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REVIEWS

Chemical Crystallography in India—From Naphthalene to Gleevec

Bipul Sarma[a] *and Ashwini Nangia*[b]

Abstract | The method of choice to determine the structure of matter at atomic resolution and at the molecular level is X-ray crystallography. Max von Laue discovered X-ray diffraction by crystals (1912) and William Henry Bragg and William Lawrence Bragg complemented the theory with the design of an X-ray spectrometer and the famous Bragg's Law (1913). India has been an integral part of the history and development of X-ray diffraction since the work of Kedareswar Banerjee on direct methods in solving the crystal structures of naphthalene and anthrancene in the 1930s. A vertical take-off of the subject of chemical crystallography to crystal engineering happened in the last two decades. Today chemical crystallography and crystal engineering have spread horizontally into the allied fields of materials science, drug design, pharmaceutical development, nanomaterials, gas storage and solar energy devices. This account summarizes the evolution of X-ray diffraction from a fundamental technique to understanding structure–property relationships to the next challenges in studying the microstructure of crystalline solids.

1 Introduction

X-ray crystallography is extensively used to determine the three-dimensional structure of atoms and molecules in crystals. X-ray diffraction is the method to obtain detailed and precise structural information about the arrangement of molecules in crystalline matter. Diffraction is the orderly pattern of spots due to the periodic arrangement of atoms, molecules or ions in the crystal lattice. The diffraction pattern provides details of atomic arrangement, their chemical nature, and the intermolecular interactions between the molecules or ions. The integration of advances in computer-controlled diffractometers has made X-ray data collection and processing a highly automated process. The correct and accurate determination of the X-ray crystal structure of a small molecule is a fairly routine and fast exercise today. As a result, single crystal studies are increasingly becoming more common and easily doable even for non-specialist scientists. Thus, organic and inorganic chemists, materials scientists, medicinal and pharmaceutical chemists, are able to obtain information about structure and bonding to interpret the properties of the solids of interest. This article is written more as an opinion viewpoint rather than a rigorous scientific review. We have kept the number of references cited to a bare minimum, because the material is easily accessible in an internet search, and moreover, this article is best read as a chronological evolution of X-ray diffraction and chemical crystallography in the country: from the use of direct methods to solve the X-ray crystal structure of naphthalene to the high profile Gleevec case on polymorphs of imatinib mesylate.

2 Historical Background

The start of modern scientific research in India began at the beginning of the 20th century. The setting up of universities and institutes during the 1920–1940s period was the base of India's scientific system. Sir C. V. Raman's ground breaking experiments at the Indian Association for the Cultivation of Science (IACS), Calcutta on the scattering of light (Raman Effect) led to the award of

[a]*Department of Chemical Sciences, Tezpur University, Tezpur 784 028, India.*
sarmabipul@gmail.com

[b]*School of Chemistry, University of Hyderabad, Central University PO, Gachibowli, Hyderabad 500 046, India.*
ashwini.nangia@gmail.com

