine	Melting any crystalline form and slowly cooling the melt	[93]
Lovostatin	Melting under nitrogen, rapid cooling to 20°C below the glass transition point	[94]

der diffraction pattern. Dialer and Kuessner [95] found that when sucrose was milled in a vibratory ball mill, the ordered crystal was transformed into a glass-like structure. The increase in surface energy of milled sucrose, as measured by heat of solution, could not be accounted for by an increase in surface area alone. Hence milling disrupts the crystal lattice and imparts the excess free energy and entropy associated with amorphous substances.

Particle size reduction can be achieved using a variety of methods. Sometimes it is helpful to carry out the particle size reduction at reduced temperatures, such as at 4°C or at liquid nitrogen temperature, -196°C. In other instances, grinding with an excipient has been employed as a means of obtaining amorphous materials. Cyclodextrins and microcrystalline cellulose have been used for this purpose. It is also possible that the use of polymeric excipients may inhibit crystal growth when the amorphous solid is dissolved in water. Table 5 contains a list of compounds that have been obtained in amorphous, or partly amorphous, form by milling.

Table 5 Amorphous Pharmaceuticals Obtained by Milling

Compound	Method used	Reference
Cimetidine	Milling	[96]
FR76505	Grinding in a ball mill	[97]
Cephalexin	Grinding in an agate centrifugal ball mill for 4 hours	[98]
Indomethacin	Grinding for 4 hours at 4°C in a centrifugal ball mill; grinding the γ -form at 4°C	[57,99]
(E)-6-(3,4-Dimethoxyphenyl)-1-ethyl-4-mesitylimino-3-methyl-3,4-dihydro-2(1H)-pyrimidinone	Grinding in a stainless steel shaker ball mill for 60 minutes	[100]
9,3'-Diacetyl-midecamycin	Mixed grinding with polyvinylpyrrolidone or polyvinylpyrrolidone + hydroxypropylmethylcellulose for 9 hours	[101]
Chloramphenicol stearate	Milling in a Pulverisette 5 grinder (Fritsch) (agate mortar and balls) with colloidal silica or microcrystalline cellulose	[102,103]
Calcium gluceptate	Milling in a Pulverisette 2 grinder (Fritsch) (agate mortar and balls) for 4 hours	[104]
Chloramphenicol palmitate	Milling in a Pulverisette 0 grinder (Fritsch) (agate mortar and balls) for 85 hours	[105]
Aspirin	Grinding with adsorbents under reduced pressure	[106]
Ibuprofen	Grinding with β -cyclodextrin	[107]
	Roll mixing with β -cyclodextrin	[108]
Hydrocortisone acetate	Grinding with crystalline cellulose	[109]

Table 5 Continued

Compound	Method used	Reference
Digoxin	Milling in a Glen Creston Model M270 ball mill for 8 hours	[110]
	Comminution of 1 g at 196°C for 15 minutes in a freezer mill	[111]
Amobarbital	Ball-milling with methylcellulose, microcrystalline cellulose, or dextran 2000	[112,113]
Acetaminophen	Ball milling for 24 hours with α - and β -cyclodextrin	[114]
6-Methyleneandrosta-1, 4-diene-3,17-dione	Co-grinding with β -cyclodextrin for 2 hours	[115]

C. Spray-Drying

In the pharmaceutical industry, spray-drying is used to dry heat-sensitive pharmaceuticals, to change the physical form of materials for use in tablet and capsule manufacture, and to encapsulate solid and liquid particles. This methodology is also used extensively in the processing of foods [116]. In the spray-drying process, a liquid feed stream is first atomized for maximal air spray contact. The particles are then dried in the airstream in seconds owing to the high surface area in contact with the drying gas. Spray-drying can produce spherical particles that have good flow properties, and the process can be optimized to produce particles of a range of sizes required by the particular application. The process can be run using either aqueous or nonaqueous solutions. Examples of pharmaceuticals obtained in the form of amorphous powders by spray-drying are found in Table 6.

D. Lyophilization

Lyophilization (also known as freeze-drying) is a technique that is widely employed for the preparation of dry powders to be reconstituted at the time of administration. It is a particularly useful technique in the

Table 6 Amorphous Pharmaceuticals Obtained by Spray-Drying

Compound	Method used	Reference
YM022	Spray-drying a methanol solution	[117]
α -Lactose monohydrate	Spray-drying in a Buchi 190	[118]
	Spray-drying a solution or suspension	[119]
4'-O-(4-methoxy-phenyl) acetyltylosin	Spray drying a dichloromethane solution	[120]
Salbutamol sulfate	Spray-drying of an aqueous solution in Buchi 90 spray dryer	[121]
Lactose	Spray-drying an aqueous solution	[118,122]
Furosemide	Spray-drying from a 4:1 chloroform: methanol solution at 50 and 150°C inlet temperature	[123,124]
Digoxin	Spray-drying an aqueous solution containing hydroxypropyl methylcellulose	[125]
Cefazolin sodium	Spray-drying from a 25% aqueous solution with an inlet temperature of 150°C and an outlet temperature of 100°C	[126]
9,3'-Diacetyl-midecamycin	Spray-drying of aqueous solution in the presence and absence of ethylcellulose	[127]

case of compounds that are susceptible to decomposition in the presence of moisture but that are more stable as dry solids. The physical form, chemical stability, and dissolution characteristics of lyophilized products can be influenced by the conditions of the freeze-drying cycle. In most pharmaceutical applications, lyophilization is performed on aqueous solutions containing bulking agents, and these often are chosen so as to form a coherent cake after completion of the freeze-drying process. However, lyophilization also can be employed to convert crystalline materials into their amorphous counterparts. The lyophilization process usually consists of the three stages of freezing, primary drying,

and secondary drying. For the preparation of amorphous materials, rapid freezing is employed so as to avoid the crystallization process. Both aqueous solutions and solutions containing organic solvents have been lyophilized. The primary drying phase involves sublimation of frozen water or vaporization of another solvent. This step is carried out by reducing the pressure in the chamber and supplying heat to the product. The secondary drying phase consists of the desorption of moisture (or residual solvent) from the solid.

Recently, excipients of various types have been employed in frozen solutions so as to inhibit crystallization. Cyclodextrins appear to be particularly useful for this purpose, although it is generally necessary to employ rapid freezing to liquid nitrogen temperatures to ensure that the freeze-dried product is noncrystalline. When α -cyclodextrin, which has a larger cavity than does β -cyclodextrin, is frozen at a relatively slow rate, it will cocrystallize with compounds such as benzoic acid, salicylic acid, *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, and methyl *p*-hydroxybenzoate [128]. However, rapid freezing of a methyl *p*-hydroxybenzoate solution containing α -cyclodextrin at a benzoate/cyclodextrin ratio of 0.33 yields an amorphous solid after freeze-drying [29].

β -Cyclodextrin and its derivatives have been shown to form amorphous lyophilized products with a number of compounds, principally nonsteroidal antiinflammatory agents. Examples from the literature of excipients and pharmaceuticals prepared as amorphous materials by lyophilization are given in Table 7.

E. Removal of Solvent from a Solvate or Hydrate

Solids can sometimes be rendered amorphous by the simple expedient of allowing solvent molecules of crystallization to evaporate at modest temperatures. If the solvent merely occupies channels in the crystal structure, the structure often remains intact, but when the solvent is strongly bonded to molecules of the host, the structure frequently will collapse when the solvent is removed and one obtains an amorphous powder. A few examples of amorphous solids obtained in this manner are found in Table 8.

