Preliminary understanding of experiments on single crystal X-ray crystallography

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A R T I C L E I N F O

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A B S T R A C T

X-Ray crystallography is an advanced technique that reveals the structures of crystals in atomic scales. This technique also enables one to accurately determine the atomic connectivity in terms of bond lengths, bond angles, and torsion angles (molecular geometry) of the molecules in the solid state. Geometrical insights into the presence of various non-covalent interactions in the crystalline state is also obtained. Furthermore, the *absolute configuration*, charge density distribution and the associated dynamics of the molecule in the solid state is also unequivocally established from *high-resolution* experiments performed on crystals of the compound of interest.

Introduction

The determination of the composition and subsequently the crystal structure of a substance are of immense significance as it has implications in all fields of sciences and engineering. In particular the role of structure is related to the observed physical, chemical, biological and material property of the substance of interest. The composition of a solid is decided by the different elements present in it. The identity of the individual atoms in molecules in a substance cannot be established by visible light radiation as the size of individual molecules are 100–10000 times smaller in comparison to the wavelength of visible light which falls in the range of 400–700nm. X-Rays are the ideal choice to resolve the identity of the atoms present in a molecule in a crystal having an order of few angstroms. The X-rays are indeed scattered by atoms but these cannot be recombined physically to form an image using conventional lenses (Fig. 1) [1].

X-Rays were first observed in 1895 by Wilhelm Conrad Röntgen and are electromagnetic waves having high energy (124 eV to 124 keV) and very short wavelength (10pm to 10nm). In 1912, it was suggested by Max von Laue, while Friedrich and Knipping performed the experiment on the phenomenon of interference in X-ray waves when these are passed through a crystal [2]. The X-rays suffer from the event of diffraction, when these are incident on crystals because the wavelength of X-rays is in magnitude comparable to the lattice parameters of crystals. In 1913, W. H. Bragg and his son W. L. Bragg proved the diffraction of X-rays using an alternate method and gave an equation for the observed diffraction. The equation is called Bragg's law and is given as:

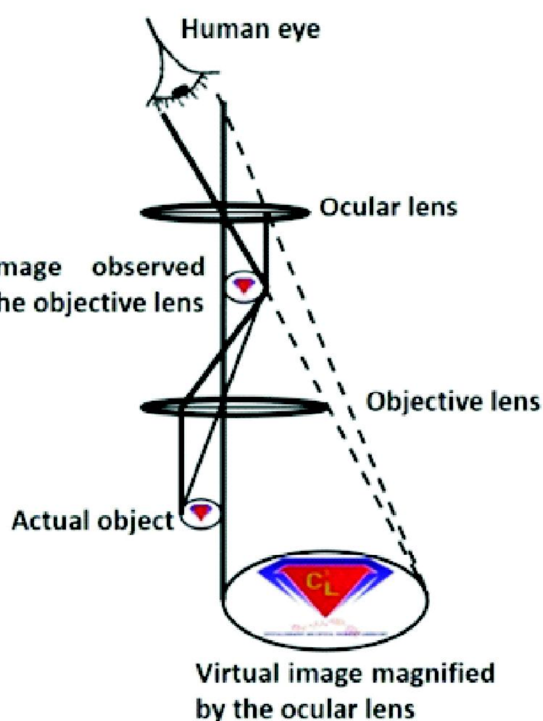

Fig. 1. Working principle of microscopy.

