Chapter 2

X-ray Crystallography

2.0 X-ray Crystallography

2.1 Early history of Crystallography

Crystallography is the experimental science of determining the arrangement of atoms in solids. In older usage, it was the scientific study of crystals. The study of the theory of crystallography has drawn the attention of many scientists since a very long time. The first contribution was from Kepler (1611); when he recognized that the origin of the shape and symmetry of snowflakes is due to the internal arrangement of the building elements of water [1]. This observation may be considered as the start of scientific crystallography. Stensen laid the foundation by pointing out that, despite the fact that, though the crystals vary physically in appearance, they share the same angles between corresponding sides. Neumann showed that the laws of symmetry that hold good for the external faces even holds good for the properties exhibited by the crystals. The contributions of Huygens and Ha¨uy considered the building blocks of crystals. Based on the atomic theory of Seeber, Bravais proved the existence of 14 lattices which are currently named after him. Later Fedorov and Schoenflies, with their investigations using group theoretical approach, proved the presence of 230 space groups. Discovery of Xrays by Wilhelm Conrad R¨ontgen in the 19th century, set the stage for the modern science of X-ray crystallography. Max von Laue was the first to propose that crystal behaves as a diffraction grating to X-rays just as a normal grating acts on visible light [2]. This encouraged two of his students Walter Friedrich and Paul Knipping who used X-rays for their first diffraction experiment [3] Later, William Henry Bragg (1915) and his son William Lawrence Bragg [4] used this technique to determine the structure of a crystal which fetched them the Nobel prize. By this time the stage was set for X-ray crystallography which saw a rapid development of theoretical ideas and techniques to unwind the structures of a wide range of crystalline substances, both natural and synthetic. The first complete crystal structure determinations made were that of Sodium chloride (NaCl), Potassiumchloride (KCl), Potassium bromide (KBr) and Potassium iodide (KI).
Now a days, many spectroscopic techniques provide molecular structural information. IR spectroscopy can tell which chemical groups are present in a small molecule; mass spectrometry shows molecular connectivity and nuclear magnetic resonance (NMR) provides some information about solution structure for both small molecules and proteins. But the most information-rich technique is X-ray crystallography. Less than one century old, this technique has developed quickly to a state where, given a suitable crystal, the structure of a small molecule can be routinely determined in a matter of a couple of hours, and the structure of a macromolecular system in a couple of days, depending on the nature of the system. X-ray diffraction is regarded as the most powerful tool to analyse both the geometrical nature of the three dimensional array of molecules within a crystal and also the nature of the molecules themselves [5]

During the twentieth century, different types of diffraction have been found. Neutrons and electrons, which would be considered particles according to classical mechanics, can be diffracted [6] The differences between X-ray, neutron and electron diffraction by crystals are such that these three techniques complement one another to a remarkable degree. Each of these techniques provides a particular stream of information which the others are incapable of yielding.

### 2.2 X-ray diffraction by crystals

A crystal is a homogeneous solid [7, 8] and is defined as a three dimensional periodic arrangement of atoms or molecules which can be conceptually regarded as

\[
\text{crystal structure} = \text{lattice} \ast \text{motif}
\]

where the motif is the structural unit and specific entity which is repeated regularly in space and lattice is conceptually array of points in space which defines the geometrically relationship between the motif in structure. The symbol \( \ast \) implies convolution of the lattice with motif. The two dimensional plane lattice and three-dimensional space lattice are defined in terms of crystallographic unit vectors and crystallographic unit cells.

The conventional crystallographic unit cell is a parallelepiped defined by the space lattice of a crystal which serves to display the symmetry of the lattice in a convenient manner. In the unit cell of a crystal, the atoms are distributed in a certain manner, and so there exists some electron density function \( \rho(r) \) which describes the location of the
electrons within the atoms of the unit cell. The electron density function will have large value at positions corresponding to atomic nuclei, and a relatively low value between nuclei. If we can find the electron density function, then we may infer the positions of atoms from the electron density maxima and derive data pertaining to the bond lengths and bond angles. All the fundamental structural information of the contents of the unit cell is contained within the electro density function, and hence we wish to determine this function from X-ray analysis.

The three- dimensional ordering of the crystalline state allows us to obtain clear diffraction at, giving us readily understandable information on the nature of the crystal and molecules which it contains. Waves can contain directional information only through their wave vector $k$, implying that the diffraction pattern may be represented as a function of $k$, $F(k)$.

If the structure of the obstacle is represented by the amplitude function $f(r)$, it is found that the diffraction pattern function $F(k)$ and amplitude function $f(r)$ are Fourier transform mates:

$$F(k) = \int_{all \, r} f(\mathbf{r})e^{i\mathbf{k}\cdot\mathbf{r}}d\mathbf{r}$$

$$F(\mathbf{r}) = \int_{all \, \mathbf{k}} f(\mathbf{k})e^{i\mathbf{k}\cdot\mathbf{r}}d\mathbf{k}$$

The amplitude of the diffraction pattern resulting from the scattering of X-rays by a distribution of the electrons characterized by an electron density function $\rho(\mathbf{r})$ is

$$F(\Delta \mathbf{k}) = \int_{all \, \mathbf{r}} f_\epsilon \rho(\mathbf{r})e^{i\Delta \mathbf{k}\cdot\mathbf{r}}d\mathbf{r}$$

where $\rho(\mathbf{r})$ represents the contents of the unit cell of a crystal, the limits of the integral corresponds to those values of $\mathbf{r}$ which define the unit cell. $(\Delta \mathbf{k})$ is the scattering vector, which describes the change in the direction which has occurred as a result of the diffraction. Since $f_\epsilon$ is constant we have
The ratio $F(\Delta k)/f_e$ represents the relative scattering ability of the contents of the unit cell as compared to a single electron. This can be referred to as $F_{rel}(\Delta k)$. The relative intensity of the diffraction pattern of the unit cell is given by $|F_{rel}(\Delta k)|^2$.

In an X-ray diffraction experiment, we sample the diffraction pattern in space and obtain values of the time average of the intensity $|F(k)|^2$, corresponding to as many values of $k$ as we care to measure. Assuming that this allows us to find the corresponding values of $F(k)$, we may obtain a complete set of $F(k)$ data. We can then calculate $f(r)$ and the structure of the crystal is solved. It can be seen from the equation above that $F(k)$ is in general a complex quantity. Since we cannot measure complex numbers in any real experiment, the only measurable quantity is $F(k)$ can be written in the amplitude-complex exponential form as

$$F(k) = |F(k)|e^{i\delta}$$

$\delta$ is a phase factor. It is clear that the observed quantity $|F(k)|^2$ contains no information about $\delta$ and this ambiguity is known as the *phase problem*.

### 2.2.1 Significance of the diffraction pattern

The diffraction pattern gives the information about the structure of a crystal.

1. The position of the main peaks gives information on the lattice
2. The shape of each main peak gives information on the overall object shape.
3. The set of intensities of all the main peaks gives information on the structure of the motif.

### 2.2.2 Bragg’s law

Sir W.H. Bragg and Sir W.L. Bragg in 1913, derived Bragg’s Law [9].