Table 7 Amorphous Pharmaceuticals Obtained by Lyophilization

Compound	Method used	Reference
Lactose	Lyophilization of a 5% Aqueous Solution	[130]
MK-0591	Lyophilization	[131]
Raffinose	Lyophilization of a 10% aqueous solution frozen at -45°C	[132]
Sucrose	Lyophilization of 10% aqueous solutions	[133]
Dirithromycin	Freeze-drying from methylene chloride solution	[134]
Cefalexin	Aqueous solution frozen at -196°C , then freeze-dried	[135]
	Lyophilization of a saturated aqueous solution	[136]
Calcium gluceptate	Freeze-drying from 2% aqueous solution	[137]
Griseofulvin	Freeze-drying of solutions of griseofulvin or of solutions of mixtures of griseofulvin and mannitol in dioxane or 1:1 dioxane-water with fast freezing in liquid nitrogen	[138]
Tolobuterol hydrochloride	Freeze-drying of aqueous solution	[139]
E1040	Freeze-drying of aqueous solution	[140]
Glutathione	Freeze-drying of a 5% aqueous solution	[141]
Aspirin	Freeze drying of an aqueous solution in the presence of 1.0% hydroxypropyl- β -cyclodextrin	[142]
Ketoprofen	Freeze-drying in the presence of heptakis-(2,6-O-dimethyl)- β -cyclodextrin	[143]
	Freeze-drying with β -cyclodextrin (rapid freezing with liquid nitrogen)	[144]
Glibenclamide	Freezing at liquid nitrogen temperature, freeze-drying over 24 hours	[145]

Table 7 Continued

Compound	Method used	Reference
Naproxen	Colyophilization (223K and 0.013 torr) of naproxen and hydroxyethyl- β -cyclodextrin, or hydroxypropyl- β -cyclodextrin	[146]
Sodium ethacrylate	Rapid freezing of an aqueous solution to -50°C , followed by freeze-drying	[147]
<i>p</i> -Aminosalicylic acid	Colyophilization of <i>p</i> -aminosalicylic acid in aqueous solution with pullulan	[148]
Ceftazidime	Freeze-drying a nearly saturated aqueous solution of the free acid	[149]
Cefaclor	Freeze-drying from a nearly saturated aqueous solution	[149]
Cephalothin sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefamandol sodium	Freeze-drying from a 25% aqueous solution	[149]
Cefazolin sodium	Freeze-drying an aqueous solution at low temperature	[149]
Nicotinic acid	Freeze-drying in the presence of β -cyclodextrin (fast-freezing); and heptakis (2,6-O-dimethyl)- β -cyclodextrin	[150]

F. Precipitation of Acids or Bases by Change in pH

If the level of supersaturation is carefully controlled, it is often possible to avoid crystallization when a water-soluble salt of a weak acid is precipitated with a base, or when a water-soluble salt of a weak base is precipitated with an acid. When crystalline iopanoic acid is dissolved in 0.1 N NaOH, and 0.1 N HCl is added, an amorphous powder is precipitated [43]. A similar phenomenon is observed in the case of the precipitation of piretanide [155]. Another example in this genre is the

Table 8 Amorphous Pharmaceuticals Obtained by Solvent Removal

Compound	Method used	Reference
Tranilast anhydrate	Dehydration of the monohydrate over P_2O_5	[151]
Raffinose	Lyophilization and heat drying of the pentahydrate	[132]
Erythromycin	Heating the dihydrate for 2 hours at 135°C in an oven, and then cooling to room temperature	[152,153]
Calcium DL-pantothenate	Drying the methanol:water 4:1 solvate <i>in vacuo</i> at 50–80°C	[154]

precipitation of amorphous calcium carbonate, which occurs when a calcium chloride solution is combined with a sodium carbonate solution at 283K [156].

G. Miscellaneous Methods

Earlier during the discussion on the preparation of polymorphs, the doping of crystals was mentioned as a technique for encouraging the formation of one type of polymorph over another. Similarly, if a dopant is employed at levels that will disrupt the crystal lattice, the substance can be made to solidify as an amorphous material. Duddu and Grant [157] observed changes in the enthalpy of fusion of (–)-ephedrinium 2-naphthalenesulfonate when the opposite enantiomer, (+)-ephedrinium 2-naphthalenesulfonate, was added as a dopant.

When *m*-cresol was added to a suspension of insulinotropin crystals grown from a normal saline solution, the crystals were immediately rendered amorphous. It was postulated [158] that the *m*-cresol molecules diffused into the crystals through solvent channels and disturbed the lattice interactions that ordinarily maintained the integrity of the crystal. When zinc acetate or zinc chloride was added to the suspension, the zinc ion stabilized the crystal lattice so that the subsequent addition of *m*-cresol did not alter the integrity of the crystals.

Sometimes solvents exert a similar effect. When a small amount of ethyl acetate is added to a calcium chloride solution prior to addition

of sodium fenoprofen, the calcium fenoprofen that precipitates has a low degree of crystallinity [159]. Similarly, when calcium DL-pantothenate is precipitated from methanol or ethanol solution by the addition of acetone, ether, ethyl acetate, or other solvents, the precipitate obtained is found to be amorphous [154].

V. SUMMARY

The pharmaceutical development scientist who is assigned the task of demonstrating that a substance exhibits only one crystalline form, or that of discovering whether additional forms exist, can utilize the techniques outlined in this chapter as a starting point. Upon completion of this program, one can certainly conclude that due diligence has been employed to isolate and characterize the various solid-state forms of any new chemical entity. One should always be aware that nuclei capable of initiating the crystallization of previously undiscovered forms might be lurking around the laboratory, ready to confound the investigator should their effects become known. In addition, the phenomenon of "disappearing polymorphs" can come into play, and techniques that formerly yielded the same crystals every time may subsequently yield crystals of another, more stable form. In the future, the use of computer simulations of alternative crystallographic structures will suggest how much laboratory work might be required to isolate the polymorphs or solvates of a given compound. Until then, the empirical approach remains superior.

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6

Methods for the Characterization of Polymorphs and Solvates

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Crystalline Polymorphism of Organic Compounds

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Crystal polymorphism is encountered in all areas of research involving solid substances. Its occurrence introduces complications during manufacturing processes and adds another dimension to the complexity of designing materials with specific properties. Research on polymorphism is fraught with unique difficulties due to the subtlety of polymorphic transformations and the inadvertent formation of pseudopolymorphs. In this report, a summary of thermodynamic, kinetic and structural considerations of polymorphism is presented. A wide variety of techniques appropriate to the study of organic crystalline polymorphism and pseudopolymorphism is then surveyed, ranging from simple crystal density measurement to observation of polymorphic transformations using variable-temperature synchrotron X-ray diffraction methods. Application of newer methodology described in this report is yielding fresh insights into the nature of the crystallization process, holding promise for a deeper understanding of the phenomenon of polymorphism and its practical control.

Keywords: Crystal polymorphism, Pseudopolymorphism, Crystallization.

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1

Introduction

The protean nature of a chemical substance, reflected in its ability to crystallize in different structural arrangements (polymorphs), has since its discovery [1] been a source of both fascination and frustration for chemists. At a given temperature and pressure, only one polymorphic form of a substance is thermodynamically stable, all other forms being metastable. Since the rate of transformation of metastable polymorphs to the stable one may be slow, it is quite common to encounter several polymorphs of a single compound under normal laboratory conditions. Organic compounds tend to form different polymorphs owing to weak, non-directional intermolecular interactions which exist in the solid state. When a compound can be isolated in different polymorphic modifications, each with the potential of possessing unique properties (solubility, density, melting point, enthalpy of fusion, chemical reactivity, electrical conductivity, to name a few), the chemist, pharmaceutical chemist, or chemical engineer is presented with a degree of flexibility of choice for a particular application. Balanced against this flexibility, however, are the considerable practical difficulties that can arise, both in ensuring reproducible preparation of a specific polymorph and, during the lifetime of its application, preventing its spontaneous transformation to an undesirable form. Since free energy differences between polymorphic forms of a given substance are generally around a few kJ mol^{-1} [2] and the process of crystallization is affected by many physical parameters (e.g. nature of the solvent, cooling and stirring rates, temperature, pressure, presence of impurities), minor variations in preparative conditions can tip the balance in favour of crystallization of a polymorph which is not necessarily the thermodynamically stable one. This element of unpredictability in the outcome of the crystallization process has serious implications for solids design in crystal engineering [3], where the required specificity of molecular organization in the crystalline state is crucial.

Various aspects of organic crystalline polymorphism and its occurrence in the fine chemicals, pharmaceuticals and other industries have been the subjects of several recent reviews. With varying degrees of overlap, these reviews can be roughly grouped into the following, according to their focus: thermodynamic and kinetic aspects [4-7], structural aspects [2, 3, 8-17], methodology [18-27], the crystallization process [28-34] and polymorphic control [35, 36]. The reader is referred to the above for a comprehensive view of what is a rather pervasive phenomenon in chemistry and whose pursuit is currently enjoying an upsurge of interest from solid-state researchers [5].

This report describes some recent developments in the understanding of the thermodynamic, kinetic and structural aspects of organic crystal polymorphism with an emphasis on the application of newer methodology used for its study, since this is one of the areas in which significant progress has been made in recent years. Numerous examples of polymorphic systems are described to illustrate the applications of both older and newer techniques for their investigation. These include studies of pseudopolymorphism manifested by hydrates and solvates of the parent organic molecule. Finally, the crucial question of

control in polymorphism is briefly addressed with a view to illustrating current strategies and their implications for the design of solids.