$$n\lambda = 2d\sin\theta \quad (1)$$

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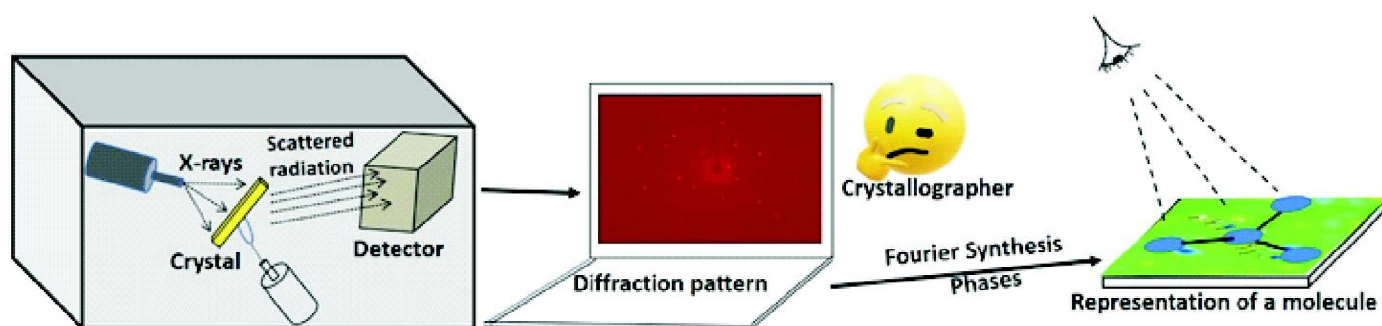

Fig. 2. Schematic representation of X-ray diffraction.

These two discoveries were breakthroughs in the science community and opened the dimensions for a new field of research i.e., X-ray crystallography which can be applied to all forms of matter. Thus, the X-ray diffraction experiments are based on the accurate measurement of the diffraction intensities on different types of detectors and treating them mathematically (using the concept of Fourier transforms) for the process of structure determination (Fig. 2).

Every diffraction spot corresponds to a diffraction intensity (I_{hkl}) and is also termed as a "reflection". Every reflection is characterized by a set of three integers, hkl, which are referred to as Miller indices. These are the symbolic representation of the various lattice planes present in a given crystal and indicates its location in the diffraction pattern or in reciprocal space. The diffraction pattern in the reciprocal space is related to the electron density in the unit cell in real space by Fourier transform. The geometrical event of diffraction is very sensitive to the interplanar spacing d (Equation-1 above). This quantity is a complex function of h, k, l, a, b, c, α, β, γ where in the last six quantities refer to the lattice parameters of the unit cell. The selection of the unit cell is a very important exercise that must be done carefully after the measurement of a few strong intensity reflections. The geometry of the unit cell, into which all the atomic contents are "essentially packed" is classified into seven crystal systems and fourteen Bravais Lattices. The arrangement of the atoms in crystals is constrained by the space group symmetry which consists of different symmetry elements, namely, rotational axes, mirror planes, screw axis, glide planes, and crystallographic point of inversion. These include different geometrical operations, namely rotation, reflection, simple translation or a combination of these operations. There are 230 3D space groups. The experimental determination of the space group is also done after completing the experiments on the single crystal during the stage of data processing.

The X-ray diffraction pattern consists of the superposition of scattered waves with varying amplitude and phase. If the unit cell contains N atoms, the intensity of the combined wave is given as [3]:

$$I(hkl) = \left(\sum_{j=1}^{N} f_j \cos\phi_j\right)^2 + \left(\sum_{j=1}^{N} f_j \sin\phi_j\right)^2 \quad (2)$$

where, f_j is called atomic scattering factor for j^{th} atom that is defined as the ratio of the amplitude scattered by j^{th} atom in a particular direction to the amplitude scattered by a free classical electron in the same direction.

$$I(hkl) = A^2 + B^2$$

Where, $A = \sum_{j=1}^{N} f_j \cos\phi_j$ and $B = \sum_{j=1}^{N} f_j \sin\phi_j$

$$I(hkl) = A^2 + B^2 = (A + iB)(A - iB); \text{ Where } i = \sqrt{-1}$$

Each reflection in diffraction pattern is associated with a structure factor F (hkl), which is defined as the ratio of the amplitude scattered by the contents of a unit cell in a particular direction to the amplitude scattered by a free classical electron in the same direction.