W. L. Bragg explained the law by modeling the crystal as a set of discrete parallel planes separated by a constant parameter $d$. (Figure 2.1) It was proposed that the
incident X-ray radiation would produce a Bragg peak if their reflections off the various planes interfered constructively. The interference is constructive when the phase shift is a multiple of $2\pi$; this condition can be expressed by Bragg’s law,

$$\lambda = 2d \sin \theta$$

where $\lambda$ is the wavelength of x-rays, $\theta$, the glancing angle, $d$, inter planar separations and $n$, order of diffraction. The above equation can also be represented as

$$\lambda = 2d_{hkl} \sin \theta$$

where $n$ is contained in $d_{hkl}$ and $(hkl)$ refers to the Miller indices of the reflecting plane.

2.2.3 Ewald sphere and reciprocal lattice

Ewald sphere or the sphere of reflection [10], proposed by Paul Peter Ewald (1969), is the geometrical interpretation of Bragg’s law and Laue equations. Since, the wavelength of the radiation remains unchanged during diffraction, the locus of all possible diffracted wave vectors will sweep out a sphere about a point C which is the position of the crystal. This is called the Ewald sphere (Figure 2.2). The radius of this sphere is of the order of $1/\lambda$.

The aim of the Ewald sphere is to determine which lattice planes will result in a diffracted signal for a given wavelength, $\lambda$ of incident radiation. The incident plane wave falling on the crystal has a wave vector $K_i$ whose length is $2\pi/\lambda$. The diffracted plane wave has a wave vector $K_f$. If no energy is gained or lost in the diffraction process, then $K_f$ has the same length as $K_i$. The difference between the wave vectors of diffracted and incident wave is defined as scattering vector $\Delta K = K_f - K_i$. Since $K_i$
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and $K_f$ have the same length the scattering vector must lie on the surface of a sphere of radius $2\pi/\lambda$. This sphere is called the Ewald sphere.

![Ewald construction](image)

Figure 2.2: Ewald construction

The incident plane wave falling on the crystal has a wave vector $K_i$ whose length is $2\pi/\lambda$. The diffracted plane wave has a wave vector $K_f$. If no energy is gained or lost in the diffraction process, then $K_f$ has the same length as $K_i$. The difference between the wave vectors of diffracted and incident wave is defined as scattering vector $\Delta K = K_f - K_i$. Since $K_i$ and $K_f$ have the same length the scattering vector must lie on the surface of a sphere of radius $2\pi/\lambda$. This sphere is called the Ewald sphere.

The reciprocal lattice points are the values of momentum transfer where the Bragg diffraction condition is satisfied and for diffraction to occur the scattering vector must be equal to a reciprocal lattice vector. Geometrically this means that if the origin of reciprocal space is placed at the tip of $K_i$, then diffraction will occur only for reciprocal lattice points that lie on the surface of the Ewald sphere.

The concept of reciprocal lattice is very helpful in explaining the X-ray diffraction phenomenon. When a crystal is rotated, it can be assumed that the reciprocal lattice associated with it also rotated. The Bragg’s law can be rearranged as

$$\sin \theta_{hkl} = \frac{1/d_{hkl}}{2/\lambda}$$

2.2.4 The structure factor

The structure factor is the central concept in structure analysis by diffraction methods. Its modulus is called the structure amplitude. The structure amplitude is a function of the indices of the set of scattering planes $h$, $k$ and $l$ and is defined as the amplitude of
scattering by the contents of the crystallographic unit cell, expressed in units of scattering. The complex form of the structure factor means that the phase of the scattered wave is not simply related to that of the incident wave. However, the observable, which is the scattered intensity, must be real. It is proportional to the square of the scattering amplitude. The structure factor is directly related to the distribution of scattering matter in the unit cell which, in the X-ray case, is the electron distribution, time-averaged over the vibrational modes of the solid. The structure factor may be represented as a complex vector:

$$F(hkl) = A(hkl) + iB(hkl)$$

where $A(hkl)$ and $B(hkl)$ are the real and imaginary components of $F(hkl)$.

The magnitude or length of the vector $|F(hkl)|$ may then be represented as:

$$|F(hkl)| = \left\{ [A(hkl) + iB(hkl)] \times [A(hkl) - iB(hkl)] \right\}^{1/2}$$

$$= \left[ A(hkl)^2 + B(hkl)^2 \right]^{1/2}$$

Alternatively, $F(hkl)$ may be expressed as an exponential quantity:

$$F(hkl) = |F(hkl)| \exp[i\alpha(hkl)]$$

where $|F(hkl)|$ is the amplitude of the scattered wave and $\alpha(hkl)$ is its phase angle. From Figure 2.1.4 it may be seen that:

$$A(hkl) = |F(hkl)| \cos \alpha(hkl) \quad \& \quad B(hkl) = |F(hkl)| \sin \alpha(hkl)$$

$$\tan \alpha(hkl) = \frac{|B(hkl)|}{|A(hkl)|}$$

$|F(hkl)|$ may be calculated directly from the measured intensity $I(hkl)$ for a reflection, since

$$I(hkl) = K|F(hkl)|^2$$
where $K$ is a constant. However, the phase angle $\alpha(hkl)$ cannot be measured experimentally and must therefore be obtained indirectly through a variety of numerical techniques. The central problem in the solution of a crystal structure is the assignment of phase angles to each reflection in the data set. The solution of the phase problem is considerably simplified for crystals that possess crystallographic centers of symmetry, since, to a first approximation, the imaginary components $B(hkl)$ are zero for centrosymmetric space groups and the phase angles are therefore restricted to values of $0^\circ$ or $180^\circ$. A structure is considered solved when a set of phase angles has been found that allows the atoms to be located and the experimental diffraction pattern to be matched to the calculated diffraction pattern. Since the electron density in a crystal varies continuously and periodically in three-dimensional space, the electron density $\rho(xyz)$ at a point with fractional coordinates $x, y, z$ in a unit cell of volume $V$ may be expressed as a three dimensional Fourier series:

$$\rho(xyz) = V^{-1} \sum_{h} \sum_{k} \sum_{l} |F(hkl)| \cos[2\pi(hx + ky + lz) - \alpha(hkl)]$$

If both the amplitude $|F(hkl)|$ and the phase $\alpha(hkl)$ of each reflection are known, the electron density within the unit cell of the crystal can be calculated directly. On the other hand, if the positions of the atoms in the unit cell are known, both the structure factor and the phase for each reflection may be calculated from the structure factor equation:

$$F(hkl) = \sum_{j} f_j \exp [2\pi(hx_j + ky_j + lz_j)]$$

where $f_j$ is the atomic scattering factor for the atom $j$ and $x_j, y_j, z_j$ are its fractional coordinates. In an actual structure determination both forms of the Fourier transform equations are utilized to arrive at a model structure from which the observed diffraction pattern can be reproduced [11].