2:

Crystal Polymorphism – Theoretical Principles and Practical Implications

2.1

Background – The Role of Polymorphism in the Production of Materials

Many of the inconsistencies encountered in product performance in the chemical, chemical engineering, pharmaceutical, food and related industries can be attributed to polymorphism. An important example is inconsistent behaviour of drug substances upon dissolution which may have a direct influence on bioavailability. This arises because different polymorphic forms of the same drug may have solubilities which differ by an order of magnitude [24]. Inadvertent production of the 'wrong' polymorph at the crystallization stage following synthesis or at any of the intermediate processing stages can therefore result in pharmaceutical dosage forms which are either ineffective or toxic [37, 38]. Spontaneous polymorphic transformations mediated by solvents is common [4] and liquid preparations of metastable drugs frequently lose their effectiveness due to precipitation of less soluble, thermodynamically more stable polymorphs or pseudopolymorphs. A case in point is the antiprotozoal agent metronidazole benzoate which, when stored as an aqueous suspension below 38°C is metastable, leading to precipitation and growth of the insoluble monohydrate [39, 40].

Dunitz and Bernstein [5] have recently documented several cases of "vanishing" polymorphs. These are usually metastable forms which, despite their thermodynamic instability, may have crystallized preferentially due to more rapid nucleation. Such metastable forms may persist and be used for many years before being "displaced", when a thermodynamically more stable form is prepared. Attempts to regenerate the original polymorph are frequently met with failure. Specific compounds with such a history include e.g. 1,2,3,5-tetra-O-acetyl- β -D-ribofuranose, benzocaine picrate and xylitol. This disturbing phenomenon extends to pseudopolymorphs. A previously known monohydrate of the antibiotic ampicillin has not been obtained since the appearance of the trihydrate [24]. A possible explanation for this behaviour is that after minute particles of the stable polymorph enter the environment, they eventually become widely disseminated ("planetary seeding" [5]) and serve as nuclei promoting crystallization of their own kind exclusively.

Manufacturing processes including crystallization scale-up, drying, heating, compression and milling can induce polymorphic transformations [24] and it follows that careful quality control is necessary at all stages to monitor undesirable changes. Systematic investigation of a compound to determine whether it is prone to polymorphism, as well as the nature of the polymorphism (enantiotropic or monotropic) [23], is routine practice in pharmaceutical pre-formulation studies. Identification of the different polymorphic forms of a drug substance, determination of their chemical and physical properties, thermodynamic

stabilities, and temperatures and rates of interconversion are essential for ensuring drug preparations with reproducible behaviour [24]. Already, legislation requiring drug manufacturers to provide information relating to the occurrence (or apparent absence) of polymorphism in their products has been introduced [41]. Demonstrating the absence of a tendency to polymorphism is not easy; most substances when investigated for a sufficiently long time will reveal more than one polymorph [42].

Successful preparation of crystals of organic compounds having special properties (e.g. second-harmonic generation, metallic conductivity) may hinge on polymorphism, only one polymorph of the compound in question displaying the desired property. Bernstein has recently described representative systems which clearly illustrate the relationship between a polymorphic structure (a crystal architecture characterised by well-defined molecular interactions) and the unique physical properties which that structure confers on the solid material [13].

These remarks serve to emphasise some of the more important practical implications and consequences of polymorphism. Overcoming the problems encountered requires a deeper understanding of the processes of nucleation, crystal growth and polymorphic transformation. Several recent studies relating to these topics are reviewed in the next section.

2.2

Crystallization and Polymorphic Transformations – Thermodynamic and Kinetic Considerations

Crystallization of a specific polymorph from a melt, solution or vapour, commences with nucleation, i.e. the formation of a critical "embryonic" nucleus which is the structural blueprint for subsequent development and growth of the macroscopic crystal. The factors determining nucleation rate (e.g. the associated Gibbs free energy of activation, molecular volume, interfacial energy) generally differ for polymorphs of the same substance [4]. Since, in a supersaturated solution, nuclei of all possible polymorphs of the dissolved substance may be imagined to exist [36], the outcome of crystallization is kinetically complicated by competitive nucleation processes. Thermodynamic considerations of polymorphic crystallization include Ostwald's law of stages [4, 43], according to which, at high supersaturation, the first form which crystallizes is the thermodynamically least stable (most soluble) form. This form subsequently dissolves and transforms into a more stable one. The cycle continues until only the thermodynamically stable (least soluble) polymorph remains. The practical implication is that it should be possible to isolate the different polymorphs of a given compound at different levels of solution supersaturation and hence exercise some control over the crystallization process.

As regards polymorphic transformations in general, two types are distinguished, namely enantiotropic and monotropic [23]. These can be described in terms of the Gibbs free energy G , which has a minimum value for the thermodynamically stable phase of a polymorphic system and larger values for metastable phases and is such that the polymorph with the higher entropy will tend

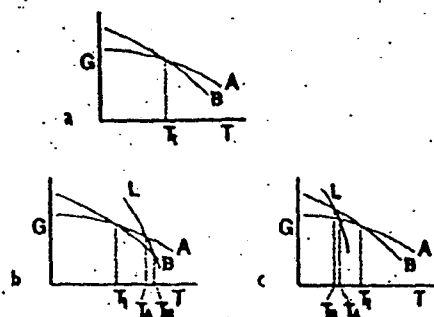


Fig. 1a-c. Gibbs free energy vs temperature for: a a dimorphic system, exhibiting; b enantiotropy; c monotropy

to become the stable form at higher temperature (species B in Fig. 1a). Above T_i (the transition temperature), B is the stable polymorph while A is metastable and vice versa at temperatures below T_i . In an enantiotropic system (Fig. 1b), the free energy curve for the common liquid phase L intersects the A and B curves at $T > T_i$. In this case, the lower melting form (A) is stable at $T < T_i$, the higher melting form is stable at $T > T_i$, and the transition between the two forms is in principle reversible. Since transition temperatures in practice are often in the range 20–200°C, one practical implication of enantiotropy is that conversion of one polymorph into another may be favoured during routine manufacturing processes [24]. On the other hand, for a system displaying monotropy (Fig. 1c), curve L intersects those for A and B below T_i and the higher melting form (A) is always the thermodynamically stable one. Thus, below the melting point, only one form is stable and the other metastable. In practice, if a desired metastable polymorph is obtained during manufacture, it can revert to the stable polymorph under suitable conditions (e.g. in suspension, via solvent-mediation, or during compression). It follows that to prepare a specific polymorph and be aware of its possible fate during handling, it is advantageous to know the transition temperatures and thermodynamic stabilities of all the forms that may appear in the system [24].

The general considerations above highlight the importance of nucleation and the role of environmental conditions (e.g. solvent, temperature) in the crystallization of polymorphs as well as their interconversions. These areas continue to be the subject of intense interest especially in the context of polymorphic control in crystallization.

Some fundamental aspects of the nucleation process have been investigated by molecular dynamics (MD) methods. In a recent review [44] the advantages and limitations of molecular cluster models in simulating the dynamics of nucleation and phase changes have been discussed. In this approach, molecular dynamic simulations are correlated with experimental nucleation rates extracted from electron diffraction patterns of molecular supersonic jets. The dynamics of freezing of ammonia, CCl_4 and water, and the phase transformations of *t*-butyl chloride have been analysed. A useful feature of the MD computational

approach is visual representation of phase transformations. Figure 2 illustrates MD-derived images of a crystalline cluster of 188 molecules of *t*-butyl chloride at various stages of freezing. MD simulations show that when sufficiently super-cooled, the tetragonal phase spontaneously transforms to a lower temperature, ordered monoclinic phase. Diffraction patterns computed from the MD molecular packing were consistent with experimental neutron powder patterns for this phase.

Other investigators [45] have recently designed a numerical model to describe nucleation and growth of polymorphs with the aim of calculating the temporal sequences of precipitation and phase transformation in metastable solutions of polymorphic substances. Another group has recently modelled the formation and aggregation of polymorphs in continuous precipitation [46]. Consideration was given to the simultaneous growth and agglomeration of two different polymorphs as well as the case of nucleation of a single polymorph which subsequently transforms into a second one. The results indicated that the ratio of the nucleation rates, the ratio of the growth rates, and the aggregation tendencies determined polymorphic product composition as well as particle size distributions. This study is important since simultaneous precipitation of different polymorphs is encountered frequently in industrial crystallizations.