The F (hkl) may be represented as a complex vector as

$$F(hkl) = A(hkl) + iB(hkl) \quad (3)$$

$$I(hkl) = (A + iB)(A - iB)$$

$$\therefore I(hkl) = F(hkl)\, F^*(hkl) \quad (4)$$

$$F(hkl) = (A + iB) = \sum_{j=1}^{N} f_j \cos\phi_j + i\sum_{j=1}^{N} f_j \sin\phi_j$$

$$F(hkl) = \Sigma_{j=1}^{N} F_j \text{ Where } F_j = f_j \cos\phi_j + f_j \sin\phi_j = f_j e^{i\phi_j}$$

$$F(hkl) = |F(hkl)| e^{i\phi(hkl)} \quad (5)$$

$$|F(hkl)| = \sqrt{A^2 + B^2} \quad (6)$$

$$\phi(hkl) = \tan^{-1}\frac{B}{A} \quad (7)$$

To determine the precise structure, many reflections of accurate

intensity, are required. The crystal is rotated in the X-ray beam, and only in certain well-defined orientations the Bragg condition is fulfilled and events of diffraction occur from the different lattice planes (d varies). The concept of Ewald sphere is very important in diffraction. It is a geometrical construct of Bragg's law in reciprocal space (inverse of direct space) to interpret the diffraction pattern. If the crystal at the goniometer head is considered as an origin and a sphere with a radius of $1/\lambda$ is drawn, the diffraction condition is satisfied when the reciprocal lattice points touch the surface of the sphere [4]. In the direct space, if the magnitude of hkl indices increases, the magnitude of interplanar distances (d_{hkl}) decreases. Hence, the scattering angle will also increase (the maximum possible angle for scattering is 180°) and this leads to the problem of peak overlap which is difficult to resolve. The consideration of the reciprocal space overcomes this problem [3].

When the crystal is rotated in different orientations, a large number of such reciprocal lattice points intersect the sphere and diffraction occurs. The (000) reflection corresponds to F(000) [structure factor with all the atomic contents of the unit cell at the origin] is always calculated as this could not be measured properly because the X-ray beam is traversing the horizontal from one side of the sphere while the transmitted beam is collected to the other side of the sphere and will be contaminated with the undiffracted direct beam. This results in inaccurate measurement of this reflection. This (000) reflection is considered as the origin of the reciprocal space [5].

Primary requirements and steps involved in an X-Ray diffraction experiment:

Single Crystal X-ray diffraction is the most powerful and widely used technique for the determination of crystal structure. The routine X-ray diffraction experiment consists of the following steps:

1. Growing a suitable crystal
2. Mounting the crystal
3. Screening for diffraction quality
4. Unit cell determination
5. Collecting complete set of data with high redundancy and high resolution
6. Background subtraction and process of data reduction
7. Solving the structure (determination of the initial structure model)
8. Crystal Structure Refinement
9. Completion of the structure

1. Crystallization

Growing suitable crystals is a primary and very important exercise and this process is called crystallization [6]. This leads to the formation of a solid with three-dimensional periodicity wherein the position of the molecules is in accordance with the requirements of space group symmetry. The process of nucleation and growth are the two important and distinct steps of crystallization. The basic concept behind crystallization is achieving metastability and then the formation of the crystalline phase from the mother phase [7]. Solvent evaporation, vapor diffusion, sublimation, and, melt cooling are the most adapted crystallization techniques (Fig. 3) used for growing crystals of small molecules for regular X-ray diffraction experiments [8,9].

2. Mounting, selection and screening of crystals for X-Ray diffraction experiments

A suitable single crystal is selected after checking in a

Fig. 3. Different crystallization techniques. (A) Sublimation, (B) Slow evaporation, (C) Melt crystallization apparatus, (D) Melt crystallization inside the chamber.