Nobel prize for Physics in 1930. IACS was a purely indigenous institution for scientific research established in 1905 by Mahendra Lal Sircar. At about the same time and at same institution, research on the structure elucidation of molecules began. An article appeared in Nature (1930)[1] on the determination of the X-ray crystal structure of naphthalene and anthracene using direct methods by the then Director of IACS, Kedareswar Banerjee. He established the foundations of X-ray crystallography research in India. He worked with Sir William Henry Bragg to propose the extremely powerful approach for a solution to the phase problem, known as direct methods. Apart from crystal structure determination, which in those days was a tremendously difficult task, Banerjee focused on crystal physics, specifically crystal optics, X-ray scattering from crystals, organic solids and polymers, etc. His significant contributions to the progress of science in the country led the creation of new research institutes which have carried forward the scientific tradition. Pioneering work by the physicists of that time expanded the system of schools, laboratories and institutes in different corners of the country. The biology school was started by G. N. Ramachandran at the University of Madras, who was a student of C. V. Raman. Ramachandran is recognized for the triple helical structure of collagen (1954) and the textbook famous Ramachandran Plot of protein structures (1963). Other schools, such as those at Banaras Hindu University (BHU), National Physical Lab (NPL), and Delhi University by A. R. Verma, in National Aeronautical Laboratory (NAL Bangalore) by S. Ramaseshan, and at the Bhabha Atomic Research Centre (BARC, Bombay) by R. Chidambaram were established during the 1960–1980s. The Universities of Allahabad, Madras, and Calcutta became the alma mater of many an early breed of scientists. Education and research in science and engineering fields picked up rapid pace after the setting up of the Indian Institutes of Technology (IITs) in the 1960s. Fast forward about 50 years, and the Indian Institutes of Science Education and Research (IISERs) and several Central Universities across the country are the centres of crystallography teaching and research. Celebrations of 100 years of X-Ray diffraction was kicked off in the year 2013 with symposia popularizing X-ray crystallography at the National Chemical Laboratory, Pune and by the Kerela State Council for Science, Technology and Environment, Thiruvanthapuram. The National Crystallography meeting at New Delhi in November 2013 closed the centenary year and simultaneously kicked off the celebrations for the International Year of Crystallography in 2014.

3 CCD X-Ray Diffractometer

X-ray crystallography is an experimental technique which exploits the fact that X-rays are diffracted by the electrons in crystals. It is not an imaging technique, rather it uses the scattered X-rays of proper wavelength in determining the electron cloud of an atom of comparable size. Additional phase information is extracted from the diffraction data and a model is then progressively built into the experimental electron density, refined against the data, and the result is quite an accurate molecular structure. The first automated single crystal X-ray diffractometers became available in late 1960s. Immediately after, crystallographers developed the conventional methodology to perform single crystal X-ray data collection by utilizing monochromatic beam in four circle diffractometer with point detector. The introduction of area detector technology (CCD) in the 1990s brought about revolutionary improvements: (i) the data collection time was drastically reduced, (ii) the ease of diffractometer operation became automated, (iii) the sensitivity and accuracy was improved many-fold, and (iv) it became possible to collect reflections on smaller crystal. Single crystal X-ray diffraction using CCD area detectors (charge coupled device) were made available by 1995 to the scientific community. A major development of tuneable wavelength beamline and synchrotron X-ray sources brought yet another significant revolution into structural chemistry and biology, and opened new opportunities for structure-aided drug design. Synchrotron radiation sources were installed and commissioned at the Bhabha Atomic Research Center, Mumbai and the Raja Ramanna Center for Advanced Technology, Indore (Indus beamlines) in the last two decades.

4 Crystal Engineering

A crystal structure represents an energy minimum resulting from the attractive and repulsive intermolecular interactions and van der Waals interactions, which have varying strengths and directional preferences. Understanding the nature and strength of intermolecular interactions, i.e. isotropic and non-directional dispersive component (C···C, C···H, H···H interactions) that is determined by the shape, size and close packing, and the anisotropic and directional hydrogen bonds, charge transfer interactions, halogen interactions, and heteroatom interactions (e.g. O–H···O, N–H···O, C–H···O, C–H···N, O–H···π, halogen···halogen.

etc.), is essential for crystal design and structure–property correlations. Hydrogen bonding is the most reliable and directional force that has a fundamental role in crystal engineering which helps us to design target crystal structures. This is because the properties of crystalline materials are largely defined by the intermolecular interactions. The interaction motifs for designing crystals are termed as supramolecular synthons, defined by Desiraju in 1995.[2] Supramolecular synthons are structural units within supermolecules that can be formed and/or assembled by known or conceivable synthetic operations involving intermolecular interactions. He gave a general definition to the term crystal engineering (1989) which is widely accepted by the scientific community, "the understanding of intermolecular interactions in the context of crystal packing and the utilization of such interactions in the design of new solids with desired physical and chemical properties". The concept of the supramolecular synthon, which are repeating structural units in crystal structures that are able to guide the rational design and retrosynthesis of supramolecular architectures based on a small number of recurring hydrogen bond patterns, is a useful guide in the design of crystalline solids with target architectures and properties. The subject crystal engineering deals with a variety of solid-state forms (Figure 1) such as host-guest complexes, network solids, pharmaceutical salts, hydrates, cocrystals, polymorphs, non-linear optical and magnetic materials, photo-luminescence, gas storage MOFs and solar cell devices.

