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A review on x-ray crystallography and it's applications

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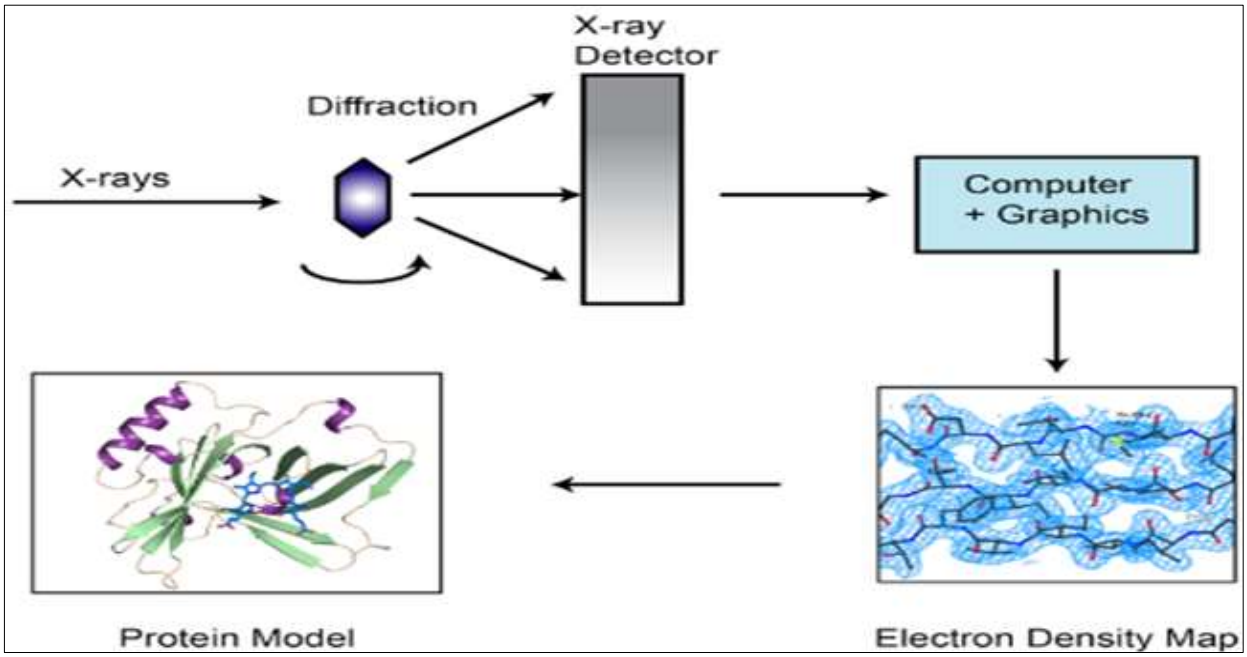
Abstract
The internal arrangement of molecules in the solid form determines a matter's inherent properties. Thus, the design of functional materials and the understanding of the correlation between structure and property require an understanding of the three-dimensional structure of matter. X-ray crystallography has been the preferred technique for precisely determining molecular structure at the atomic level.
Electronics, minerals, geosciences, materials science, medicines, and other fields saw rapid growth due to the structural knowledge derived from crystallographic research. Understanding the structure of active pharmaceutical ingredients (APIs) is necessary for the synthesis of novel chemical entities intended for use in the creation of new drugs as well as for the rational design of drugs. In the last twenty years, X-ray crystallography has been essential to the use of X-rays to examine crystals is known as X-ray crystallography (XRC). X-ray crystallography (XRC) is the principal technique used to visualize and discover the detailed structure of molecules, particularly those related to living systems. The process involves subjecting a well-organized crystal of a substance to X-rays, and then obtaining the structural information from the spots that are created on a film as a result of this impact. The structure of the majority of milk proteins has been found via X-ray crystallography, which has been widely and significantly utilized in the analysis of liquid milk, milk powders, milk stones, polymorphism of milk fat, and linking the structure of these proteins with potential roles. X-ray crystallography helps analyze the active pharmaceutical ingredients (APIs) in pharma industries, it also used in Nano science, Forensic sciences, Electronic device materials making. Protein stracture analysis.