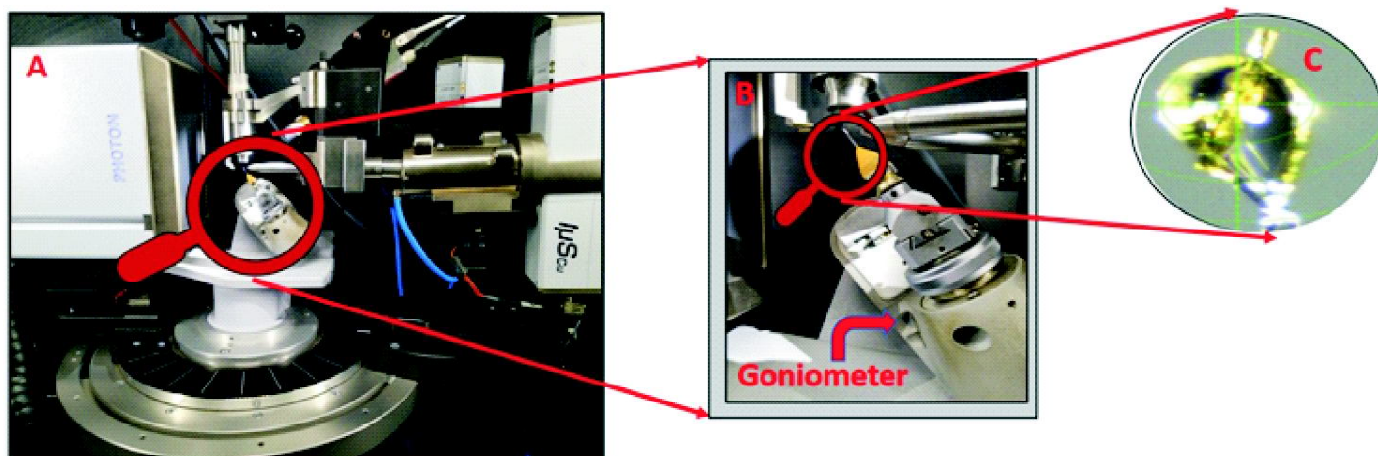

Fig. 4. (A) Optical setup of X-ray diffractometer, (B) Goniometer, (C) Crystal mounted on goniometer head.

polarising microscope equipped with an analyser. This is then mounted on the goniometer head as shown in Fig. 4. The optimum centering of the crystal is achieved by rotating the goniometer head which allows for the alignment of the crystal in such a way that the X-ray beam falls at a specific point on the crystal during the entire process of data collection.

An initial exposure time is considered depending on the size of the crystal and the atoms present in the compound. For thick sized crystals and / or heavy atoms present (Atomic number > 16), low exposure time is required. The initial screening of the crystal involves recording of a still image (Fig. 5) and checking for the quality of the diffraction pattern. The diffraction spots must be spherical, without any streaks and this allows one to determine the quality of the crystal for performing further experiments.

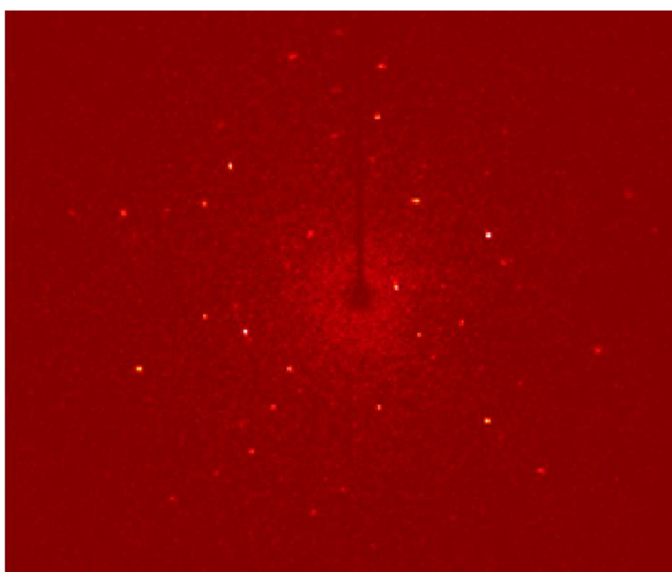

Fig. 5. Still image for pyrrolidine dithiocarbamate-copper(II) (Cu[PDTC]$_2$) [10].