Polymorphism is extremely important in the pharmaceutical industry, brought out by the high profile legal battle between Glaxo vs. Novopharm on polymorph I and II of anti-ulcer drug ranitidine hydrochloride (Zantac), and the accidental appearance of a stable, less soluble polymorph of the anti-retroviral drug ritonavir (Norvir) of Abbott. Closer home, a legal battle on the polymorphs of the anti-cancer drug imatinib mesylate (Gleevec) between Novartis and the Indian Patent Office was decided in 2013 (discussed later). Therefore, a complete characterization of all possible polymorphs of a drug is considered an essential and obligatory step in the pharmaceutical industry. The best drug formulation must have the desirable properties of good solubility, bioavailability, stability, filterability, compaction, and tableting. X-ray diffraction and the crystal structure is therefore a central technique in polymorphism.

4.1 *Crystal engineering post 2000s*

Progress updates in the areas of polymorphism, crystal engineering and retrosynthesis directed supramolecular materials were reviewed in Indian publications, e.g., see the Platinum Jubilee issue of the Indian Academy of Sciences (2009)[3] and the Indian National Science Academy (2010), special of J. Chem. Sci. (2010), and a special issue of J. IISc. (2007),[4] etc. The history of crystallography

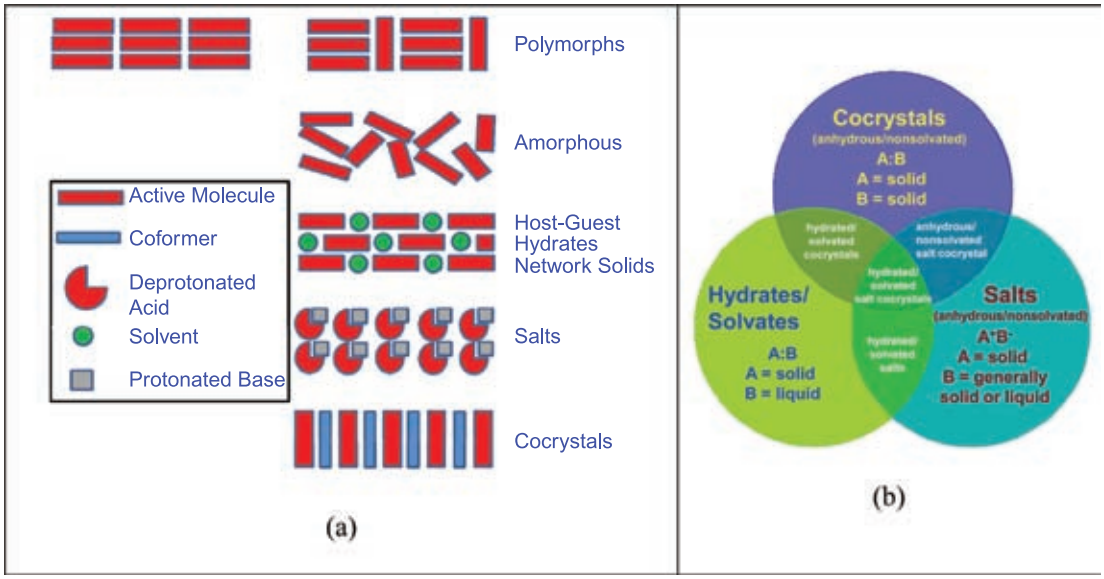

Figure 1: (a) Different crystalline forms that can exhibit different physiochemical and material properties. Graphic is taken from ACS journal Cryst. Growth Des. **9** 2950 (2009). (b) A revised representation of different solid state pharmaceutical forms which evolved at the Indo-US bilateral meeting held near New Delhi in February 2012. Graphic is taken from ACS journal Cryst. Growth Des. **12** 2147 (2012).

in India was described in full detail in a dedicated volume of the IUCr Newsletter (2007).[5] The last two decades have been the golden years for X-ray crystallography and structural chemistry in the country.