Keywords: X-ray crystallography, crystals, detector, electron density maps, resolution

Introduction
Because of their short wavelengths, X-rays can see molecules' molecular structures and individual atoms. Any structure can only be viewed if electromagnetic light with a wavelength comparable to its dimensions is employed. Proteins should be in the order of (1010 m). The wavelength of X-rays (1 to 100) approximates this; thus, the use of X-ray crystallography technology is effective for examining the structure of biological macromolecules.
Crystals are not required for X-ray investigation; any ordered (or partially ordered) array of molecules can generate meaningful X-ray data. However, crystals are the best samples. As a result, crystals have become a requirement for X-ray diffraction. experiments. For many proteins, the production of appropriate crystals is rate limiting [1]. The most critical component in producing diffraction quality crystals is the high purity of protein preparations utilized for crystallization.
Diffraction-based crystallography has developed into a popular and effective experimental method during the past 100 years for figuring out the three-dimensional structures of both tiny and large molecules [2].
All matter on Earth is composed of atoms connected by interatomic connections. Matter's properties are determined by its precise interatomic bonding. To better comprehend material qualities, researchers studied how atoms are organized to create molecules and the intermolecular interactions that bind them into solids. X-ray crystallography is the most effective method for determining the atomic locations, intermolecular interactions, and overall structure of a molecule at atomic resolution [3].
Since its original application for determining the crystal structure of NaCl, X-ray diffraction has earned essential importance in many branches of science for understanding the structure-property correlation [4].
X-ray crystallography is an analytical method in which the crystalline atoms cause a beam of incident X-rays to diffract into numerous distinct directions. This method is used to determine the atomic and molecular structure of a crystal.

X-rays electromagnetic radiation with wavelengths ranging from 0.1 to 100, created by blasting a target (usually a metal such as Cu or Mo) with fast electrons. Chirality is a geometric trait that some molecules and ions possess. A chiral molecule or ion cannot be superimposed on its mirror counterpart (Greek: chair = hand). One of numerous structural factors that cause chirality in organic and inorganic compounds is the existence of an asymmetric carbon center.



History of X- ray crystallography

Diffracted by single crystals in 1912, earning him the Nobel Prize in Physics in 1914 [5]. The following year, W.L. Bragg developed the renowned diffraction law known as Bragg's law, which proved the use of a diffraction pattern to determine the crystal structure of NaCl [6]. Instrumentation to observe diffraction patterns was designed in the pioneering manner by his father, W. H. Bragg. The diffraction pattern was analyzed using Bragg's law (called only after the son, W. L. Bragg), and the father and son Braggs were awarded the Nobel Prize in Physics in 1915 for their collaborative and independent research. X-ray diffraction's significance and range of applications in science have resulted in 29 Nobel Prizes being given out thus far. The United Nations has recognized the successful integration of crystallography into other scientific domains by designating 2014 as the International Year of Crystallography [7]. For this reason, it's crucial to highlight a few revolutionary findings that increased the importance of crystallography. The diamond structure established the relevance of crystallography in understanding material properties after the first crystal structure of NaCl was found by X-ray diffraction. Carbon atoms in the crystal lattice are arranged in a repeating tetrahedral pattern, which is the basis for the hardness of diamond (Fig. 1) [8].