### 2.3 Instruments used

The data collection for the structure solution in crystallography started with using photographic films. But to improve the accuracy of intensity measurement for getting
more refined structure and to deal with the protein structure where very large number of reflections involved, the alternative for photographic film, the single crystal diffractometer is developed. Now a day, this instrument which measures by direct counting of the diffracted photons, is used in all of the data required to solve small molecule structures and also in macromolecular crystallography. The most important advantage is better precision since the film has much higher level of background scattering.

A single–crystal diffractometer consists of an X-ray source, an X-ray detector, a goniostat that orients the crystal so that a chosen X-ray diffracted beam can received by the detector, and a computer that controls goniostat and detector movements and perform the mathematical operations required positioning the crystal and detector in the desired orientations. Modern diffractometers use the equatorial geometry in which the diffracted beams are always measured in a horizontal plane defined by the incident X-rays a rotation of the detector about an axis passing through the crystal. The detector can only move on this plane and it forms an angle of $2\theta$ with the incident beam. The electronic detectors incorporate the sensitivity of the diffractometry with the efficiency of film methods. The advantage of these is:

1. The data collected is more precise
2. A large number of intensity measurements can be made in less time
3. Intensity measurements can be saved in a computer readable form hence saves time.

X-ray diffraction is the most powerful technique used to elucidate the three-dimensional structure of molecules. In the present studies, data were collected using different diffractometers in various research institutes across India are given below:

i) Bruker SMART APEX II Single crystal X-ray diffractometer
ii) Bruker SMART CCD area- detector diffractometer
iii) Oxford Diffraction Xcalibur diffractometer.
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2.3.1 Bruker SMART APEX II Single crystal X-ray diffractometer

The Bruker SMART APEX II Single crystal X-ray diffractometer is four circled diffractometer. This is the most accurate means of collecting intensity data. (Figure 2.3).

This is the view of the goniometer of the Bruker SMART APEX II Single crystal X-ray diffractometer. The detector is the large box on the left side of the instrument. The crystal detector distance is adjustable on the 2θ arm. Here the distance is set at 56 mm. The source is on the right side and the crystal is at top of the copper mounting-pin in the center of the image. The mounting pin is coincident with the 0 axis. The angle χ is fixed at 54.74° and the χ block containing the φ drive is mounted on the ω circle, which is concentric with 2θ.

The scattering angle 2θ defines the position of the counter with respect to the incident beam. Since it has three circles which specify the location of the counter, it is named as four circle diffractometer. Figure 2.4 shows schematic diagram of four circle diffractometer.

Figure 2.3: Bruker SMART APEX II Single crystal X-ray diffractometer
Table 2.1: Bruker SMART APEX II configuration.

<table>
<thead>
<tr>
<th>SMART APEX</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data collection details</td>
<td>Combination of omega and phi scans</td>
</tr>
<tr>
<td>Data Reduction</td>
<td>Integration with the SADABS; Sheldrick 2004 and SADABS Bruker 2001.</td>
</tr>
<tr>
<td>X-ray Source</td>
<td>Mo Ka</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>X-ray Generator</td>
<td>50kV, 40mA Max.</td>
</tr>
<tr>
<td></td>
<td>20kV, 5mA Min.</td>
</tr>
<tr>
<td>Monochromator</td>
<td>Graphite</td>
</tr>
<tr>
<td>Collimator</td>
<td>0.3mm, 0.5mm</td>
</tr>
</tbody>
</table>

The working of the instrument is to position the crystal in the incident beam, so that the plane defined by the incident beam and diffracted beam under observation is horizontal. The counter is then positioned so as to detect the desired diffraction beam. The three coordinates $\phi$, $\chi$, and $\omega$ of the crystal are read from the appropriate mounting circles, and the scattering angle $2\theta$ is measured from the counter circle. For each diffraction maximum, the crystal position and the corresponding Bragg angle are recorded. The photon counter reading is fed directly into the computer where the intensity corrections are calculated, so that the computer output is the setting of the crystal, the reciprocal lattice indices $hkl$ corresponding to the observed diffraction maximum, and the appropriate structure factor magnitude. The data collection process starts with the mounting of a crystal on the diffractometer and physically centering by using microscope.

![Schematic diagram of a four circle diffractometer](image)

Figure 2.4: Schematic diagram of a four circle diffractometer
2.3.2 Oxford Diffraction Xcalibur diffractometer

Xcalibur systems are single crystal diffractometers that use the property of X-ray diffraction to determine the crystal structure of materials. They are intended for use with single crystals of chemical substances (inorganic, organic or organo-metallic), mineralogical and biological samples. Xcalibur systems may also be used in the analysis of powder samples. Intended samples should have a maximum unit cell dimension of 100 Å in any direction for small molecule Xcalibur diffractometers and 500 Å for macromolecular Xcalibur PX systems.

The Xcalibur system consists of

1. A kappa geometry, 4-circle diffractometer
2. A CCD area detector
3. An instrument cabinet with electronics rack
4. A stand for optional equipment like high or low temperature attachments
5. System software installed on PC workstation
6. A water chiller for the CCD detector
7. A water chiller for the X-ray tube and X-ray generator.

The diffractometer and CCD area detector are mounted inside a cabinet. The cabinet experiment area is mounted on top of the electronics rack. Water chillers for the CCD detector and the X-ray generator are positioned to the side of the instrument. The PC workstation is located close to the instrument to allow a clear view of the diffractometer and convenient access to it. The diffractometer consists of an X-ray tube, a 3-axis Kappa goniometer (omega, kappa and phi axis) for sample orientation, and a detector arm (theta axis), which has a universal mount capable of supporting any Oxford Diffraction CCD area detector or scintillation point detector. The CCD area detector and the point detector are used to measure the X-quanta diffracted from the sample.
The X-rays are generated by a sealed tube, which is mounted on the goniometer and powered by the high voltage X-ray generator. The X-ray optics consist of a high speed shutter located next to the tube shield, a monochromator for selecting a specified bandwidth from tube spectrum and a collimator for limiting beam divergence. The sample can be viewed with the vide microscope, which is attached to the stand doming the instrument. The CCD area detector works according to the following principle:

Table 2.2: Oxford diffractometer configuration.

<table>
<thead>
<tr>
<th>Oxford Diffractometer</th>
<th>Configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data collection details</td>
<td>Combination of omega and phi scans to maximize reciprocal space coverage to at Least 80° 20.</td>
</tr>
<tr>
<td>Data Reduction</td>
<td>Integration with the CrysAlis PRO RED software provided by Oxford Diffraction</td>
</tr>
<tr>
<td>X-ray Source</td>
<td>Mo Kα</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Scan speed</td>
<td>0.05deg/sec, frame width 1 degree, total frames 1328.</td>
</tr>
<tr>
<td>Monochromator</td>
<td>Graphite</td>
</tr>
<tr>
<td>Collimator</td>
<td>0.3mm, 0.5mm</td>
</tr>
</tbody>
</table>

The X-rays enter the detector through a Beryllium window to the vacuum-sealed detector unit. A scintillation screen transforms the X-ray photons to light, which is...
conducted via a fiber optic reduction taper towards the scientific grade CCD chip. The CCD signal is digitized to 18-bit resolution by a correlated double sampling circuit with analog-to-digital converter located in the detector head. The data transfer via a fibre optic data link to the frame buffer located in the PC workstation. The control program reads the data from the frame buffer to the PC workstation and stores it for further data analysis to the hard disk.