A mathematical phase-field model for the kinetics of isothermal polymorphic crystallization has recently been proposed [47], according to which crystallization involves rapid relaxation of the metastable state followed by nucleation and growth of the polycrystalline phase. Computer simulations were used to obtain results which could be tested experimentally using X-ray scattering experiments. Growth rates of different polymorphic polymers have also been investigated [48]. Simultaneous development of spherulites of different polymorphs occurs at different rates under isothermal conditions. From observation of interspherulitic boundaries between the α - and γ -forms of polypivalolactone,

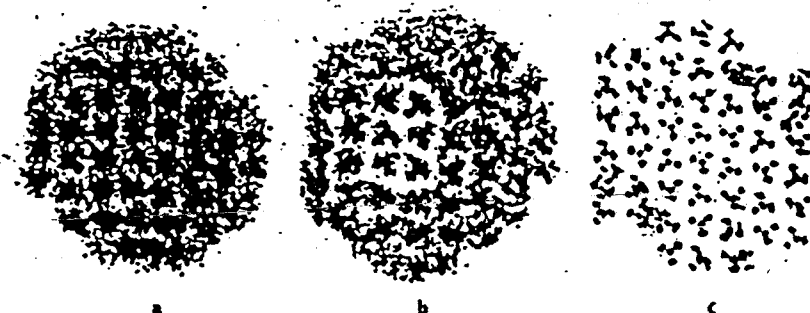


Fig. 2a-c. Images of a crystalline cluster of *t*-butyl chloride molecules at various stages of cooling, looking down the threefold molecular axis: a orientationally disordered tetragonal phase at 130 K; b nucleus of monoclinic phase growing in tetragonal phase at 80 K; c ordered monoclinic phase at 50 K after transformation. Surface molecules tend to be disordered at all temperatures. (Reprinted with permission from [44], copyright 1995 American Chemical Society)

their relative growth rates could be determined. The thermodynamics and kinetics of crystallization of large molecules from solution have been discussed [49]. Modelling of the crystallization of protein molecules indicated that the concepts and numerics of colloid stability theory are appropriate.

Recent studies of polymorphic transformations in organic crystals mediated by melt, solution and interface have been reviewed [4]. Interface-mediated transformation has only recently been recognized as a distinct mode of polymorphic transformation. It involves nucleation and growth of a new polymorph through mass transfer across the interface connecting single crystals of two different polymorphs and it differs from solid-solid transformations in that a microscopic solution layer is required as an interface. Transformations mediated by melt, solution and interface are usually more rapid than solid-solid transitions; for the latter, the activation energy is larger due to the fact that nucleation and growth of the new phase within a second phase involve diffusion and structural rearrangement at the reaction interface. The fundamental thermodynamic relationships governing polymorphic solid state transitions have been reviewed [6]. It has also been pointed out that the mechanisms of polymorphic transitions in molecular crystals are largely unknown, though order-disorder transitions are understood in reasonable detail [5]. An important technique for studying solid-solid transformations is thermal analysis and a review of the basic thermodynamic principles for interpreting thermal analysis data for both polymorphic and pseudopolymorphic systems has appeared [23]. Kinetic and thermodynamic aspects of the thermal decomposition of inclusion compounds (a special class of pseudopolymorphs) have also recently been discussed [50].

Theoretical and experimental studies of the role of solvent on polymorphic crystallization and phase transformations abound in the literature of the last few years and some pertinent examples are described here. For solvent-mediated transformations, the driving force is the difference in solubility between different polymorphs. An important earlier paper on the kinetics of such phase transformations [51] described a model featuring two kinetic processes in solid to solid phase changes via a solution phase, namely dissolution of the metastable phase and growth of the stable one.

The effect of solvent on the crystallization of polymorphs has recently been investigated [43] using as a model compound the antibacterial sulphathiazole whose four known polymorphs are well characterised. The study, whose express intention was to test the Ostwald law, involved crystallization of the pure polymorphic forms of the drug, solubility measurements and crystallizations from various solvent systems. Systematic variation of supersaturation was employed in an attempt to crystallize each of the four forms of sulphathiazole, as predicted by the Ostwald law. Solubility studies showed Form I to be the most soluble form, followed in order by Forms II, IV and III. The supersaturation crystallizations using acetone, acetone-CHCl₃ (3:2), *n*-propanol and water, revealed that only the acetone-CHCl₃ system yielded results in accord with theory, Forms I, III and IV being isolated from it by varying the supersaturation. Crystallization from *n*-propanol, for example, yielded only Form I at all supersaturation levels. Thus, the finding that some solvents selectively favour the crystallization of a

particular form (or forms) indicated that, while supersaturation is an important factor determining crystallization of polymorphs, the solvent may play a dominating role which is not thermodynamic in nature, but rather kinetic. The proposed mechanism, namely selective adsorption of different solvent molecules on specific faces of particular polymorphs, is consistent with that suggested earlier [52] for the effects of additives and solvents on crystal morphology. Such specific adsorption might result in inhibition of nucleation of certain polymorphs or retardation of their growth, allowing other, thermodynamically less favoured, polymorphs to crystallize instead.

The mechanism of a solvent-mediated transformation may change with complete change of solvent, or more subtly, when a gradual change in polarity is effected by dilution of the original solvent with another. The effects of the solvent systems water [53] and ethanol/water [54] on the crystallization of L-histidine polymorphs have been investigated. In aqueous solution at the isoelectric point, it was found that both the A and B polymorphs precipitate with a nearly constant ratio over a wide concentration range. However, slow transformation from B to A (which does not occur in the absence of solvent) was observed and pure A could eventually be isolated. This transformation involves smooth growth of the stable A polymorph and dissolution of the metastable form B. The ratio of the rate constants for the appearance of A and the dissolution of B indicated a growth-controlled mechanism for the transformation. In subsequent experiments investigating the effect of added ethanol, however, it was found that the fraction of polymorph A in precipitates decreased rapidly with increasing volume fraction of ethanol in the mixture, and pure B could be obtained when this fraction was 0.4. An explanation for the change in growth mechanism with added ethanol, based on the decreased concentration of polymorph A, was proposed [54].

This type of behaviour is not confined to polymorphs but may extend to pseudopolymorphic forms such as hydrates and solvates. A recent case of solvent-mediated phase transformation involved polymorphic and pseudopolymorphic forms of thiazole carboxylic acid [55], where the transformation is again sensitive to the composition of the mixed solvent. Three forms of the compound are known, an anhydrous form, a 0.5 hydrate, and a 1.5 hydrate. In 50–80% solutions (% = vol. % MeOH-H₂O), transformation of the 1.5 hydrate to the 0.5 hydrate was observed while transformation to the anhydrous form occurred in 85–100% solutions. No transformation occurred in 0–30% solutions. Detailed study of a solvent-mediated polymorphic transition has also been carried out for the antiulcerative agent cimetidine [56] for which seven polymorphic forms are known. An important feature of this study was the systematic use of seed crystals to induce crystallization at different supersaturation ratios.

The possibility of relating solvent effects to polymorphic crystallization at the molecular level may be realized when the individual crystal structures of the polymorphs are known. An analysis of this kind was carried out for the anti-inflammatory drug piroxicam [57] which was found to crystallize as the α -polymorph from proton donor and basic solvents, but as the β -polymorph from non-polar solvents. On the assumption that crystallization of the drug requires

release of solvent molecules from acceptor and donor sites on the molecule; it was possible to reconcile the observed intermolecular hydrogen bonding arrangements in the crystals (infinite chains in α -, cyclic dimers in β -) with probable sites of solvation on the piroxicam molecule in solvents of different polarities. In this way, formation of the α - and β -polymorphs from different solvent systems could be rationalised. Combination of polymorphic crystal structural data with appropriately detailed solvation models could be a useful adjunct to the existing methods of rationalising or predicting the outcome of polymorphic crystallization from solution.

2.3

Polymorphism – Structural Considerations

Dunitz has recently developed the theme of the crystal as an ordered supramolecular entity [2]. From this perspective, different polymorphic modifications of a given compound may thus be regarded as "supramolecular isomers". In discussing the possible structural arrangements occurring in crystals of polymorphs, a convenient distinction may be made [3] between rigid molecules (e.g. planar chloro-aromatics) and those with conformational flexibility (e.g. *N*-benzylideneanilines). In the former case, different polymorphic structures frequently display common features such as similar intermolecular directional contacts, layer stacking and unit cell dimensions which are related in a simple way. Conformational polymorphism, the existence of different conformers of a flexible molecule in the various crystal structures [58], may be expected to occur when the conformational energy minima differ by less than about 8 kJ mol^{-1} . If the energy barriers separating these minima are sufficiently low, these conformers may co-exist in solution and slight variations in crystallization conditions may lead to their individual isolation as conformational polymorphs [11]. Systems of this type have been exploited to study both the influences of the crystalline environment on molecular conformation as well as the properties of molecules which depend strongly on conformation [13]. An example of conformational polymorphism in which the structural arrangements are dictated by non-directional van der Waals forces only is shown in Fig. 3. The molecule in question is probucol, a drug used to control blood-cholesterol levels. Here, intermolecular hydrogen bonding between hydroxyl groups in the crystals is prevented owing to intramolecular steric crowding of these groups by neighbouring *t*-butyl substituents. The molecules adopt distinctly different conformations in the two polymorphs [59], the more symmetrical conformation approaching point symmetry C_{2v} .