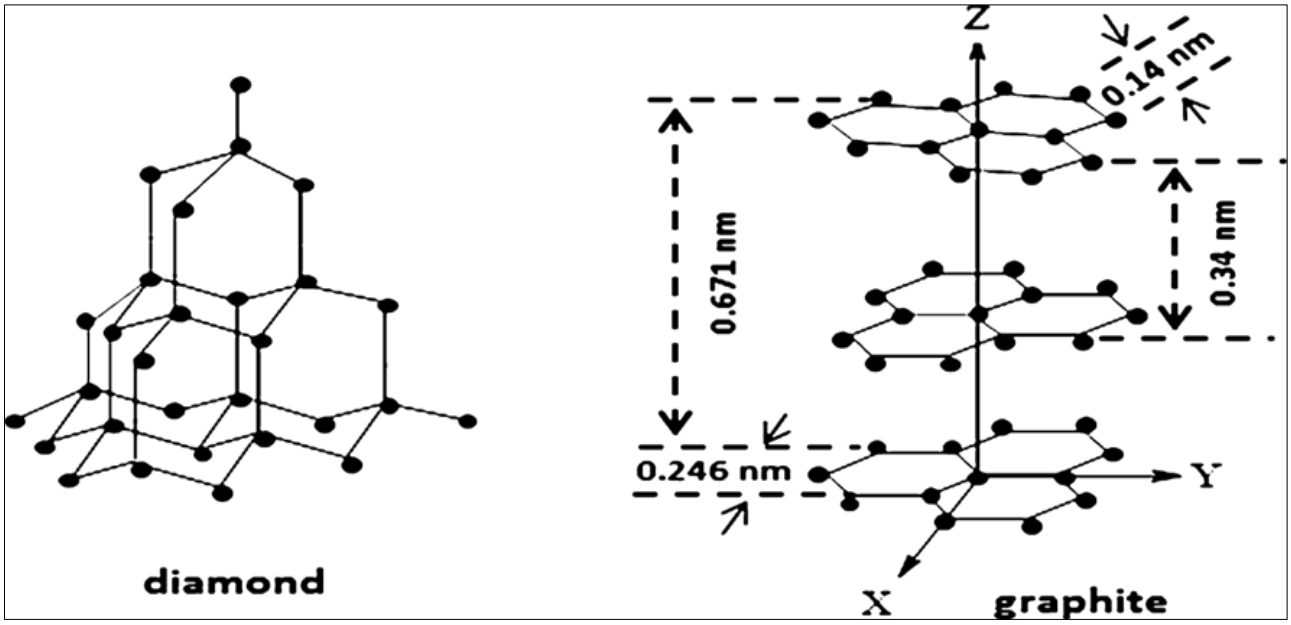


Fig 1: Diamond (left) and Graphite (right), two allotropes of carbon, display distinct physical properties, which are rationalized on the basis of crystal structure analysis

Modern developments: structure from powder diffraction

All crystalline materials occur as microcrystal-line powders, and producing single crystals appropriate for structural analysis via single-crystal X-ray diffraction is a difficult process. In these cases, X-ray powder diffraction provides an alternate method for crystalline powder material characterisation[9]. X-ray powder diffraction has evolved as one of the most promising characterization methods for qualitative and quantitative analysis of powder materials throughout the last century. The technique is non-destructive and has been used to assess phase purity in an array of materials from geology, polymeric, environmental, forensic, and pharmaceutical sciences [10] (Fig. 2).