The Cambridge Structural Database (CSD) is a digitized repository of over 6,00,000 crystal structures as in 2013. An author search of this database highlights the Indian contribution to the overall area of small molecule crystallography with organic molecules and ligands. Over 30 research groups from India are actively publishing in structural chemistry and crystal engineering topics. Led by the pioneering work of G. R. Desiraju (Univ. of Hyderabad up to 2008 and then from IISc), several chemists were quick to realize the opportunities from systematic studies of crystal structures. A. Nangia (UoH), J. N. Moorthy (IIT Kanpur), Parimal Bharadwaj (IIT Kanpur), T. N. Guru Row (IISc), S. Natarajan (IISc), V. Chandrasekhar (IIT Kanpur and TIFR Hyderabad), P. Dastidar (IACS), Tanusree Kar (IACS), Alok Mukherjee (Jadavpur Univ.), K. Biradha (IIT Kharagpur), Gopal Das (IIT Guwahati), J. B. Baruah (IIT Guwahati), Pradyut Ghosh (CSMRI and IACS), Manabendra Ray (IIT Guwahati), P. S. Mukherjee (IISc), Sandeep Verma (IIT Kanpur), Rajesh Gonnade (NCL Pune), A. Ramanan (IIT Delhi), R. Murugavel (IIT Bombay), R. N. Mukherjee (IIT Kanpur), T. P. Radhakrishnan (UoH), K. C. Kumaraswamy (UoH), Samar Das (UoH), Tapas Maji (JNCASR), Arvind Bansal (NIPER Chandigarh), P. Venugopalan (Panjab Univ. Chandigarh), among others have contributed to the field for over a decade. Now younger chemists are quickly making their mark, Binoy Saha (Pondicherry University), Rahul Banerjee (NCL Pune), C. M. Reddy (IISER Kolkata), Raju Mondal (IACS), N. J. Babu (IICT Hyderabad), T. S. Thakur (CDRI Lucknow), B. Sarma (Tezpur University), A. R. Choudhury (IISER Mohali), D. Chopra (IISER Bhopal), among others. The diversity of structures and their total numbers deposited in the CSD by crystallographic groups in India is really impressive.

Two main themes seem to have emerged in India. Those working with organic molecules and ligands have focused on the application of synthons in crystal engineering for exploring pharmaceutical cocrystals and polymorphs and salts. The metal–ligand groups have largely exploited the design of large cavities and engineered pores for gas storage and energy materials. It is a formidable task to select examples from among the large number of papers published in the last five years as Authors' Selects. We present only two examples, the first is an advancement in crystal engineering strategy and the second about its application in making improved pharmaceuticals. Both papers appeared in 2013. Tothadi & Desiraju[6] presented a general design method to engineer ternary cocrystals using hydrogen bonds and halogen bonding (Figure 2). Thus, cocrystallization of a 2:1:1 stoichiometric mixture of 4-nitrobenzamide : oxalic acid : and 1,4-diiodobenzene gave the desired three-component assembly mediated by the acid–amide heterosynthon (H bonds) and the nitro–iodo synthon (X bonds). The generality of this termolecular assembly was demonstrated by a variety of dihalides and diacids. One should point out to the general reader that the designed assembly of three different chemical species in the crystal lattice by deliberate placement of complementary functional groups had no general solution up to the present time.

