CHAPTER I
INTRODUCTION
The present thesis incorporates the results of studies on crystal and molecular structures of the three biologically important compounds, namely, (i) tryramine hydrochloride, (ii) copper iminodiacetate dikydrate and (iii) copper propionate.

The brilliant researches, particularly in the field of molecular biology, pursued during the last few decades have explored and established the vital role of some key molecules, so far the life processes are concerned. These key molecules appropriately termed as biomolecules, may be both large and small. In general the functions of large molecules that is biomacromolecules are structure dependent that is they perform specific diversified functions through their extensive range of conformations. The functions of the small molecules, on the other hand, are predominantly reaction dependent and the functions depend not so much on their structure as on their reserve. Most of the energy essential for the life processes is supplied through highly specific and complex reactions of diverse nature involving the small molecules.

Biomacromolecules or biopolymers are conveniently considered under three fundamental types of long chain molecules and their hybrids such as polysaccharides, polymucleotides and polypeptides. They all are composed of linear aggregation of some basic units and depending on these they may be homopolymer e.g. polysaccharides or heteropolymers e.g. nucleic acids and proteins. There are two types of nucleic acids deoxy ribonucleic acid (DNA) and ribonucleic acid (RNA) and the structure of DNA is a typical example of molecular structure suggesting the molecular mechanism. Few products of
natural origin are as versatile in their behaviour and properties as are the amino acids, and a few have such a variety of biological duties to perform.

The amino acids are participants in crucial metabolic reactions on which life processes depend, the essential constituents of protein molecules whose biological and chemical specificities are determined in part by the number, distribution and spatial interrelations of amino acids of which they are composed. They reveal at once uniformity and diversity; uniformity in that they are constituents of proteins and hence of living systems, diversity, because each possesses a different side chain which confers upon it unique properties distinguishing it physically, chemically, and biologically from others. In this duality, the array of amino acids is a partial reflection of the larger biological world.

As mentioned earlier, most of the small molecules are responsible for energy supply and regulation of various biological processes in living systems. The two energy rich molecules, ATP and ADP, for example, supply large amount of energy through a great variety of highly complex chemical reactions. Some small molecules have also been found to control the enzyme activity by being incorporated to them and thereby altering their shape and size. The structure of these molecules play a dominant role on the chemical reactions in which they are involved. It is well established that a specific biological function is associated with a particular three dimensional structure and conformation.
The spatial disposition of a molecule which can be assumed through rotations about single bonds may be defined as conformation and while a molecule may have a number of conformations, the allowed ones are those compatible with the free energy and stereochemical considerations. Quite a number of methods may be applied for the determination of conformations and X-ray crystallographic approach is one of the most effective method for studying the same. The X-ray method may not lead to the unique conformation relevant to a particular biological activity, but it surely provides reliable information regarding the stable molecular conformations.

A class of compounds commonly known as sympathomimetic amines illustrates that the biological activities of small molecules depend largely on their structure. In the year 1898 Lewandewasky (1) recognised that the effects of stimulation of sympathetic nerves were in many ways similar to those effected by injecting adrenal extract. A few years later Elliot (2) reported that the sympathetic nerves on stimulation released a small quantity of adrenaline like substance identified as noradrenaline.

In 1910 Berger and Dale (3) examined the physiological activity of some synthetic amines related to adrenaline and found that these amines mimic the property of adrenaline and hence coined the term sympatho-mimetic amines.

In order to understand intimately the structure-function relationship of some sympathomimetic amines and also to examine whether it is possible to throw some light on the unique structural requirement of these substances responsible for their specific activities, structural investigations on tyramine hydrochloride have been undertaken and which incidentally forms a
part of a major research programme of our laboratory. The first part of the dissertation deals with the detailed account of the crystal and molecular structure determination of tyramine hydrochloride.