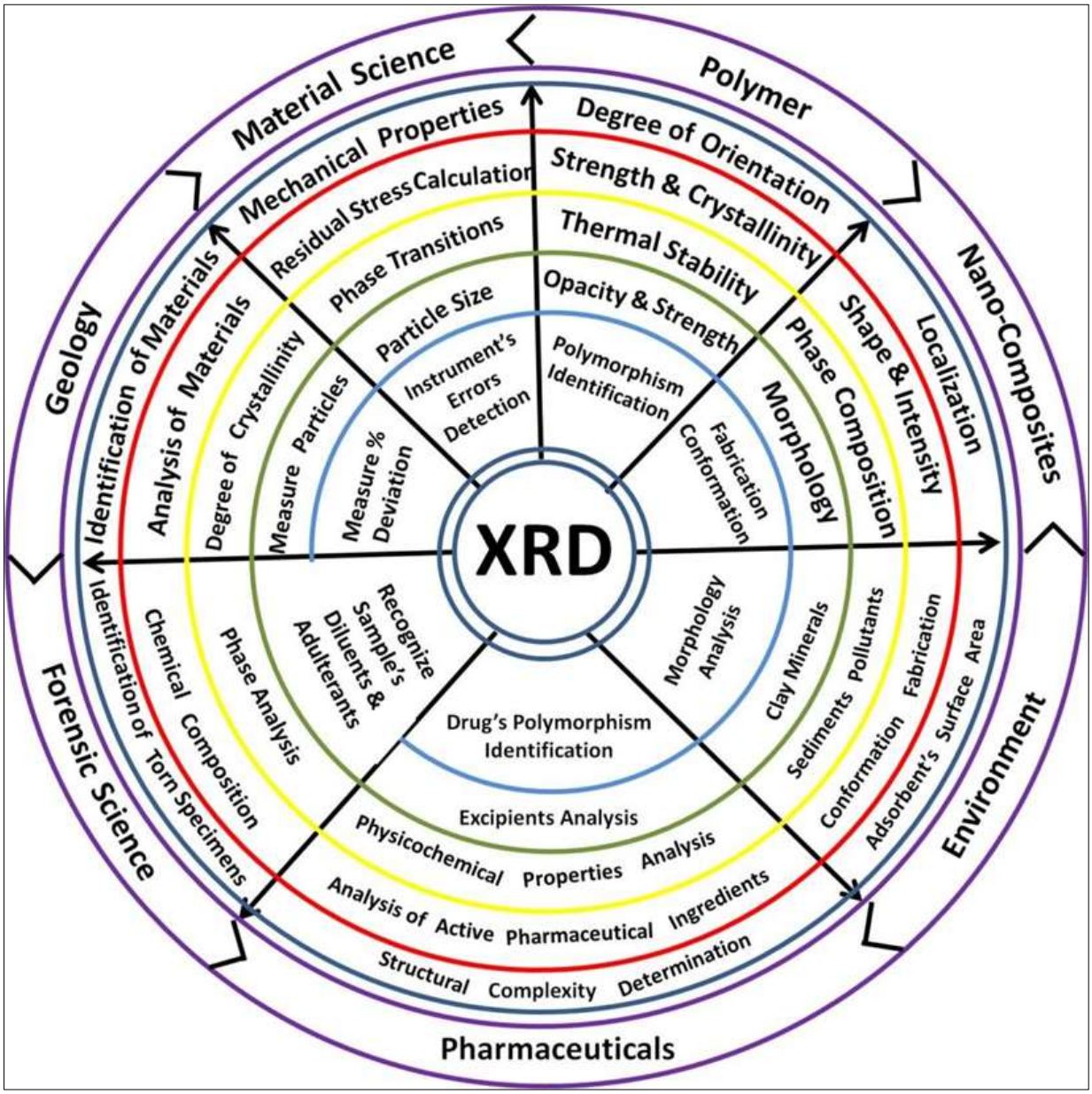


Fig 2: Analytical applications of powder X-ray diffraction in various fields

Mark the start of employing X-ray powder diffraction to characterize materials. It was predominantly utilized for phase identification in mineralogy, metallurgy, and solid-state chemistry for almost 50 years, and its application to more difficult problems, such as structure refinement and structure determination, was limited to simple high symmetry systems. H. Rietveld's pioneering discoveries heralded a paradigm shift by expanding the scope of powder techniques from simple high-symmetry structures to complicated low-symmetry structures. By the 1970s, there had been a number of novel structures from powder data. These initiatives, however, were mostly based on trial-and-error procedures. Since the availability of high- resolution powder diffract meters, such as synchrotron X-ray and pulsed neutron sources, the discipline of structure determination has evolved from an art to a science. The initial issue in structure determination is indexing the diffraction pattern, which was easily accomplished using high-resolution data. The powder pattern may then be decomposed into an approximate collection of resolved, integrated intensities, allowing the traditional structure-solving methods of the period to be applied. This was a resounding success in the mid-1980s. The Patterson methods were used to solve the unknown structure of iron (III) arsenate (FeAsO4) using high-resolution neutron data [11]. Powder X-ray diffraction has also found many applications in pharmacology [12].

Active pharmaceuticals are frequently subjected to a variety of techniques during the Preformulation and formulation stages of drug development. As a result, powder X-ray diffraction plays an important role in evaluating stability and potential phase con-variants throughout manufacturing and formulation development. Many times, the solids produced by these procedures are not exposed to further crystallization, and powder diffraction is the only way to determine the crystal structure. Many pharmaceutical compounds' crystal structures have been identified using powder diffraction data, including polymorphs of fanaserine, talmisartan, fluticasone propionate, and tetracaine hydrochloride, to mention a few.