Figure 4 shows representative hydrogen bonded (N-H...O, C-H...O) layers of planar nitrofurantoin molecules occurring in the α - and β -polymorphs [60], which are triclinic and monoclinic, respectively. In this system, the molecular conformations in the two polymorphs are indistinguishable but the symmetries of their intermolecular hydrogen bonding schemes differ significantly. The common molecular conformation shown here occurs in five modifications (two polymorphs and three pseudopolymorphs) of this compound [61]. The three-dimensional crystal structures of the polymorphs result from close stacking

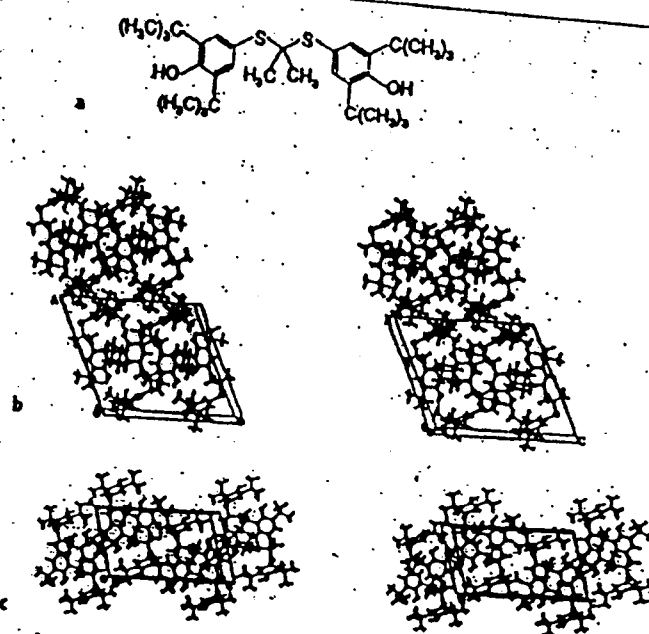


Fig. 3. a Molecular structure of probucol; b stereoview of the crystal structure of Form I; c stereoview of the crystal structure of Form II. (Adapted from [59] with permission)

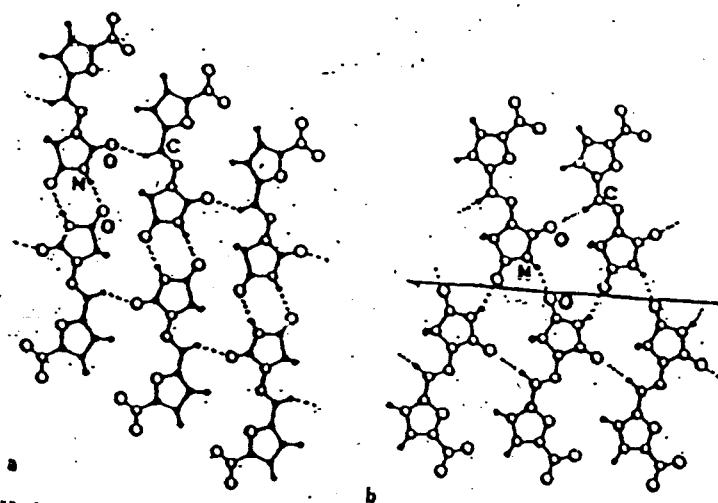


Fig. 4. a, b. Hydrogen bonded layers in nitrofurantoin polymorphs: a α -form; b β -form (Adapted from [60] with permission)

(~ 3.2 Å) of the respective layers shown in Fig. 4. This example conveys an idea of the variations in polymorphic structural arrangements that are possible with molecules containing hydrogen bonding functionalities.

During the last few years, the development of graph set analysis [16] has greatly facilitated the visualisation and comparison of polymorphic structures. Here, hydrogen bonding networks are classified as belonging to one of four distinct patterns, each specified by a designator (G in general): intramolecular (S), chains (C), rings (R), or other finite patterns (D). Further specification of the number of donor (d) and acceptor (a) atoms as well as the total number of atoms (n) comprising the pattern yields a concise and informative description of the hydrogen bonding arrangement, $G_d^a(n)$. A simple illustration of the use of graph set descriptors is given in Fig. 5 for a polymorph of thalidomide (space group $C2/c$, $Z=8$) [62]. Alternating hydrogen bonded ring motifs exist in this polymorphic structure as a result of bifurcated hydrogen bonding involving the N-H group. In contrast, the other known racemic modification of thalidomide (space group $P2_1/n$, $Z=4$) [63] contains only centrosymmetric dimers of the type $R_2^2(8)$. Application of graph set analysis to three polymorphs of iminodiacetic acid [64] has led not only to facile comparison of the crystal structures, but has also provided a basis for concise description of the polymorphic transformations occurring in that system. Thus, e.g. transformation of one polymorph of the acid into another is simply described as a conversion of $R_2^2(4)$ into $R_2^2(8)$. It has been pointed out that graph set analysis of hydrogen bonded systems also provides a means for seeking systematic correlations between the resultant patterns that demonstrate "hydrogen bond pattern functionality" [16]. This concept may be useful in the prediction of crystal structures as well as for the design of materials with desired supramolecular features. Extension of the graph set approach to encompass intermolecular atom-atom interactions other than hydrogen bonding for the classification of polymorphic crystal structures is envisaged as a natural and desirable development and more widespread use of this classification can be expected in future.

Elucidation of detailed polymorphic structural features and structural changes accompanying polymorphic transformations relies heavily on the single crystal X-ray diffraction technique. The inability to produce single crystals in

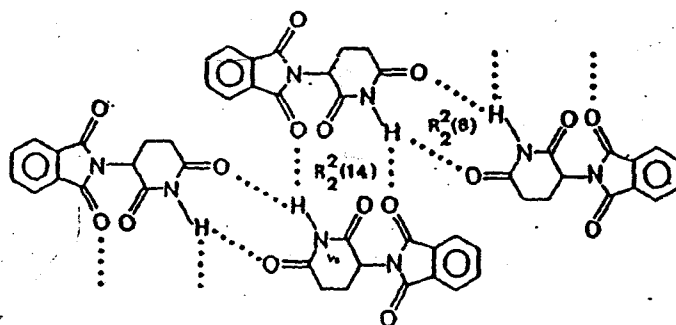


Fig. 5. Graph set notation for hydrogen bonded ring motifs in a polymorph of thalidomide

the laboratory (especially for metastable polymorphs) has hindered progress in research on polymorphism. If, however, a polymorph is available in a powdered crystalline form and a computational method exists for predicting possible three-dimensional crystal structures, then the latter can be used to generate computed X-ray powder patterns for comparison with the experimental pattern. While the scope of the present review does not permit detailed exposition of the approaches and algorithms employed in crystal structure prediction, a summary of some recent developments, especially as they relate to polymorphism, is in order. The studies of polymorphism and crystal structure prediction are indeed two facets of the same topic [65]. Additional powerful motivations for predicting crystal structures include a better understanding of the crystallization process as well as the design of new solid materials. The reader is referred to several recent treatments of crystal structure prediction [66-70]. Comments on some of these methods and their applications follow.

In the atom-atom potential (AAP) method as implemented in the program PROMET [66], symmetry operations (e.g. a screw axis, centre of inversion) appropriate to the chosen space group, are applied to a molecule in fixed conformation (possibly optimised previously by a molecular mechanics calculation) to generate molecular clusters. Following a search for the most stable clusters on the potential hypersurface (calculated using empirical atom-atom potentials), translation is applied to generate one or more periodic structures. Packing energies are computed and acceptable structures are optimised. This is a very useful means of generating a series of polymorphic structures for a given molecule. In this approach, consideration is usually given to only the most populous space groups (93% of organic molecules being confined to 18 space groups [71]) thereby running a small risk of an incorrect choice. If an experimental X-ray powder pattern of the material is available, the correct crystal structure may be identified by comparison with computed powder patterns from the candidate structures generated by PROMET. In a study using this procedure [72] several literature cases were selected, for each of which the crystal structure of one polymorph had been determined, and mention was made of the existence of a second polymorph whose crystals were unsuitable for complete structural elucidation by X-ray analysis. Only unit cell and space group data were available for these undetermined polymorphs. Each starting molecular conformation was assumed to be the same as that in the corresponding fully characterised polymorph and was submitted to the PROMET procedure. In all cases, satisfactory structures were generated, with predicted unit cell data in good agreement with the experimental ones and with packing energies in the expected ranges. These results represent authentic crystal structure prediction, assisted by partial X-ray data. The method is therefore an alternative to direct methods of structural solution and also implies that, provided cell and space group information can be acquired, full structure determination without diffraction data is feasible. The authors of this study are less optimistic as regards true *ab initio* crystal structure prediction for numerous reasons, among them that packing energies for polymorphs of the same compound are always very similar, rendering the choice of the correctly computed structure difficult in the absence of other data, and that the occurrence of polymorphs containing mole-

cules in different conformations is common. The point was also made that the correct energy ordering of polymorphic structures by computational methods may bear no relation to the experimental situation, where kinetic factors may determine which polymorph actually crystallizes under given conditions.