2.4 Data reduction

The process followed to extract relative structure factor amplitude from the raw integrated intensities is known as the data reduction. In order to determine the complete crystal structure, the vital thing is to understand the relationship between the intensity of a beam diffracted from a set of $hkl$ planes and the atoms that make up the planes themselves. The relative amplitude of the diffraction spot associated with the reciprocal lattice point $hkl$ is given by the structure factor $F_{hkl}$.

The structure factor $F_{hkl}$ is affected by certain physical and geometrical factors. One needs to account for these influences by means of corrections to be applied. They are Lorentz factor, polarization factor, absorption coefficient, extinction coefficient, radiation damage and temperature factor.

2.4.1 Lorentz factor

The Lorentz factor used to correct diffraction intensities by taking into consideration the varying time by which different Bragg reflections take to pass through the Ewald sphere. The time that each diffraction spot spends near enough to the Ewald sphere to give rise to a diffracted beam is then found to be dependent and is termed as Lorentz factor.

The Lorentz factor $L_{hkl}$ is defined by

$$L_{hkl} = \frac{1}{\lambda} \frac{\omega}{v_n} = \frac{1}{\sin 2\theta}$$

where, $\lambda$ is the wavelength of the incident radiation, $\omega$ is the angular velocity with which the crystal rotates, $v_n$ is the velocity component perpendicular to the Ewald sphere surface and $2\theta$ is the scattering angle.
2.4.2 Polarization factor

The incident X-ray beam is unpolarized, which means that the electric vector is in a random direction. The diffracted beam will be partially or completely polarized. Intensity of the diffraction maxima depends on the scattering angle. Hence, the correction for the polarization, i.e., the polarization factor, turns out to be

\[ P_{hkl} = \frac{1 + \cos^2 2\theta}{2} \]

where \( 1+\cos^22\theta \) denotes the polarization factor, \( \sin2\theta \) describes the change in irradiated volume of a crystal as a function of \( 2\theta \). The correction to be applied to the measured intensity is \( 1/p \). Since we use graphite crystal monochromator in a diffractometer the incident beam on the crystal itself will be partially polarized. Hence polarization factor is:

\[ P = (p_t) \frac{(\cos^22\theta_m + \cos^22\theta_{hkl})}{1 + \cos^22\theta_m} + (1 - p_t) \frac{\cos2\theta_m + \cos^22\theta_{hkl}}{1 + \cos2\theta_m} \]

where \( \theta_m \) is the Bragg angle for monochromatizing crystal and \( p_t \) is the perfection factor for monochromatizing crystal.

2.4.3 Absorption correction

The absorption correction is the reciprocal of the transmission correction and is the amount by which the measured intensity should be artificially boosted in order to make the absorption correction. According to Beer-Lambert’s law, absorption reduces the intensity of the X-ray beam travelling through a given material by an amount which depends on the material and the length of the path travelled.

If \( I_0 \) is the intensity of incident beam after traversing the thickness \( t \) of the crystal, the intensity of the beam becomes

\[ I = I_0 e^{-\mu t} \]

where \( \mu \) is the linear absorption coefficient.

If the compound has very large absorption coefficient for the radiation used, the raw intensities require absorption correction. Among the methods used for absorption correction the numerical method [12] and \( \psi \)-scan method [13] are very promising. This is a scan of diffraction data measured as the crystal rotates about the diffraction...
vector. The intensities can be corrected for absorption as a function of the orientation of the crystal when the reflection under consideration was measured.

2.4.4 Extinction correction

Extinction corrections are related to mosaic spread. Corrections for this effect are generally applied after refinement, which involves finding a factor needed to adjust the most intense Bragg reflections so that they agree with those calculated. Darwin, who first theoretically investigated the effects of extinction, subdivided the general attenuation of intensity due to this cause into two categories viz., primary extinction and secondary extinction.

**Primary extinction:** Primary extinction occurs only when the crystals are ideally perfect. One of the interesting aspects of the geometry of X-ray reflection is that the ray which is reflected at the Bragg angle by a set of planes is directed at just the correct angle to be reflected back again into the direct beam by other planes of the same stack. Thus, each plane of the crystal redirects a small fraction of the reflected rays reaching it back into the primary beam. It is evident that radiation scattered by an electron differs in phase from that of the primary beam by $\pi/2$. It follows that the rays which are twice reflected differ in phase from the primary beam by $\pi$. For this reason, the twice reflected rays instead of strengthening the primary beam actually weaken it by destructive interference. As the primary beam penetrates the crystal further and further, it is continuously attenuated in this manner.

**Secondary extinction:** Secondary extinction is more pronounced at low $\sin \theta/\lambda$ values [14]. The reduction of the intensity of the primary beam due to this kind of previous reflection was called by Darwin secondary extinction. Correction for the secondary extinction is considered at the end of the refinement given by the equation

$$|F_c| = K|F_{rel}|(1 + g|I_c|)$$

where K is the scale constant and $g$ is secondary extinction coefficient. Lowering the temperature of the crystal will reduce this effect.
2.4.5 Radiation damage

There may be changes in the ordered structure of crystalline material caused by interaction with X-rays. The changes in crystalline structure may result in either beneficial or detrimental modifications of properties. Radiation damage causes Bragg reflection to change intensity as the function of time. It involves movement of atoms so that each Bragg reflection is changed in a specific way, depending on crystal structure. Radiation damage may be detected by monitoring a set of reference reflections that are measured at regular intervals throughout the data collection.

2.4.6 Anomalous scattering

The scattering factor \( f \) for an atom is roughly proportional to the number of electrons that it possesses. However for wavelengths, that approximate those for which the atom strongly absorbs radiation the scattering factor undergoes a change due to anomalous dispersion. The dispersion not only affects the magnitude of the factor but also imparts a phase shift in the elastic collision of the photon. The scattering factor can therefore best be described as a complex number [15].

When the energy of incident X-ray beam is close to that at which it will give rise to a change in the quantum state of the electron, the expression of Thomson scattering will not be valid. Due to perturbation of electron distribution, spherical symmetry and centrosymmetry are destroyed. Then the atom is said to be an anomalous scatterer. The Fourier transform of atomic scattering factor \( f_j \) is no longer real and takes the form

\[
f_j = (f_j)_c + A f' + i A f''
\]

where \((f_j)_c\) is the form factor calculated on the assumption of spherical symmetry and is corrected by a real part \( A f' \) and an imaginary part \( A f'' \) both of which may be calculated theoretically. When \( f_j \) is real,

\[
F(k) = F^*(-k)
\]

where \( F^* \) is the complex conjugate of \( F \). This is the well known Friedel’s law.

The intensity of X-rays

\[
I(k) = I^*(k)
\]

When anomalously scattering atom is present, Friedel’s law breaks down and
Friedel’s law helps to identify the existence of the inversion center and also in the solution of phase problem.