Different solid forms and their role in drug development

Pharmaceuticals are often made up of one or more APIs that are then compounded into a suitable final form that includes inert components. APIs are the most valuable materials in terms of inherent value. Because of the convenience of manufacture and the benefits of stability, the bulk of pharmacological products on the market are developed in the form of solids. Among the many crucial difficulties in drug development, addressing poor physicochemical qualities such as solubility, stability, dissolution rate, hygroscopicity, and permeability is critical. If a given solid form has any physicochemical issue(s), medication
developers frequently explore for alternative solid forms, such as amorphous forms, polymorphs, salts, solvates/hydrates, and cocrystals [13].

Crystalline solids feature a regular arrangement of molecules with repeating units in three dimensions, whereas amorphous materials lack long-range structure (Fig. 3). Amorphous solids have favorable pharmacological features such as faster dissolution rate and bioavailability than crystalline solids, however they are not as commonly marked as crystalline solids due to their low physical stability and potential for crystallization [14].

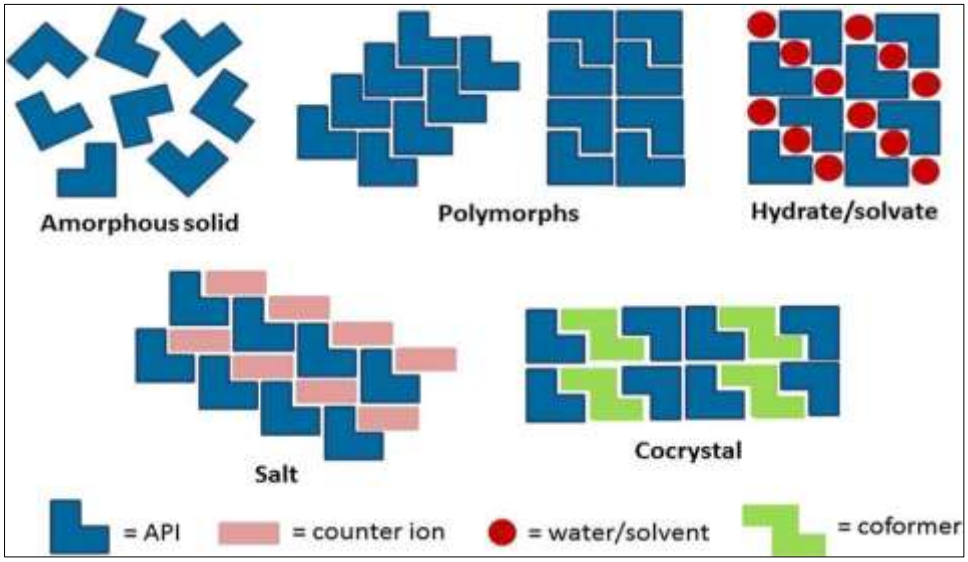


Fig 3: Most common solid forms of pharmaceuticals

Since the polymorphic form is the only other crystalline form available in the pure form of the medicinal ingredient, developing it is a promising path. But stability is a big worry since metastable (unstable) polymorphs tend to change to stable form, which affects shelf-life and medication performance. Polymorphic versions only slightly improve solubility. Crystalline materials with water and solvents, respectively, embedded in their crystal lattice are known as hydrates and solvates. Despite the fact that hydrates and solvates are used in the development of some current pharmacological products, their low thermal stability, variable stoichiometry, and restricted advantages with respect to physicochemical qualities make them fewer desirable solids.

Principle

In the apparatus, the sample is put on a goniometer, which is used to position the crystal in specific orientations for analysis from numerous angles. If the sample is impure and the crystal structure is unclear, it may be necessary to purify it before examination. An X-ray tube generates X-rays, which are then filtered to be monochromatic, meaning they have a single wavelength frequency. The atoms in the crystal refract the X-rays, which are elastically scattered onto a detector. Because they are elastically distributed, they have the same energy as the incident X-rays directed at the sample. This produces a 2D diffraction pattern for the crystal.