The AAP approach, with some variations, has recently been applied with varying degrees of success to the prediction of several other structures including, in order of increasing molecular complexity, high pressure solid phases of benzene [73], the three polymorphs of sulfanilamide [74], and the low and high temperature phases of poly(*p*-hydroxybenzoic acid) [75].

An *ab initio* molecular packing analysis procedure (program mpa) which avoids any prior assumption of space group symmetry has recently been described [76]. Only the molecular structure and the force field are required as inputs and the program finds intermolecular energy minima for packed arrangements for any given number of molecules comprising the asymmetric unit. Space group symmetry operations are predicted in this procedure and successful application to the crystal structures of urea and benzene were reported. It is significant that the energy minimisation for the urea structure converged to the correct space group ($P4_2/m$) which has a frequency of occurrence of only 0.39%.

Quantum mechanical methods have also been applied to crystal structure prediction. A recent example involved the use of *ab initio* crystal field methods with the SM (supermolecule) model and the PC (point charge) model applied to the three known polymorphs of glycine [77]. Comparison of the optimised structures with published X-ray structures for these forms indicated that the quantum-mechanically based SM model employing a 15-molecule cluster produced results in better agreement with experiment than the PC model which describes the crystal environment purely electrostatically.

Despite the varying degrees of success attainable by such computational methods, a survey of the recent literature on this subject seems to indicate that crystal structure prediction by theoretical methods is more rapid, has greater chances of success, and consumes far fewer computing resources when coupled with other techniques which provide additional experimental data for the crystal in question. (Examples of such combined studies are discussed in Sect. 3.2). At the same time, it can be argued that successful *ab initio* crystal structure prediction (i.e. assuming only the molecular structure as given) by whatever means possible in the future, would represent a very significant advance in the understanding of the fundamentals of the crystallization process. Regarding the feasibility of crystal structure prediction in general, some philosophical and technical points have been discussed, together with excellent practical recommendations for a programme of experimental and theoretical studies for elucidating the basic principles of organic solid-state chemistry [78]. This is seen as a prerequisite to the solution of the problem of crystal structure prediction.

Some new insights into the nature of organic crystal polymorphism have been gleaned from a recent systematic analysis [79] of data for polymorphic structures retrieved from the Cambridge Structural Database. A total of 345 crystal structures were reduced to 163 clusters (a cluster referring to a group of two or more polymorphs of the same compound). These clusters comprised 147

with 2, 13 with 3, and 3 with 4 partners. Differences in molecular properties (P) (e.g., density, molecular volume, packing coefficient, AAF-calculated packing energies and other thermodynamic properties) were computed between cluster members (i, j) as $\Delta P = P_j - P_i$ or $\Delta P = 100(P_j - P_i)/P_i$ ($j > i$) for a total of 264 data points. Both monovariate and bivariate statistical analyses were performed on the data, yielding several revealing trends and correlations. Histograms of differences in properties between polymorphic pairs, shown in Fig. 6a–c, indicate respectively that differences in crystal packing energies, densities and lattice-vibrational entropies for polymorphs are rather small while Fig. 6d reveals that an appreciable proportion (actually 18%) of polymorphs have $Z' > 1$ (where Z' is the number of molecules in the crystal asymmetric unit). Some further important conclusions drawn from this study are as follows: both calculated and experimental values for the relative stability of crystal polymorphs are currently subject to large uncertainties; polymorphs with $Z' > 1$ are as stable, or even more stable than those with $Z' = 1$; higher crystal density is, as expected, found to

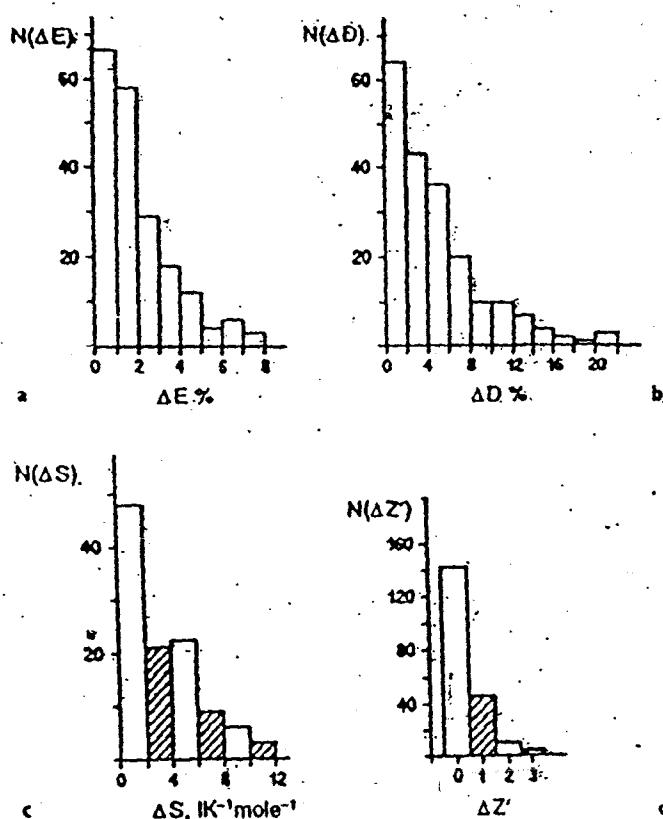


Fig. 6a–d: Histograms of differences in properties between polymorphs: a ΔE (packing energy, %); b ΔD (density, %); c ΔS (lattice-vibrational entropy, $\text{J K}^{-1} \text{mol}^{-1}$); d $\Delta Z'$ (no. of molecules in the asymmetric unit). (Reprinted with permission from [79], copyright 1995, American Chemical Society)

correlate with higher packing energy. Finally, from the observation that 24 % of the polymorphic pairs analysed comprised both a centrosymmetric and a non-centrosymmetric partner, it was concluded that the link between molecular properties and centrosymmetry of crystals is evidently weak. The reader is referred to this seminal study [79] for the full exposition of the above conclusions and their justification, as well as further perceptive observations on the phenomenon of polymorphism.

3

Methodology for the Study of Crystal Polymorphism

3.1

Review of Preparative Methods

Research on the polymorphism of a new molecular entity normally commences with experimental screening which can indicate the occurrence of more than one crystalline form of the substance. An inexpensive method of such testing is hot stage microscopy (HSM), which has been used very extensively and effectively by a leading proponent [80] for many years to provide preliminary indications of the presence of crystalline polymorphic and pseudopolymorphic (solvated), as well as glassy (amorphous) forms, all of which may have practical utility. Pseudopolymorphic forms are molecular adducts containing solvent of crystallization and have been classified [37] as stoichiometric solvates and non-stoichiometric inclusion compounds possessing channel, layer or cage (clathrate) structures. Procedures for detecting the existence of multiple forms by HSM have been outlined [37,81]. These include, for example, the observation of solid-solid transformation upon heating the substance, observation of transformation (spontaneous or mechanically induced) following the freezing of a melt, and detection of gas evolution as bubbles from pseudopolymorphs immersed in silicone oil during heating (indicative of a solid \rightarrow gas + solid transformation). Once the existence of multiple forms is established, practical methods for the preparation of specific forms on a larger scale may be explored. Frequently, recrystallization of the compound from solvents or solvent mixtures spanning a wide polarity range is effective in producing several of the different forms in sufficient quantity for complete characterisation by the analytical methods to be discussed. Most pseudopolymorphs are prepared by crystallization of the parent organic compound from the respective solvent, whereupon the latter becomes incorporated in the new crystal. Recrystallization from a mixed solvent system may yield a pseudopolymorph containing either or both solvents. Exposure of the parent organic compound to vapours may also result in the formation of pseudopolymorphs (as occurs e.g. when anhydrous drugs react with atmospheric water to form hydrates). This is exemplified by the drug indomethacin, for which four polymorphic forms (I-IV) have been identified [82]. Crystallization of the commercially available Form I from over fifty solvents yielded Forms I, II, IV and mixtures of these, as well as a number of pseudopolymorphs [83]. To ensure subsequent reproducibility in the preparation of specific forms by crystallization, careful attention to the details of solvent purity, degree of solu-

tion agitation, temperature, supersaturation and the rate of cooling of the solution is necessary. Detailed methods for isolating metastable polymorphs from the melt or from solution have been reviewed [81].