The authors conclude that "A fine balance of interactions (H bonds and X bonds) and solubilities (of the components) is therefore needed to get a ternary cocrystal, the design of which

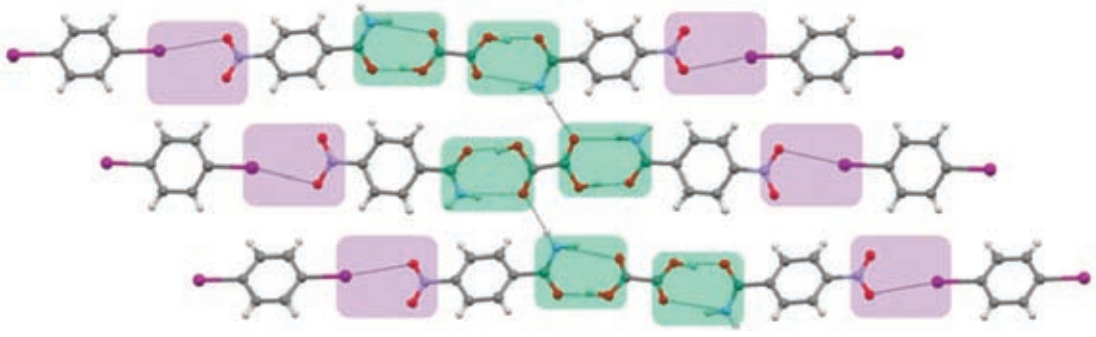

Figure 2: A designed ternary cocrystal of 4-nitrobenzamide with oxalic acid (green synthon) and diiodobenzene (pink synthon) in 2:1:1 ratio. The packing diagram is extracted from RSC journal (ref. 6).

remains one of the big synthetic challenges in crystal engineering of molecular organic solids". The second example is again about the subtlety of interaction strengths and close packing to give a cocrystal, solid solution or eutectic. Cherukavada and Nangia[7] summarized guide rules about the product nature in multi-component assembly (Figure 3) based on the analyses of a few pharmaceutical case studies: "When the adhesive interactions dominate, the result is a cocrystal; when the adhesive and cohesive interactions are balanced and there is size/shape match, the product is a solid solution; and when the cohesive interactions take over for size/shape mismatched components, the product is a eutectic." A direct bonus of this learning point is that (i) the success rate of cocrystal experiments may improve by providing alternate pharmaceutical materials as eutectics, and (ii) eutectics will then become a part of mainstream crystal engineering.

Both these recent studies mean that a digital 0/1 type approach to crystal engineering may well have worked with modular model systems, but real applications will require a holistic understanding of the crystal structure[8] and its constituent intermolecular interactions.

4.2 *PXRD analytical technique*

Single crystal X-ray diffraction is certainly the gold standard in structure determination. However, single crystals can be difficult to crystallize in a routine manner. Structure solution from powder data (SDPD, e.g. see the papers from Alok Mukherjee group of Jadavpur University) is an attractive solution to obtaining 3D coordinates matching in accuracy with those of a single crystal X-ray structure. In a typical powder XRD measurement it is not possible to know the orientations of the reciprocal vectors. The solution gives only their lengths and an approximate (guess) unit cell and space group assignment of Miller indices. The PXRD technique, despite its current limitations to structure determination in a routine way, which will hopefully be addressed soon with advances in diffraction technology and structure solution software, is the most preferred method to study phase transition, anisotropic stress, high temperature gradients, etc. PXRD is in many ways the gold standard for crystalline form of fingerprinting in the pharmaceutical industry. The growth of X-ray powder diffraction in India (see Table 1) owes, in a large measure to the rise of the generics pharmaceutical industry.

Thanks to the availability of XRD instruments in academic and industrial labs, generic pharmaceutical companies are now able to properly characterize and quantify their products in terms of polymorphic purity, hydration state, crystalline form, regulatory compliance, etc. The nature of projects being handled by pharmaceutical scientists has advanced over the years. Whereas a common objective about a decade ago was "how to grow a given polymorph selectively", a challenge these days seems to be "how to stabilize a novel polymorph without transformation to the stable modification". Surely new opportunities are opening up with analytical support from X-ray diffraction.