3. Unit cell determination

A large number of reflections are required for a complete data collection while a subset of strong reflections is sufficient for estimating the six lattice parameters which helps to determine the crystal system. To determine the unit cell, two methods, a real space method (also called auto indexing) and a reciprocal space method are used. In these methods, the recorded diffraction data is extracted from the different portions of the reciprocal space. A list of possible unit cells are generated from the diffraction pattern and three shortest non-coplanar vectors are searched. Using these vectors, a trial unit cell is proposed and hkl indices are also assigned to every reflection used for the determination of the unit cell. This allows one to determine the lattice symmetry and then the unit cell is proposed [5]. These reflections are recorded using one of the various methods such as the (1) Laue method, in which a stationary crystal is irradiated with polychromatic X-ray radiation and the diffraction pattern is recorded on a stationary detector, (2) In the rotation method, monochromatic X-ray radiation is used, crystal is rotated while the detector is kept stationary, (3) In the Weissenberg and precession method, both the crystal and detector are moved and monochromatic X-ray radiation is used [3]. The intensities of reflections are measured by integrating the brightness of each spot recorded in the diffraction pattern. These measured intensities are compared with calculated intensities based on the structural model of the crystal. The lower the difference between measured and calculated intensities, more accurate will be the structural model.

Using the concept of symmetry operations,the intensities of reflections are compared. These reflections are either related by certain symmetry or may have zero intensity. The zero intensity is the effect of symmetry operations having translational

components (lattice centring, glide planes and screw axis). Based on these comparisons, a possible space group may be assigned to the given crystal system [1].

4. Data collection

After evaluation of the six lattice parameters for the substance, it is classified into one of the seven crystal systems and the lattice symmetry possessed by the unit cell is also established. Once the unit cell parameters are known, the strategy to collect final data is optimized so that the data completion should be to the greatest extent possible (ideally 100%) and to a resolution of 0.7 Å in d. The time required for data collection depends on the exposure time given for every frame and the total number of frames to be collected. The amount of unique data to be sampled depends on the symmetry of the Bravais lattice. The diffraction pattern is always centrosymmetric regardless of the lattice symmetry and, if a significant amount of anomalous scattering is not present, it follows Friedel's law, I (h, k, l) = I (–h, –k, –l). Hence even the lowest symmetry space group P1 has two-fold redundancy in the complete data. Out of 32 crystallographic point groups, only 11 ($-1, 2/m, mmm, 4/m, 4/mmm, -3, -3m, 6/m, 6/mmm, m-3, m-3m$) correspond to the symmetry of a diffraction pattern, because the other remaining 21 point groups lack an inversion centre. These 11 points groups are known as the Laue classes.

For the triclinic system, only hemisphere data is required, for the monoclinic system only 1/4th (quadrant) of the full sphere data is required while in orthorhombic lattice only 1/8th (octant) of the full sphere is required for data collection. As the symmetry in a crystal system increases, the unique set of reflections decreases so the time of data collection is reduced. But the preliminary data collected for unit cell determination should be analysed carefully otherwise collecting the diffraction data in higher symmetry in a given crystal system may lead to reduced number of measured reflections if in reality it belongs to a system of lower symmetry.

5. Background subtraction

One frame of data is collected while keeping the X-ray shutter closed so that no signal can be recorded through X-ray diffraction and only background noise is collected. The data-acquisition time for this frame must match that which is proposed for the data collection on your crystal. For background correction, this dark frame is subtracted from each measured frame [5].

6. Data reduction

Different geometrical factors affect the measured intensities. This also includes absorption of X-Rays by crystals. The correction is required in the measured intensities to enable an accurate structure determination. This processing of reflection data is called "data reduction" in which the measured intensities (I_{meas}) and estimated standard deviations ($\sigma_{I_{meas}}$) are converted to observed intensities (I_{obs}) and estimated standard deviations ($\sigma_{I_{obs}}$) or more specifically to the observed structure amplitude ($|F_0|$) or (F_0^2) and corresponding σ_{F_0} or $\sigma_{F_0^2}$.