Desolvation of a pseudopolymorphic form by controlled heating leads to the formation of a polymorph or a mixture of polymorphs of the parent compound, hence providing an additional route to isolation of such species. For example, for indomethacin quoted above, twelve pseudopolymorphs with the general formula indomethacin · (solvent)_n ($n = 0.2 - 1.1$) were isolated [83] and their products of desolvation were characterised. Whereas mixtures of Forms I and II resulted from heating the benzene, CCl₄, CHCl₃ and toluene pseudopolymorphs, pure Form II was obtained from desolvation of the acetone pseudopolymorph. The most soluble (and pharmaceutically desirable) polymorph, Form IV, was obtained in a pure state by desolvation of the pseudopolymorph containing methanol.

A technologically very important and potentially beneficial feature of some polymorphs obtained in this way is the possibility that they may acquire altered rheological or other properties (flowability, texture, particle size distribution, compressibility) when compared with the same polymorphic crystalline forms obtained by direct crystallization from solution. Two examples of considerable pharmaceutical relevance may be cited, namely those of lactose and paracetamol. The lactose used as an excipient in pharmaceutical tablets and capsules is α -lactose monohydrate. It was found that thermal dehydration of this species or desiccation of α -lactose containing methanol yielded a stable product with superior binding properties and excellent flowability [84]. Tablets prepared by compaction of the stable product had an overall porosity nearly equal to those of tablets prepared with the original materials. The very poor compression abilities of paracetamol prompted an investigation of solvation/desolvation as a process for preparing pure paracetamol with improved properties [85]. A crystalline hemisolvate of paracetamol was prepared by cooling a hot-saturated solution of the drug in dioxane. Desolvation of this species yielded pure paracetamol with significantly improved technological properties including flowability, die filling, and hardness/pressure profile. A crucial point is that this improvement was not attributable to the production of a new polymorphic form of the drug; the X-ray powder diffraction pattern of the desolvated material was the same as that of the commercially available, monoclinic polymorph of paracetamol. However, detailed examination with scanning-electron microscopy revealed that desolvation produces material with an unusually porous, sintered-like texture which lends itself to compression more readily than other forms of the drug. Interestingly, solvation/desolvation using other oxygen-donor solvents (e.g. acetone, cyclohexanone) yielded paracetamol in the same (monoclinic) form but failed to produce paracetamol crystals with the required texture. An earlier review on polymorphism [37] lists several drug pseudopolymorphs whose desolvation leads to significant particle-size reduction (or micronization) of the ensuing polymorphic crystallites. This usually results in improved dissolution and tableting properties of the polymorph.

Mechanical grinding and compression of compounds represent another possible route to polymorphs. In the former case, the local pressures induced by

the mechanical stress may initiate the transformation of the original polymorph into another crystalline form. From an industrial viewpoint, grinding and compression are attractive processes, being relatively inexpensive and requiring no solvents. In addition, complete polymorphic conversion may be effected in very short times (several minutes in some cases). Recent examples include the production of polymorphic Form I of sulphathiazole by planetary ball-milling of Form III [86], the transformation of metastable Form I of caffeine into the stable Form II by either grinding or compression [87] and production of Form I of probucol by manual trituration of Form II [59]. The role of grinding in the design of pharmaceutical dosage forms has been investigated and the effects of increased specific surface areas and enhanced solubilities following mechanical action have been noted [88]. The same group reported the effects of environmental temperature and compression energy on the polymorphic transformations of the antidiabetic chlorpropamide [89] and discussed the relation between the polymorphic transformation pathway during grinding and the physicochemical properties of bulk powders of cephalexin, chloramphenicol palmitate and indomethacin [90]. Local temperature variations during grinding may also play a role in effecting polymorphic transformation and it is desirable to separate the two influences. This was recently achieved with cortisone acetate by cryogrinding at 78 K, during which the monoclinic form transformed to an orthorhombic form in ten minutes purely by mechanical effects [91].

Prolonged mechanical grinding of a crystalline compound may produce material in an amorphous (or glassy) state which, due to the lack of long-range internal order of the constituent molecules, displays a broad melting temperature range and a diffuse X-ray diffraction pattern. A discussion of polymorphism without reference to amorphism would represent serious neglect of an important aspect of phase behaviour. The amorphous state represents the thermodynamically least stable form of the compound, which consequently has a tendency to revert to a more stable form. The amorphous material is also the most soluble form of the compound and this property is used to advantage in pharmaceutical preparations in cases where the solubility of the crystalline form of the drug is low, leading to poor systemic absorption. A well known example is the antibacterial novobiocin acid, for which the solubility of the amorphous (and therapeutically active) form is ten times that of the crystalline (inactive) form [92]. However, use of an amorphous form in a suspension may require addition of another component to suppress spontaneous transformation to a thermodynamically more stable form. For novobiocin acid preparations, the additives methylcellulose and polyvinylpyrrolidone are successful in this respect. It seems likely that the amorphous state may attain even greater practical significance in view of the recent reference to "amorphous polymorphism" [14], i.e. the existence of more than one distinct amorphous phase of the same substance. This phenomenon, which has been studied by computer simulation, evidently occurs in substances where the thermodynamic behaviour of the liquid state exhibits liquid-liquid phase separation or a tendency towards it.

In concluding this discussion of the methods of preparing polymorphs, several examples may be quoted of observations or procedures which, owing to their novelty or confinement to only one particular compound, cannot be

described as general. However, since systematic study of the factors which might be involved in effecting or affecting polymorphic transformation in these cases could in principle find wider application, brief mention of such examples is made here. The effect of explosion on polymorphs of chitin and chitosan has recently been studied [93]. Explosion of α -chitin resulted in no change, but explosion of hydrated chitosan of low crystallinity yielded a product with increased crystallinity as well as a small amount of the anhydrous phase. The effect of an electric field on polymorphic transformation in certain classes of compounds may be a phenomenon warranting systematic investigation. Strong temperature-dependence of the $\beta \rightarrow \alpha$ polymorphic transition of isotactic polypropylene (containing additives) has been observed when the system is exposed to an electric field [94]. The effects of neutron-irradiation on the kinetics of polymorphic phase transitions for several inorganic compounds have been reported [95], but similar studies using organic compounds as substrates are lacking. It has, however, been demonstrated that ionizing radiation can induce a polymorphic transformation in an organic compound. A polycrystalline sample of the β -polymorph of a diacetylene nitroxide has been reported to undergo transition to the α -polymorph on irradiation with $\text{CuK}\alpha$ or ^{60}Co sources [96]. X-ray powder diffraction was used to monitor this phase transformation.

Finally, the role of serendipity in producing polymorphs may be mentioned. Two recent cases involved unsuccessful attempts to produce molecular complexes by reaction of two components in solution, resulting instead in the precipitation of crystals of one component in a desirable polymorphic form. Attempts to grow crystals of methotrexate by various techniques failed [27]. However, tetragonal crystals of the compound were obtained from a solution containing methotrexate and thymidine, prepared for the purpose of obtaining a co-crystal of these components. Similarly, attempted complexation between 5-sulphamethoxydiazine and *p*-aminosalicylic acid failed, the solution of these species in a 1:1 molar ratio producing instead large crystals of Form II of the sulphonamide [92]. Interestingly, this polymorph of the drug is the biologically most active form and is usually crystallized from ethanol followed by rapid cooling of the solution to -12°C . This yields small particles with little or no geometrical form [99]. A possible explanation for the crystallization of Form II during attempts to produce the complex has been discussed [98].

3.2

Review of Investigative Methods

Having outlined the methods of preparation of polymorphs and pseudopolymorphs, this report now focuses on the methodology used to study these forms. The discussion commences with a survey of some well established and still widely used techniques, each of which is illustrated by one or more applications. This is followed by a survey of newer methodology which is being used to probe organic crystal polymorphism.