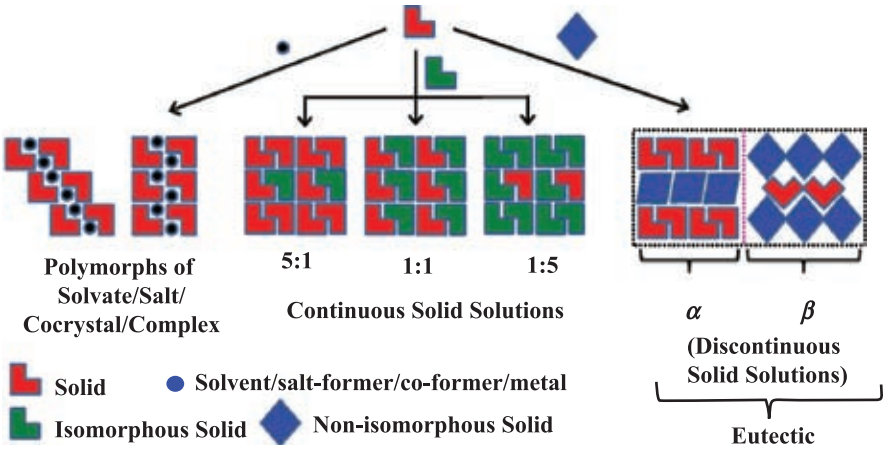

Figure 3: The combination of isomorphous solids gives rise to continuous solid solutions and solids in which the adhesive interactions outweigh the cohesive ones lead to cocrystals. With weak adhesive, strong cohesive and a geometric misfit, the product is a eutectic. Cartoon representation is extracted from RSC journal (ref. 7).

Table 1: The number of PXRD instrument installations in India over the last decade.

Year	Academic Institutes	Industrial R&D	Total
2000	25	7	32
2001	26	9	35
2002	25	8	33
2003	27	11	38
2004	32	21	53
2005	29	17	46
2006	33	17	50
2007	66	25	91
2008	55	10	65
2009	64	17	81
2010	64	15	79
2011	53	14	67
2012	68	25	93
2013	60	25	85
Total	**627**	**221**	**848**

4.3 *The Gleevec patent case*

The INN of Gleevec is imatinib mesylate, or 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl] benzamide methanesulfonate. It is sold as film-coated tablets containing imatinib mesylate equivalent to 100 mg of imatinib free base. Imatinib is a protein-tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, the constitutive abnormal tyrosine kinase created by the Philadelphia chromosome abnormality in chronic myeloid leukemia (CML). The main contention in the patent battle is about two polymorphs of imatinib mesylate, α and β, from several known forms. α crystals are of needle morphology and hygroscopic, and pose difficulties in manufacturing and tableing. β crystals are of uniform morphology and easy to handle and also thermodynamically stable. The free base imatinib is protected by US patent 5,521,184 granted on May 28, 1996 (the so-called Zimmermann Patent after the inventor Jürg Zimmerman). Subsequently, a US patent was awarded for the β crystalline form (No. 6,894,051 dated May 17, 2005). β crystals exhibit superior crystalline behavior, are non-hygroscopic, and constitute a pharmaceutically acceptable stable salt. Whereas the drug was submitted for approval taking priority of the Zimmerman patent, the marketed crystalline form of Gleevec (also Glivec) is the latter patent on the β form.