### 2.4.7 Temperature factor

Usually the atoms in the crystal are considered to be rest. But, the atoms actually vibrate about a mean position. The extent of vibration depends on the temperature. Because of the finite size of the electron cloud about the nucleus, the intensity scattered by an atom falls off with increasing \( \sin \theta / \lambda \). Thermal vibration of the atom has the effect of smearing out the electron cloud in a larger volume, consequently enhancing the fall-off rate. These vibrations can be considerable even at room temperature. This vibration has considerable effect upon the intensity of a diffracted beam. The chemical bonds link the atomic vibrations throughout the crystal and the thermal motion of any atom in a crystal is generally assumed to be independent of the vibration of the others.

The effect of thermal vibrations is to reduce the effective scattering power of an atom. If the atom is oscillating, then the expression for \( f_j \) must be modified to take an account of this. The temperature corrected atomic factor is defined as

\[
(f_j)_T = f_j \exp[-B_j (\sin^2 \theta/\lambda^2)]
\]

in which \( \theta \) is the Bragg angle and \( \lambda \) is the wavelength of the incident radiation. \( B_j \) is called the temperature factor and its value depends on the atom and the temperature at which diffraction takes place. The equation accounts for the thermal motion by introducing a negative exponential. The term \( \sin^2 \theta/\lambda^2 \) is necessarily positive and \( B_j \) is always positive. Since \( B_j (\sin^2 \theta/\lambda^2) \) must be dimensionless, the dimensions of \( B_j \) are [length]\(^2\). The value of \( B_j \) is given by

\[
B_j = \frac{8\pi^2}{3} u^2
\]

where \( (u^2) \) represents the mean square displacement of the \( j^{th} \) atom. This equation shows that the value of \( B_j \) is necessarily positive. Hence, \( (f_j)_T < f_j \). When \( \theta \) is zero, \( (f_j)_T = f_j \). Also the value of the exponential quantity decreases quite rapidly as \( \theta \) increases causing \( (f_j)_T \) to fall off sharply as compared to \( f_j \).
Chapter 2

2.5 Structure solution with Fourier synthesis

2.5.1 The phase problem and estimation of $\rho(xyz)$

X-rays are scattered by the electrons of the atoms in the crystal. To locate the position of an atom it is necessary to calculate the electron density function $\rho(xyz)$ in the unit cell. The diffraction amplitude due to the contents of the unit cell would be the Fourier transform of $\rho(xyz)$ obtained as

$$\rho(xyz) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} F_{hkl} e^{-2\pi i (hx + ky + lz)}$$

where $V$ is the volume of the unit cell, $F(hkl)$ is the structure factor for the particular set of $(h,k,l)$ and $(x,y,z)$ are the fractional coordinates which are the atomic coordinates expressed as the fractions of the unit cell length, and

$$F_{hkl} = |F_{hkl}| e^{i\varphi(hkl)}$$

where, $|F(hkl)|$ is the amplitude of the structure factor, $|F(hkl)|^2$ is proportional to the intensity of the reflections. $\varphi$ is the phase of the scattered beam. We can measure the amplitude which is proportional to the square root of the intensity of the reflections, but the direct measurement of the relative phase is not possible. So, the trick of crystallography lies in the finding of the phase.

$$\rho(xyz) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} |F_{hkl}| e^{i\varphi_{hkl}}$$

But it is not possible in a unique way, because from the experimental data only the magnitude of the structure factor are obtained, not the phase. Therefore, in order to compute the electron density, we must somehow derive the missing information, phase. The deviation of the correct values of the structure factor $F_{hkl}$, both magnitude and phase, from the relative intensities $|F_{hkl}|^2$ constitutes the phase problem. The phase is not detectable in our experiments as we lack instruments like detector with very fast time response, a coherent source etc.

There are two principal methods to solve the phase problem. The first one is the chemical modification method involves the chemical modification of the molecule within the crystal and the second approach is a more mathematical one in which the
relative intensity $|F_{hkl}|^2$ data are treated statistically and analytically in order to search for certain relationships such as inequalities, which have been theoretically predicted. This in turn is used to determine the phase $\varphi_{hkl}$. These mathematical techniques of solving the phase problem are called direct methods.

### 2.5.2 Patterson method

Patterson, A. L. (1934) introduced and discussed the physical significance of Fourier series which can be directly calculated from experimental intensity data [16]. This method can be applied to structures in which one or a few atoms are markedly heavier than the rest. It is possible to find the locations of the heavy atoms by use of the Patterson function which does not require a prior knowledge of the phases. The Patterson map, commonly designated $\rho(uvw)$, is a Fourier synthesis that uses the indices, $h,k,l$, and the square of the structure factor amplitude, $|F_{hkl}|^2$, of each diffracted beam. It is usual describe the Patterson map in vector space defined by $u,v,$ and $w$, rather than $x,y,z$ as used in electron-density maps. The Patterson method consists of evaluating Patterson function which can be represented with the following equation:

$$\rho(uvw) = \frac{1}{V} \sum_{hkl} |F_{hkl}|^2 \exp[-2\pi i (hu + kv + lw)]$$

If the electron density is known, the Patterson function at any point $u,v,w$ may be calculated by multiplying the electron density at a point $x,y,z$ with that at $x+u, y+v, z+w$, doing this for all values of $x, y$ and $z$ and summing the products [17]:

$$\rho(uvw) = \int \rho(xyz) \rho(x + u, y + v, z + w) dv$$

In other words, the Patterson function is the convolution of the electron density at all points $x, y, z$ in the unit cell with the electron density at points $x + u, y + v, z + w$. A peak at $u,v,w$ in the Patterson map represents a vector from the origin of the Patterson function to the point $u,v,w$. This means that if any two atoms in the unit cell are separated by a vector $u,v,w$, then there will be a peak in the Patterson map at $u,v,w$. The peaks in a Patterson map are inherently broader than those in a Fourier map for finite size of atoms. Instead of $N$ peaks in a unit cell of a Fourier map, there is
a crowding of $N(N-1)$ peaks in the same volume of the Patterson map enhancing the probability of superposition. However, when the structure contains one or more heavy atoms, the peaks due to interactions of the heavy atom stand out among other peaks of lower magnitude making interpretation comparatively easy. The Patterson technique is therefore best applicable to heavy atom structures. The difficulty with the Patterson method is that a unit cell with $N$ independent atoms will have $N^2$ inter-atomic vectors (peaks in the Patterson map). When $N$ is large as for a macromolecule, the peak overlaps make it difficult to solve the structure directly using this method [18].

2.5.3 Direct method

During a diffraction experiment only the magnitudes of $F_{hkl}$ are measured, the corresponding phases $\varphi_{hkl}$ being lost. This is true for any single reflection. It is well known that with a complete set of intensity data, the collection of $|F_{hkl}|$ values does contain enough information regarding the phases. Thus, it is possible to determine the phase directly from the set of $|F_{hkl}|$ values measured with reasonable accuracy, and hence the name direct method.