The Lorentz-polarization factor is an important geometrical correction which is carried out in data reduction. When the crystal is rotated in a different orientation for recording a large number of reflections, the various reciprocal lattice points pass through the surface of the Ewald sphere but each reciprocal point takes a different amount of time while crossing the surface [11]. So, a correction term is required which is called the Lorentz factor. For equatorial plane rotation, the measured intensities are divided by the Lorentz factor;

$$L = \frac{1}{\sin 2\theta} \text{ (2}\theta\text{ is diffraction angle).} \quad (8)$$

When the X-ray beam, an electromagnetic radiation is reflected by the diffracting planes (an imaginary concept) having a certain electron density, the electric vector component of the beam parallel to the plane is reflected by electron density on the plane without reduction of intensity and is very much independent of the angle of the incident beam. The electric vector component perpendicular to the diffraction plane is reflected by the electron density of the plane with the reduction in intensity. When the monochromator is used the electromagnetic radiation again suffers from the effects of polarization and affects the intensities [2]. There is also a correction term needed which is called polarization correction. The polarization factor is given by:

$$P = \frac{1 + K\cos^2 2\theta}{1 + K} \quad (9)$$

when monochromator is not used, K = 1.

For molybdenum source the polarization factor is negligible and it is ignored for routine experiments. The polarization corrections do not need any information about the chemical composition and physical habit of the mounted crystal.

These two factors are collectively called Lorentz-polarization (Lp) correction and given by:

$$Lp = \frac{(1 + \cos^2 2\theta)}{2\sin 2\theta} \quad (10)$$

When the X-ray beam travels through the crystal, it also suffers

from absorption. The correction in the observed intensities for each reflection is required because the absorption depends on the path length of the incident and the diffracted X-ray through the crystal and varies as the crystal orientation is changed so each reflection is affected differently [8]. For spherical crystals, the absorption correction is the same for all reflections but different for non-spherical crystals.

As the X-ray beam passes through the matter, the transmitted intensity is attenuated according to the following law:

$$I = I_0 e^{-\mu t} \quad (11)$$

Where I is transmitted intensity, I_0 is the incoming intensity, μ is the linear absorption coefficient of the material and t is the path length of the beam through the crystal. As the atomic number of the elements associated with the absorbing material is increased the absorption of X-ray also increases. Absorption also depends on the wavelength of the X-ray source, the longer the wavelength, the greater the attenuation by the material [12]. Numerical methods, semi-empirical methods, and empirical methods are some common absorption corrections [3]. Face indexing is a type of numerical method used for absorption correction in which careful measurement of the crystal size and shape and path lengths are calculated. A video recording of the crystal is required for face indexing. Indexed faces of a crystal are shown in Fig. 6.

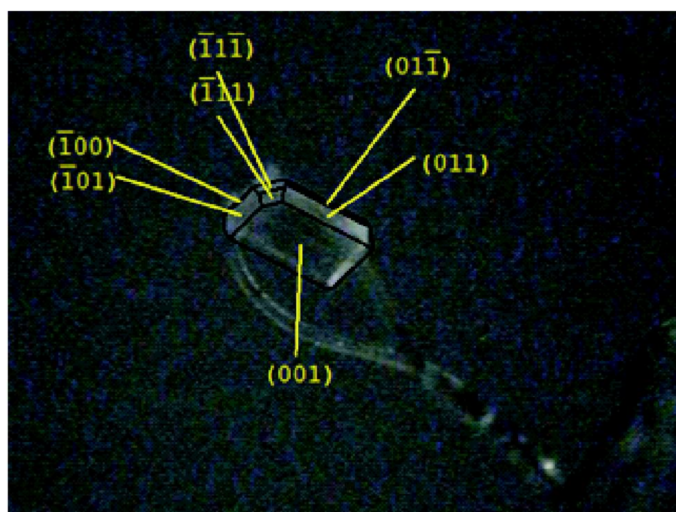

Fig. 6. Face indexing of the crystal.

Extinction, crystal decay, and thermal diffuse scattering are some other factors taken into account during data reduction as per requirements. Merging and averaging of repeated and symmetry equivalent reflections are also performed during the data reduction to produce a set of unique, corrected, and scaled set of data [1].