Novartis filed for a patent in India, not for the free base imatinib but the β polymorph of imatinib mesylate. Gleevec is available in the Indian market since 2001. After a series of litigation arguments and judgments by the Intellectual Property Appellate Board, The Madras High Court, and appeals by the Swiss drug maker Novartis, the case was finally decided at the Supreme Court in April 2013. Novartis AG vs. The Union of India & Ors. Civil Appeal Nos. 2706–2716 of 2013. Novartis argued that even as Gleevec was claimed in the Zimmerman patent (imatinib molecule), it was not fully disclosed in an enabling manner (the β polymorph), thus making a differentiation between "claims" and "disclosure". This wonderful legalese was eloquently rejected by the Supreme Court, "We certainly do not wish the law of patent in this country to develop on lines where there may be a vast gap between the coverage and the disclosure under the patent; where the scope of the patent is determined not on the intrinsic worth of the invention but by the artful drafting of its claims by skilful lawyers, and where patents are traded as a commodity not for production and marketing of the patented products but to search for someone who may be sued for infringement of the patent." The Indian courts' and patents board rulings were guided by Section 3(d) of the Indian Patents Act (Amendment), 2005: "the mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such process results in a new product or employs at least one new reactant," is not patentable. The Madras High Court had emphasized that "if the discovery of a new form of a known substance must be treated as an invention, then the Patent applicant should show that the substance so discovered has a better *therapeutic* effect." The Supreme Court heralds Section 3(d) as a "second tier of qualifying standards for chemical substances/pharmaceutical products in order to leave the door open for true and genuine inventions but, at the same time, to check any attempt at repetitive patenting or extension of the patent term on spurious grounds." This latter practice is referred to as ever-greening by innovator pharmaceutical companies to extend the patentable life term of drugs. Section 3(d) aims to prevent ever-greening by providing that only those pharmaceutical derivatives which demonstrate significantly enhanced "efficacy" are patentable. The landmark Gleevec judgment clearly shows that pharmaceutical IPR in India will be interpreted as a balance between Patent and Patient. The patients prevailed in the Gleevec verdict.

5 Conclusions

It is time to celebrate 100 years of X-ray diffraction[9] and 20 years of crystal engineering.[10] Chemical crystallography is an inter-disciplinary subject covering chemistry, physics, biology, materials and pharmaceutical science, and poised for high growth and impact in its golden period. India's contribution to the field is mainly in small molecule crystallography of organic, inorganic, pharmaceutical, optical-magnetic and microporous MOF materials. The availability of modern X-ray diffractometers in academic institutes and R&D labs has yielded rich dividends for crystal engineering and the pharmaceutical industry. This sets the stage for enabling the next round of technological advance, namely accessibility to synchrotron and neutron radiation sources for academic and industry user groups in the country.

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Bipul Sarma is Assistant Professor at Department of Chemical Sciences, Tezpur University. He received B.Sc. degree from B. Borooah College and M.Sc. (In 2003) from Cotton College, Gauhati University, Guwahati. Then he completed his Ph.D. from School of Chemistry, University of Hyderabad (In 2009). After his post-doctoral research at Illinois Institute of Technology (IIT), Chicago, USA (2010) and Massachussets Institute of Technology (MIT), Cambridge, USA (2010–2012), he joined Tezpur University in the middle of 2012. He is the author of nearly 20 publications including book chapters. He is also the recipient of Dr. K. V. Rao Scientific Society Annual Research Awards for Young Scientist 2009 under the category of Chemistry and Allied Sciences and the member of American Chemical Society (ACS). His current research focus is on organic pharmaceutical solid states chemistry, heterogeneous nucleation at the functionalized interface and organic nanocrystals.
http://www.tezu.ernet.in/dcs/

Ashwini Nangia is Professor of Chemistry at University of Hyderabad. He completed M.Sc. from IIT Kanpur (1983) and Ph.D. from Yale University (1988). He joined University of Hyderabad in 1989 and was promoted to Professor in 2002. The focus of his research is on crystal engineering topics with applications in drugs and pharmaceuticals, namely polymorphs, cocrystals, salts, and most recently eutectics. He is author of over 200 research publications and a dozen patents. He is a Fellow of all three premier National Science Academies of India. He is a recipient of JC Bose Fellowship awarded by the Department of Science & Technology.
http://chemistry.uohyd.ernet.in/~an/