Two important properties of the electron density function that are helpful in obtaining the phase information are:

- it is everywhere positive, i.e., $\rho(r) \geq 0$ (positivity)
- it is composed of discrete atoms (atomicity)

Possible phase angles are constrained by these two conditions, so that relative phase determination hinges on the mathematical expressions for Fourier series. In the total Fourier synthesis involving $F_{hkl}$ with the correct value of $\varphi_{hkl}$, these two conditions should apply.

In direct methods structure factor phases are derived directly from the observed amplitudes through purely mathematical techniques; without assuming any kind of molecular structure. When the number of structure amplitudes measured is more than ten times the number of the parameters to be determined, direct methods, which utilize the sign and phase relations, can be used to solve the structure. One such relation applicable to centrosymmetric system, where phase can only be $0^\circ$ or $180^\circ$, is that the sign of $F(hkl)$ is probably equal to the product of sign of $F(h'k'l')$ and $F(h-$

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Possible phases can be derived from systematically searching for sets of reflections (with high intensities) whose indices are related in this way. An electron density map can then be calculated. Peaks are assigned for the atoms in the structure to get the trial structure. From the trial structure the new phases are calculated. The structure can be solved by repeating this cycle. This method is mainly used for small molecules with up to roughly 50 non-hydrogen atoms.

The steps involved in direct method are [19]:

Step 1 Conversion of observed structure factor $|F_{hkl}|$ to normalized structure factor $|E_{hkl}|$ which are independent of $\theta$.

Step 2 Setting up of phase relations using triple phase relation (triplets) and four phase relations (quartets).

Step 3 Selection of few reflections, the phase of which are assigned apriori.

Step 4 Phase propagation and refinement using tangent formula.

Step 5 Calculation of best phase sets and expressing the reliability of the phases in term of Combined Figure of Merit (CFOM).

Step 6 Calculation of electron density map (E-map) with $|E_{hkl}|$ as the Fourier coefficient.

The electron density at a point with position vector $\mathbf{r}$ can be expressed as

$$
\rho(\mathbf{r}) = \frac{1}{V} \int_{a t i \Delta \mathbf{k}} F_{rel}(\Delta \mathbf{K}) e^{i \Phi(\Delta \mathbf{K})} e^{-\Delta \mathbf{K} \cdot \mathbf{r}} d(\Delta \mathbf{K})
$$

and the quantity $F(\Delta \mathbf{k})$ is given by:

$$
F(\Delta \mathbf{K}) = \rho(\mathbf{r}) e^{(i \Delta \mathbf{K} \cdot \mathbf{r})} d\mathbf{r}
$$

where the integration is carried over the unit cell volume. For a unit cell having $n$ discrete atoms this becomes,

$$
F(\Delta \mathbf{K}) = \sum_{n} f_{j} e^{i \Delta \mathbf{K} \cdot \mathbf{r}_{j}}
$$

The Phase of this quantity is given by:
This is an indirect method of estimating the values of $\phi(\Delta k)$’s.

2.5.4 Unitary and normalized structure factors

The methods used to ensure that the Fourier summation does not give a negative electron density map are mathematical in nature. In order to generalize the mathematics, a quantity unitary structure factor is defined as,

$$ U_{hkl} = \frac{F_{hkl}}{\sum_i f_i} $$

Further, unitary atomic scattering factor, $n_i$ is defined as

$$ n_i = \frac{f_i}{\sum_i f_i} $$

Therefore,

$$ U_{hkl} = \sum_i n_i e^{2\pi i (hx_i + hy_i + hz_i)} $$

This is analogous to the expression for $F_{hkl}$.

Another useful quantity is the normalized structure factor $E_{hkl}$, which is defined as

$$ \left| E_{hkl} \right| = \frac{U_{hkl}}{\langle |U_{hkl}|^2 \rangle^{1/2}} $$

where

$$ \langle |U_{hkl}|^2 \rangle^{1/2} = \frac{1}{N} \sum_{hkl} |U_{hkl}|^2 $$

in which the summation is over the $N$ values $|U_{hkl}|^2$ corresponding to all reciprocal lattice sites $hkl$. $\langle |U_{hkl}|^2 \rangle$ represents the average value of $|U_{hkl}|^2$. The $E_{hkl}$ values have an average value equal to unity, i.e., $\langle |E_{hkl}|^2 \rangle = 1$. Hence, the name normalized.

[20] The intensities from non-centrosymmetric crystal are nearer the mean value of $E_{hkl}$ than those from the centrosymmetric crystal. The centrosymmetric crystals give higher number of weak Bragg reflections than the non-centrosymmetric crystals.
2.5.5 Structure invariants and semi-invariants

Quantities which are invariant under any arbitrary change of the origin are called structure invariants. It may be easily verified that if \( h + k + l = 0 \) then \( \phi_h + \phi_k + \phi_l \) is a structure invariant. The magnitudes of \( |F_h| \) are obviously independent of our choice of origin for describing the structure. But the phases \( \phi_h \) are very much dependent on origin specification.

In all the space groups except \( P1 \) a point with some advantageous symmetry environment is chosen as origin. For example, in the centrosymmetric space group \( P\overline{1} \), the origin is at a center of symmetry. In the non-centrosymmetric space group \( P2_{1}2_{1}2_{1} \), origin is at a point half-way between the three pairs of non-intersecting screw axes. Now, with the origin chosen in the respective ways as stated, we find that in case of both the space groups mentioned, the following eight points in the unit cell

\[
(0,0,0), \left(\frac{1}{2},0,0\right), (0,\frac{1}{2},0), (0,0,\frac{1}{2}), (0,\frac{1}{2},\frac{1}{2}), \left(\frac{1}{2},0,\frac{1}{2}\right), \left(\frac{1}{2},\frac{1}{2},0\right) \text{ and } \left(\frac{1}{2},\frac{1}{2},\frac{1}{2}\right)
\]

have identical symmetry environments. Change of origin from one to another of such points may be called as allowed shift of origin. Quantities which remain unaltered under an allowed shift of origin are called structure semi-invariants. Since the structure factor magnitudes of \( |F_h| \) are structure invariants, they are expected to provide information regarding other structure invariants and semi-invariants only and not about individual phases in general. This fact will further lead to some relations involving the magnitudes and phases.

The fact that in general the magnitudes of \( \phi_h \) vary with our choice of origin even among the allowed alternatives for a space group calls for a procedure for fixing the origin towards the beginning of our phase determination procedure. This is done by assigning arbitrary phase to a requisite number (maximum 3) of reflections with indices of suitably chosen parity.