If the diffraction pattern is unclear, the material may not be pure and must be purified at this stage. Other conditions that can prevent a diffraction pattern from being created include a too-small sample (0.1 nm in each dimension), an uneven crystal structure, and the presence of internal flaws in the crystal, such as fissures.

If the crystal is found to be acceptable, the analysis and X-ray bombardment of the sample continue. The sample spins on the goniometer, resulting in a succession of 2D diffraction patterns from different sides of the sample. The intensity is recorded at each orientation, yielding hundreds of 2D diffraction patterns representing various portions of the 3D structure. From here, a computational approach examines the various diffraction phases, angles, and intensities to produce an electron density map of the material. The electron density map is used to build an atomic model of the material. The model is constantly modified to ensure that it is accurate, and after the final atomic model has been constructed, the data is entered.

Cocrystals in Pharmaceutical Design

Modern drug development: Need for better solid forms

Pharmaceutical salts are by far the most extensively employed solid forms for drug formulation development. It is believed that nearly half of all medications contain the active component in the form of a salt[15]. There are several potential advantages to salt production. Salts, for example, are known to improve solubility, dissolving rate, melting point, photo stability, process ability, flavor, and so on. A plethora of papers and publications emphasize the significance of salt production in medication development. Despite its widespread use in drug research, salt production is limited to APIs that are ionisable under normal pH circumstances. APIs in this category are found in many drug products and drug compounds in the development stages.

As a result, alternative techniques such as pharmaceutical Cocrystallization are critical for investigating the solid-form diversity of APIs. Cocrystallization has an advantage over salt production in that it can be used with non-ionizable APIs.

Pharmaceutical cocrystal are created using conformers chosen from a list [16]. Their list includes a diverse spectrum of substances such as aldehydes, alcohols, acidic substances such as carboxylic acids, amides, and sweeteners. As a result, the wide range of GRAS compounds in terms of structure and physical qualities gives an additional means of selecting a suitable conformer for an API target change. Cocrystal, along with other well-known solid forms like as salts, polymorphs, and hydrates/solvates, have shown enormous potential in facilitating formulation creation by addressing the physicochemical issues of APIs over the last decade.

Impact of cocrystals on drug development

The physicochemical properties of an API vary from one solid form to the next, which is frequently due to how the API molecules are organized in the crystal lattice of that solid form. As a result, choosing a solid form has significant clinical, legal, and regulatory ramifications. It is believed that 80% of medications now in development fall under Class II or IV of the biopharmaceutical classification system (BCS). The medications in these categories are characterized by poor solubility. To solve solubility concerns, drug formulators frequently use procedures such as micro nation, solid dispersion, encapsulation, salt creation, amorphous forms, and so on. However, there are industrial disadvantages to these methods as well as an increased risk to the stability of the formulations that are produced.

Molecular insights into cocrystal formation

Cocrystals are multi-factor crystals made of or greater strong additives in stoichiometric proportions1 [17]. The combination of solid components in a cocrystal is frequently caused by non-covalent interactions of the type hydrogen bonds. As a result, understanding intermolecular interactions between molecular components is required for a successful cocrystal design. G.R. Desi Raju's supramolecular synthon notion comes to mind in this regard [18] and M.C. Etter's hydrogen-bonding rules [19] provide light on the interaction of two or more molecules in the crystal lattice.

In a crystal structure, recurring intermolecular interactions are called Supramolecular synthons are classified further into two fundamental categories. The one involving the same functional groups is known as supramolecular homosynthon, while the one involving different but complementary functional groups is known as supramolecular heterosynthon. Because cocrystal are generated by intermolecular interactions between two or more different molecules, supramolecular heterosynthons involving functional groups of different molecules play a crucial role in cocrystal design.