A wide spectrum of analytical techniques may be used to characterize polymorphs and pseudopolymorphs in terms of their structure, spectral energies, thermodynamic stabilities, kinetics of transformation and solubility behaviour.

The choice of analytical and physicochemical methods for the characterization of polymorphs is dictated by the need to measure properties which ultimately depend on the different internal arrangements of the same molecules in these phases. When pseudopolymorphs are also considered, the range of suitable analytical techniques is significantly broadened owing to the presence in the crystal of the solvating molecule and the possibility of analysing the physical and chemical changes which may accompany both formation and decomposition of pseudopolymorphs.

Some remarks on the use of white- and polarized-light microscopy serve as an appropriate introduction to this section. With the advent of more sophisticated analytical methods, the use of microscopy has, to some extent, fallen into neglect. This is unfortunate, since investment of relatively little time and effort in studying a crystal of a polymorph or pseudopolymorph by microscopy can be invaluable, enabling one to assess the overall quality of a recrystallization, to detect crystal faults, fractures and macroscopic inclusions, and obtain preliminary information which will facilitate subsequent X-ray examination. Detection of different crystal habits (acicular, tabular, bladed; plate-like, prismatic) using white-light microscopy is not necessarily indicative of polymorphism since crystal habit depends on crystallization conditions and may vary widely for a given polymorph. However, this method is useful for distinguishing polymorphs having different colours in reflected or transmitted light. Pseudopolymorphs which undergo pseudomorphosis (i.e. loss of solvent on removal from their mother liquor) tend to form opaque, microcrystalline masses which are also discernible by ordinary microscopy. Addition of a polarizing attachment allows distinction between optically isotropic crystals and anisotropic crystals as well as the measurement of refractive indices [100]. Optically isotropic crystals belong to the cubic system and have a single value for their refractive index. The vast majority of crystalline organic compounds are optically anisotropic, having multiple refractive indices and displaying numerous optical effects which may be used to differentiate polymorphic forms. Anisotropic crystals reveal themselves by producing variable interference colours as well as regular extinction of plane-polarized light on rotation of the microscope stage. They may be uniaxial (characterized by two principal refractive indices and belonging to the trigonal, tetragonal or hexagonal systems) or biaxial (with three refractive indices and belonging to the triclinic, monoclinic or orthorhombic systems). Measurement of these refractive indices is certainly a means of identifying a polymorph unequivocally, but is seldom done for this purpose. The uniaxial or biaxial nature of the crystal is easily determined from observation of the respective characteristic interference figure when the crystal is viewed with condensed (conoscopic) light. Taken together, extinction directions, crystal morphology and uniaxial or biaxial character can facilitate the identification of a new polymorph, as exemplified by the following case from our laboratory. Carbamazepine commonly crystallizes in the monoclinic system with a prismatic habit [101]. Microscopic examination of crystal batches obtained by recrystallization of the drug from a wide range of solvents confirmed the predominance of this form. However, crystals obtained from tetrahydrofuran were acicular, yielding extinction parallel to the needle-axis and presenting a uniaxial interference figure.

Measurement of the interfacial angles of a crystal section both microscopically and using an optical goniometer yielded values of $120 \pm 1^\circ$. Thus, a new polymorph belonging to either of the trigonal or hexagonal systems was unequivocally identified. This was confirmed by subsequent X-ray photography which revealed a trigonal space group [102].

Owing to its vast depth of field, scanning electron microscopy (SEM) is widely used for observing the texture, morphology and surface features of both powders and large single crystals of polymorphs. The high vacuum used for sample observation precludes study of pseudopolymorphs containing volatile solvents but SEM micrographs of polymorphs are useful for purposes of identification provided that crystallization conditions and SEM sampling methods are carefully controlled.

Crystal density is an important technological parameter since it affects the flow properties of bulk solids. Due to the different bulk densities of polymorphs and their abilities to retain solvent, different isolation strategies are required in industry [24]. Furthermore, if mixtures of different solid phases are present in a sample (e.g. in a powdered pharmaceutical formulation), differences in component densities may lead to a heterogeneous product during processing due to phase segregation. Distinguishing polymorphs of the same compound by density measurement (determined by flotation or gas displacement pycnometry) is difficult because, as shown in Fig. 6b, differences in the densities of such species seldom exceed 5% and the experimental error of routine measurements is typically 2%. However, the latter can be reduced if special precautions are taken during flotation measurements, especially with regard to eliminating occluded air. Under these conditions, anomalously high or low measured densities may be useful indicators of the presence of pseudopolymorphs. Thus, e.g., a measured density of $1.30(1) \text{ g cm}^{-3}$ for a crystal of doxylamine succinate obtained from ethyl acetate was sufficiently different from that of polymorphic Form I ($\rho = 1.21(1) \text{ g cm}^{-3}$) to indicate the presence of a pseudopolymorph. Subsequent X-ray analysis showed the crystal to have the unexpected composition (doxylamine succinate)₁ · (succinic acid) [103].

Among the thermal methods of analysis, thermogravimetric analysis (TGA), differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have been used extensively to quantify thermal events accompanying controlled heating of polymorphs and pseudopolymorphs [18, 23]. Figure 7 shows combined TGA and DSC traces for two pseudopolymorphs of nitrofurantoin [61], containing respectively *N,N*-dimethylformamide (DMF) and dimethylsulphoxide (DMSO).

In TGA, the sample (~5–10 mg) is heated at a predetermined rate and the weight is recorded as a function of temperature. This technique cannot distinguish polymorphs of a given organic compound, but for pseudopolymorphs which lose their included solvents prior to melting or decomposition of the parent ("host") compound, the percentage weight loss may be accurately measured and used to calculate the stoichiometry of the pseudopolymorph. Data recorded from the TGA traces shown in Fig. 7 indicated a nitrofurantoin:DMF stoichiometric ratio of 1:1 and a nitrofurantoin:DMSO ratio of 2:1. A TGA trace may reflect simple one-step weight loss of included solvent or more complex

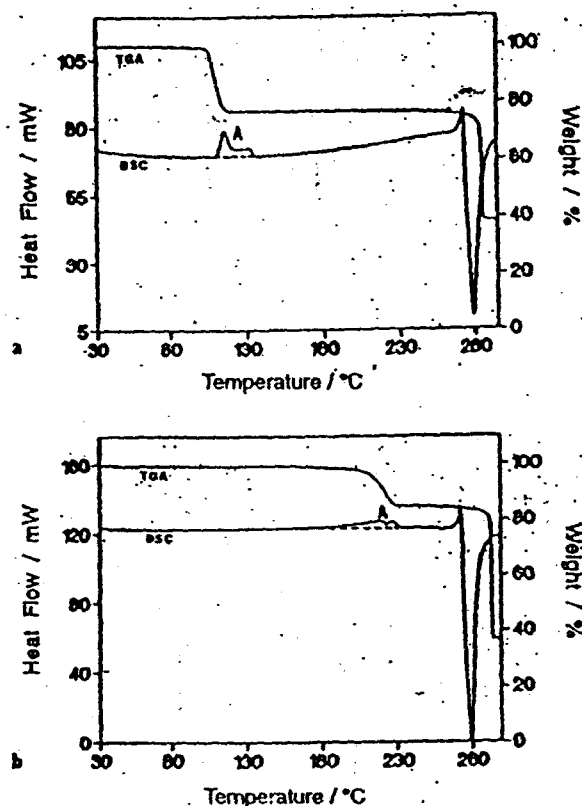


Fig. 7a, b. Combined TGA-DSC traces for pseudopolymorphs of nitrofurantoin containing: a DMF; b DMSO (Reprinted with permission from [61]; copyright 1996, Gordon and Breach Publishers)

multi-step weight losses. Coupled with TGA, evolved gas analysis (EGA) using IR or mass spectrometry is an important means of identifying gaseous products [18] and may be used to quantify both the pyrolysis products from thermal degradation of polymorphs as well as solvents released from desolvation of pseudopolymorphs. Another important application of the TGA method is the determination of the activation energy for desolvation of a pseudopolymorph from traces recorded at varying heating rates [104]. Its application to pseudopolymorphs of succinylsulfathiazole [105] and tenoxicam [106], and to inclusion compounds of synthetic hosts [50] have recently been described.

In DTA, the sample temperature (T_s) is compared with that of a reference compound (T_r) as a function of increasing temperature. The resulting plot of ΔT ($=T_s - T_r$) vs T may display endothermic peaks corresponding to desolvation (for pseudopolymorphs) or fusion (for polymorphs) and exothermic peaks representing recrystallization or decomposition processes. In the related DSC technique, the difference in energy inputs into a compound and a reference substance is plotted against T during a controlled temperature programme. In