In case of non-centrosymmetric structure, the enantiomorphism is also fixed, because, a change from one enantiomorphism to the other causes a change in the sign of the phases and consequently in those of linear combinations which are structure invariants. To fix the enantiomorphism, the phases of reflection with suitably chosen indices are restricted, often within the range from \( 0 \) to \( \pi \) and from \(-\pi/2\) to \(+\pi/2\).
2.6 Refinement of the structure

Once the model structure has been proposed by one of the methods explained earlier, it is necessary to refine the preliminary coordinates by some process. The model obtained is however an approximate one and the positional and thermal parameters describing the model together with a scale factor for the structure factors calculated on this basis have to be refined, so that the actual structure may be approached as closely as possible within the limits of experimental accuracy. An agreement between the calculated $|F_o|$ and the observed $|F_c|$ structure factors indicates the degree of refinement. The most common method of assessing the agreement is by calculating the *residual index* $R_1$ or reliability index, which is defined as

$$R_1 = \frac{\sum |F_o| - |F_c|}{\sum |F_o|}$$

the summations being over all the reflections. Also, another form of the residual used is

$$wR_2 = \left[ \frac{\sum w(F_o^2 - F_C^2)^2}{\sum w(F_o^2)} \right]^{\frac{1}{2}}$$

where $w = 1/\sigma^2(F_o)$, $\sigma(F_o)$ being the standard deviation of $F_o$. Thus $R$ measures the relative discrepancies between $F_c$ and $F_o$, so that lower the value of $R$, better is the agreement. In the ideal case $R = 0$.

The simplest approach to structure refinement involves merely the combination of the observed structure amplitudes with the calculated phases, and use of these as the coefficients in a Fourier series. [21] The resulting Fourier synthesis should not only return the atoms of the input model, but it should improve the coordinates of their location and reveal new atoms. This process is repeated until all the non-hydrogen atoms are located. This process is called cyclic Fourier refinement.

Further refinement of structure is done using the method of least-squares. The function minimized is

$$R_1 = \sum_{h,k,l} w_{hkl} (|F_o|^2 - |F_c|^2)^2$$

where $w_{hkl}$ is the weight of the observation. All possible least-squares equations are constructed and solved by the matrix method.
2.6.1 Difference Fourier synthesis

When the structure is refined using any one of the models, the remaining lighter atoms and hydrogen atoms can be located by the method of difference Fourier synthesis which is expressed in the form

$$\Delta \rho(x, y, z) = \frac{1}{V} \sum_{h} \sum_{k} \sum_{l} (|F_0| - |F_C|) e^{i2\pi(hx+ky+lz)} e^{i \varphi_c}$$

where $\varphi_c$ is the phase of $F_C$ and $x$, $y$ and $z$ are coordinates of a point in the unit cell.

From the value of maximum $\Delta \rho(x, y, z)$, the missing atoms or misplaced atoms or the atom which has been considered as a different atom can be identified. In general $\Delta \rho(x, y, z)$ for hydrogen atoms are not calculated, and are fixed at chemically acceptable positions.

2.6.2 Weighting scheme

In the process of refinement every observation should have a weight $w$ [20] associated with it, reflecting the reliability of that particular observation. The proper weight to be assigned to an observation is

$$w_i = 1/\sigma_i^2$$

where $\sigma_i$ is the standard deviation. The reciprocal of the variance is indeed a measure of its reliability, and the function minimized is

$$\sum w(|F_0| - |F_C|)^2$$

where $\sigma$ is the standard deviation and it can be shown that where $N$ is number of counts.

$$\sigma = \sqrt{N}$$

where $N$ is number of counts. In diffractometer while measuring the X-ray reflection, left background and right background counts are considered and the standard deviation turns out to be

$$\sigma = \frac{1}{2} \sqrt{L_P \left[ \frac{N_t + N_{LBG} + N_{RBG} + (0.01N_{net})^2}{N_{net}} \right]^\frac{1}{2}}$$

where $K$ is a scale constant, $L$ is the Lorentz factor, $P$ is the polarization factor and
2.6.3 Results derived from the refined structure

The most important results that can be derived from the refined structure are the distances between bonded atoms and the angles between pairs of bonds. It is also necessary to find the equation of the mean plane passing through a group of atoms supposed to lie on a plane, to check the deviations of the atoms in such a group from the plane and perhaps also to calculate the distance of other atoms from it.

For all such calculations, the fractional coordinates of any atom \(X (= x, y, z)\) with respect to crystallographic axes are transformed into coordinates \(X' (= x', y', z')\) in Å, with respect to the orthogonal coordinates whose x-axis is coincident with the crystallographic \(a\)-axis, y-axis lies in \(ab\)-plane and z-axis along \(c^*\)-direction. The transformed coordinates are given by

\[ X' = UX \]

where \(U\) is transformation matrix, given by

\[
\begin{pmatrix}
a & b \cos \gamma & c \cos \beta \\
0 & b \sin \gamma & -c \sin \alpha \sin \beta \\
0 & 0 & c \sin \alpha \sin \beta
\end{pmatrix}
\]

2.6.4 Bond lengths and bond angles

A bond length is the distance between two atomic nuclei that are joined together by some type of electronic bonding. This conformational parameter gives an idea about the nature and strength of any bond. Because atoms undergo vibration and other motions, reported bond lengths are average or equilibrium bond lengths.

If two atoms at \(x_1, y_1, z_1\) and \(x_2, y_2, z_2\) lie in a unit cell that has edges with lengths \(a, b\) and \(c\) and inter axial angles of \(\alpha, \beta\) and \(\gamma\), the square of the distance \(r\) between these two atoms is

\[
r^2 = [(x_1 - x_2)a]^2 + [(y_1 - y_2)b]^2 + [(z_1 - z_2)c]^2 \\
+ [2ab \cos \gamma (x_1 - x_2)(y_1 - y_2)] \\
+ [2ac \cos \beta (x_1 - x_2)(z_1 - z_2)] \\
+ [2bc \cos \alpha (y_1 - y_2)(z_1 - z_2)]
\]
The inter-nuclear bond angle $ABC$, is the angle between the bonds $A - B$ and $B - C$ formed by three atoms $A$, $B$ and $C$, connected in that order. If the length of $A - B = l_1$ and $B - C = l_2$ and $A \cdots C = l_3$, then $A - B - C = \delta$ may be calculated from the formula

$$\cos \delta = \frac{l_1^2 + l_2^2 - l_3^2}{2l_1l_2}$$

**2.6.5 Estimated standard deviation (esd)**

It is a method to assess the precision of a geometric quantity of interest in a structure determination. This is obtained from the least square refinement. The more precisely a measurement is made; the smaller is the *estimated standard deviation (esd)* of that measurement. The esd values contain a measure of the random error in each atomic coordinate. They do not give any indication of systematic errors with respect to unit cell dimensions, scattering factors, space group etc. The esd of individual atom coordinates in a given structure is approximately inversely proportional to the number of electrons in that atom.