GR Desiraju defined crystal engineering as "the understanding of interactions between molecules in the context of crystal packing and in the utilization of such understanding in the design of new solids with desired physical and chemical properties." This definition points out how knowledge of intermolecular interactions allows molecules detect one another [20]. Consequently, cocrystal design is guided by the techniques of crystal engineering. Cofactors for cocrystal formation are chosen using the knowledge gathered from the examination of the crystal structures deposited in the Cambridge Structural Database (CSD). Supramolecular synthons, which are typically found between the functional groups on cocrystal components, are necessary for a good cocrystal design. Therefore, the first step in designing cocrystal for a given molecule is to examine its functional groups and identify comparable functional groups that are likely to produce predictable supramolecular synthons. Consequently, the choice of conformer in a cocrystal design method strengthens the importance of the understanding of intermolecular interactions, which is frequently derived via X-ray crystal structure research.

Physicochemical properties of pharmaceutical cocrystals

When two or more different molecules recognize one other through energetically beneficial intermolecular interactions, Cocrystallization occurs. The physicochemical properties of a cocrystal are determined by the strength of intermolecular interactions and the arrangement of its constituents in the crystal lattice. Therefore, it is critical to understand the physicochemical properties by a thorough structural analysis, as this leads to a structure-property correlation and facilitates the design of subsequent crystals for fine-tuning the properties of the API. Single - crystal X-ray diffraction has shown itself to be a valuable method for the unambiguous determination of crystal structures during the past century, helping to facilitate innovative material property investigation. Structural characterization of cocrystal determines validity of the cocrystal design technique, and displays hydrogen bond preferences.

Advantages

- Non-destructive nature
- Highly sensitive.
- Widely used method to determine crystal structures.
- X-rays are not absorbed very much by air, so the sample need not be in an evacuated chamber.

Disadvantages

- X-rays do not interact very strongly with higher elements.
- Expensive.
- Model construction.
- The diffraction patterns produced by X-rays passing through a crystal contain signal and noise, and separating the two can be challenged. This noise can lead to inaccuracies.

Applications of X-ray Crystallography

Pharmaceutical Industry: X-ray crystallography helps analyze the active pharmaceutical ingredients (APIs) in medicines. It aids in drug design, optimization, and understanding drug-protein interactions.

Nano Science and Material Science: Researchers use X-ray crystallography to study nanostructures and material properties. It provides insights into crystal phases, orientations, and defects.

Forensic Science: In forensic labs, X-ray crystallography assists in identifying unknown substances. It aids in analysing trace evidence and crystalline materials.

Electronic Devices and Materials: X-ray crystallography ensures the quality control of electronic components. It verifies crystal purity, defects, and interfaces.

Protein Structure Determination: X-ray crystallography reveals the 3D structure of proteins and understanding the protein shapes is crucial for drug targeting and disease mechanisms. Determining the precise orientations and configurations of the various amino acids that make up a protein is known as protein structure determination. We can even establish the function of proteins by using X-ray crystallography to ascertain their structure.

Studies on protein interaction: The term protein interaction describes the relationship between two or more proteins. X-Ray crystallography aids in determining the relationships between the key amino acids, site of action, and orientation of proteins involved in a given reaction.

Conformational studies: These investigations focus on the spatial configurations of atoms within molecules that result from atoms freely rotating around a chemical bond. Since the arrangements dictate the structure and function of proteins, it is essential to ascertain them. An effective method for doing so is X-ray crystallography.

Determination of enzyme catalysis: Enzymes are proteins. The structure, and more especially the kind of amino acids that are present in the active sites, dictates the catalytic activity and degree of enzyme interaction.
The catalytic efficiency of enzymes can be determined and predicted with the use of X-ray crystallography.

Conclusion

X-ray crystallography has been a key tool for analyzing material structures since its discovery. Recent developments include the evolution of synchrotron radiation and structural determination using x-ray powder diffraction. Multiple hydrogen-bonding sites on the API or selected coformer can complicate and impede the process. Implementation of classic cocrystal design methodologies. Understanding a functional group's hydrogen bonding preferences in the presence of other groups can help with good crystal design. Current cocrystals design methodologies focus on identifying coformers based on functional groups on an API. However, designing cocrystals for molecules with van der Waals interaction tend to cluster tightly, making it difficult to predict cocrystal formation. The ability of X-ray crystallography to investigate the composition and properties of materials has been well recognized throughout the last hundred years. An expanding field of study is the prediction of a material's properties only from its crystal structure, and initiatives in this direction highlight the significance of X-ray crystallography.

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