7. Structure solution

The primary task in the crystal structure solution is the assignment of phase angles to each reflection in the data set. The experiment involves measurement of the intensities; however, the phase information is completely lost. In case of crystal system having a centrosymmetric space group, the j value in the equation; $I(hkl) = \left(\sum_{j=1}^{N} f_j \cos\phi_j\right)^2 + \left(\sum_{j=1}^{N} f_j \sin\phi_j\right)^2$ ranges over $N/2$ atoms in the unit cell which are not related by centre of symmetry, the imaginary component becomes zero, so the only possible phase angles are 0° and 180°. Hence, assigning the phases to the centrosymmetric space group is simplified but very complex for other non-centrosymmetric systems. The Patterson search method and, direct methods are used for solving the phase problem [5]. The trial solution of the structure means obtaining the atomic positions or the electron density distribution from the corrected diffraction data. In a unit cell of volume V, the electron density ρ (x, y, z) at a point with fractional coordinates (x, y, z) is calculated as a three-dimensional Fourier series via the following equation [3] :

$$\rho(x, y, z) = \frac{1}{V}\sum_h \sum_k \sum_l |F(hkl)|\, e^{i\phi(hkl)}\, e^{-2\pi i(hx+ky+lz)} \quad (12)$$

Generally, these methods could assign the positions of a few atoms, but not all, so only the partial structure is initially determined. This partial structure is chosen as the initial structural model which is the starting point for the complete determination of the crystal structure. By treating the forward Fourier transform equation (given above) over the initial model a set of calculated structure factors (F_C), corresponding to each of the observed structure factors (F_0) is determined.

The resemblance between the calculated and observed diffraction pattern can be examined in various ways. The most adapted assessment is a so-called R-factor or residual factor, given as:

$$R = \frac{\Sigma\, ||F_0| - F_c||}{\Sigma\, |F_0|} \quad (13)$$

The large difference corresponds to poor reliability.

Using of F^2 values which can be directly measured instead of |F| values, squaring the differences and multiplying by different weighting factors for different reflections, another widely used quantity is the weighted-residual factor:

$$wR2 = \sqrt{\frac{\Sigma\, w(F_0^2 - F_c^2)^2}{\Sigma\, w(F_0^2)^2}} \quad (14)$$

Another factor called goodness of fit (GooF or S) is given by:

$$S = \sqrt{\frac{\Sigma\, w(F_0^2 - F_c^2)^2}{(N_R - N_P)}} \tag{15}$$

N_R is the number of independent reflections and N_P is the number of refined parameters. For a properly adjusted weighting scheme, the ideal value of S is one. The wrong assignment of the space group and incorrect absorption correction affects the goodness of fit [13].

The reverse Fourier transform is carried out over the calculated amplitudes $|F_c|$ and calculated phases ϕ_c to regenerate the elector density of the initial model structure. Now, again the reverse Fourier transform is carried out with experimentally observed amplitudes $|F_o|$ and calculated phases ϕ_c, which regenerates the ρ (electron density) for the new model structure. This electron density assigns the atoms to the existing model structures and additional atoms which were not known yet. The process generated an improved model structure with additional atoms compared to the initial model [1]. This process can be repeated till all atoms are located and refined.

8. Refinement of the Structure (Initial Model)

Once all the non-hydrogen atoms are located, the model structure is required to be refined. The refinement is required to adjust the numerical parameters in the model with certain criteria so that the phasing could be improved and ultimately the computed electron density closely matches with the actual electron density distribution for the molecule of interest. The method of least-squares is used in the refinement process. For each atom sitting in the general position of the unit cell in the model, some parameters are refinable. These refinable parameters are three atomic coordinates, atomic displacement parameters, which are six in case of anisotropic model, and one in case of the isotropic model, one overall scale factor and some other factors such as the Flack parameter for non-centrosymmetric structures, and one parameter for extinction [13]. A final satisfactory and most accurate structure is determined at the end of the refinement.

Conflicts of Interest

There are no conflicts to declare.

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