**Estimated standard deviations of bond lengths**

For two atoms, $A(x_A, y_A, z_A)$ and $B(x_B, y_B, z_B)$, $\sigma_{xA}$ is the esd of $x$ for atom $A$ so on and $\Delta x = x_B - x_A$. The required esd values are obtained by least squares refinement. The esd of the bond distance between $A$ and $B$ is

$$\sigma_r^2 = \frac{1}{r^2} \left[ \left( \sigma_{xA}^2 + \sigma_{xB}^2 \right) (\Delta x + \Delta y \cos \gamma + \Delta z \cos \beta)^2 \right]$$

$$+ \left[ \left( \sigma_{yA}^2 + \sigma_{yB}^2 \right) (\Delta y + \Delta x \cos \gamma + \Delta z \cos \alpha)^2 \right]$$

$$+ \left[ \left( \sigma_{zA}^2 + \sigma_{zB}^2 \right) (\Delta z + \Delta x \cos \beta + \Delta y \cos \alpha)^2 \right]$$

**Estimated standard deviation of inter-bond angles**

The esd of the angle between the bonds $A - B$ and $B - C$ is calculated by

$$\sigma_\theta = \sqrt{\frac{\sigma_A^2}{(AB)^2} + \frac{\sigma_B^2(AC)^2}{(AB)^2(BC)^2} + \frac{\sigma_C^2}{(BC)^2}}$$
where $\sigma_A$, $\sigma_B$ and $\sigma_C$ are the e.s.d of the positions of atoms $A$, $B$ and $C$.

### 2.6.6 Torsion angles

Torsion angles (or angle of twist) of ring system or chain of atoms are the dihedral angles between planes formed by consecutive bonds. For a sequence of atoms $A$, $B$, $C$, $D$, the torsion angle of bond $B-C$ is the angle between the positive normal to the planes $(B-A, B-C)$ and $(C-B, C-D)$, where the positive direction of a normal is defined to be that which forms a right-handed set with the two vectors defining the plane. The torsion angle between groups $A$ and $D$ is then considered to be positive if the bond $A-B$ is rotated in a clockwise direction through less than 180 in order that it may eclipse the bond $C-D$; a negative torsion angle requires rotation in the opposite sense. (Figure 2.7).

![Figure 2.6: Definition of the torsion angle $\omega$](image)

Stereo-chemical arrangements corresponding to torsion angles between 0 and 90 are called *syn* ($s$), those corresponding to torsion angles between 90 and 180 *anti* ($a$). Similarly, arrangements corresponding to torsion angles between 30 and 150 or between $-30$ and $-150$ are called *clinal* ($c$) and those between 0 and 30 or 150 and 180 are called *periplanar* ($p$). The two types of terms can be combined so as to define four ranges of torsion angle; 0 to 30 *syn-periplanar* ($sp$); 30 to 90 and $-30$ to $-90$ *syn-clinal* ($sc$); 90 to 150 , and $-90$ to $-150$ *anti-clinal* ($ac$); 150 to 180 *anti-periplanar* ($ap$) [23] (Figure 2.8).
Figure 2.7: Torsion angles

Torsion angle is the parameter which is different for different conformations of single molecule. Thus, the torsion angle is the most important and informative parameter in the study of molecular conformation. The torsion angle is a very useful tool when different molecules of the same compound in the same or different structure are to be compared to study their conformations. If the conformations of two molecules of the same compound are to be different, one more corresponding torsion angles in them must be different.

2.6.7 Mean planes

The equation of a plane in orthogonal coordinate system at a distance \( p \) from the origin is:

\[
lx + my + nz - p = 0
\]

where \( l, m, n \) are direction cosines of the normal to the plane. The distance of a point \((X_i, Y_i, Z_i)\) from the plane being

\[
P_i = lx_i + my_i + nz_i - p
\]

The equation of the mean plane through a group of \( N \) atoms is obtained by minimizing the quantity

\[
R = \sum_{i=1}^{N} \omega_i p_i
\]
where

\[ \omega_i = \frac{1}{\sigma_i^2} = \frac{3}{\sigma_{x_i}^2 + \sigma_{y_i}^2 + \sigma_{z_i}^2} \]

Applying the condition,

\[ \frac{\delta R}{\delta P} = 0 \]

Then,

\[ l \sum \omega_i X_i + m \sum \omega_i Y_i + n \sum \omega_i Z_i - p \sum \omega_i = 0 \]

\[ lX + mY + nZ - p = 0 \]

where

\[ X = \frac{\sum \omega_i X_i}{\sum \omega_i}, Y = \frac{\sum \omega_i Y_i}{\sum \omega_i}, Z = \frac{\sum \omega_i Z_i}{\sum \omega_i} \]

showing that the mean plane passes through the centroid \((X, Y, Z)\) of group of \(N\) atoms. Now,

\[ R = \sum \omega_i [l(X_i - \bar{X}) + m(Y_i - \bar{Y}) + n(Z_i - \bar{Z})]^2 \]

The condition \(\frac{\delta R}{\delta l}, \frac{\delta R}{\delta m}, \frac{\delta R}{\delta n} = 0\) gives three liner homogeneous equation in \(l, m, n\) which are solved, remembering that

\[ l^2 + m^2 + n^2 + 1 = 0 \]

Thus equation for the mean plane referred to orthogonal axes as,

\[ lX + mY + nZ - (l\bar{X} + m\bar{Y} + n\bar{Z}) = 0 \]

The deviation \(P_i(i = 1\text{ to } N)\) of the \(N\) atoms from the plane may be calculated and we can also calculate

\[ X^2 = \sum_{i=1}^{N} \frac{P_i^2}{\sigma^2(P_i)} \]

where

\[ \sigma^2(P_i) = l^2\sigma^2(X_i) + m^2\sigma^2(Y_i) + n^2\sigma^2 \]
2.6.8 Structural conformations

The X-ray crystal structure determination provides the most precise and accurate details possible for the molecular composition, configuration and conformation. Consequently, molecular and structural data derived from X-ray crystallographic studies play a pivotal role in developing, understanding and controlling the biological processes [24]. Conformational analysis is the examination of the positions a molecule and the necessary energy changes it undergoes as it converts among its different conformations. Because each of the various conformations of a molecule has different properties, the conformation the molecule normally adopts has a profound influence on its physical and chemical properties. Chemists use conformational analysis to understand the behaviour of the molecules in chemical reactions. Biochemists and molecular biologists also use conformational analysis to study the ways the molecules interact with each other in living systems.

In general, all rings other than the three-membered and aromatic ones are non-planar. The origin of non-planarity is due to the balance between valence bond angle strain [25] and torsional strain. The distortion of the bond angles costs much more energy than the torsion strain. Ring strain is reflected in the stability of cyclic molecules. Strained molecules tend to undergo chemical reactions which relieve strain, such as hydrolytic ring opening. Strain could also be relieved by adopting least-strained non-planar conformations. Normally, different conformations are obtained by rotation about the bonds. At a certain temperature, one or more of the possible conformations can be observed, depending on their relative energies and the energy barriers that have to be overcome. The potential energy and the energy barriers are influenced by intra and intermolecular forces.

The six-membered rings assume varieties of conformations. The chair conformation is the most stable of the many conformations that a six-membered ring can achieve. Each carbon in the ring has two substituents. One of these will be in an axial position and the other will be in an equatorial position. A substituent in an equatorial position leads to a lower energy conformation than one with the same substituent in axial position. For completely planar molecules, all the torsion angles will be zero. Hence, the deviation of the observed torsion angles from this ideal value gives the amount of non-planarity in the structure. These factors can be effectively used to describe and compare the conformations of the molecules occurring in a quantitative way.
References