It has been observed that sympathetic drug like tyramine increases the nor-adrenaline content in the nervous outflow of several organisms in vivo. In addition, the amine is responsible for relatively rapid and brief liberation of transmitter and ultimately sympathomimetic effects.

As a result of extensive research on structure and activity of sympathomimetic amines, it has been established that phenethylamine can be considered as the parent compound of sympathomimetic amines, essentially consisting of an aromatic nucleus—a benzene ring and an aliphatic side chain with a positively charged tetracovalent nitrogen atom as shown below:

\[
\text{HO} \quad \text{C} \quad \text{C} \quad \text{NH}_2
\]

It has been established by the early workers that for a sympathomimetic amine to exhibit characteristic activity, the ethylamine side chain must be maximally extended and in most of the cases perpendicular to the ring system. The most probable conformation in the receptor site seems to be consistent with this view.

Copper iminodiacetate dihydrate, the second compound studied, is of paramount importance particularly in biochemistry and physiology. Iminodiacetic acid constitutes the functional group of a highly important and useful ion exchange resin chelex-100 where iminodiacetic acid is introduced into the matrix of a styrene divinyl benzene polymer. Chelax-100 shows
unusually high preference for copper and other heavy metals and the selectivities of chelax-100 for metals correspond to high affinity of iminodiacetic acid and resins for copper, iron, calcium and magnesium, makes it uniquely suited for removing trace metals from biochemical and physiological fluids, culture media and enzyme system. Seigel and Degens (4) and Weble and Wood (5) converted chelax-100 to the copper form and used it for the separation of a particular trace element from a large pool of heterogeneous components and used this principle for analysis of amino acids in sea water. Buist and Q'Brien (6) have used the resin copper complex for the separation of amino acids from peptide in urine. Goldstein (7) has demonstrated that ligand exchange chromatography on copper loaded chelax-100 to be extremely useful technique for the rapid separation of nucleic acid component. The resin serves as a valuable tool for the production of radioactive tracers of high specific activity using Szilard-Chalmers method for exploring biological interactions of diverse nature.

Copper propionate constitutes the third compound investigated. In the strict sense of the term, this may not be considered in the category of biomolecules. It is, however, associated with different chemical reactions in biological systems. It has long been known that fatty acids including propionic acid and their heavy metal salts possess antifungal, fungicidal and astringent properties. Peck and associates (8) reinvestigated the fungicidal properties of these compounds and attributed the antifungal activities of sweat to the fatty acid contents. To explore the structure and specific function relationship of this compound and to develop a general idea of the activity of the same in the similar compounds, structural investigations of these types of compounds were undertaken.
The role of X-ray crystallography as the most effective and potential tool for structural analysis of molecules needs no elaboration. In the year 1912 the brilliant discovery of X-ray diffraction by Von Laue provided the fundamental knowledge and paved the way for the fantastic research activities to follow. Laue established the regular internal structure of crystals and demonstrated the wave nature of X-rays by sending a beam of X-ray through a crystal. An ideal crystal is an array of atoms, molecules or ions where a basic pattern repeats itself exactly at regular intervals in space. The condition of diffraction by a crystal depends upon the intervals at which the pattern repeats in space and these patterns are considered to be a set of scattering points on space lattice. When a train of monochromatic waves sweep through a set of scattering particles arranged in a space lattice, the waves reflected by successive lattice planes will not be in phase. In 1913 W. L. Bragg, who made the historic break through in the field of structural studies by X-rays, propounded that the reinforcement of phase would take place at certain angles which satisfies the simple condition

\[ n\lambda = 2d \sin \Theta \]

Where \( \Theta \) is the glancing angle and \( \lambda \) is the wave length of radiation, \( n \) is an integer and \( d \) is the interplanar spacing. The condition, known as Bragg's law of diffraction is incidentally a simultaneously fulfilment of three Laue equations. It is often difficult to visualize diffraction relation in terms of diffraction of X-ray. The relation, however, can easily be interpreted by having recourse to reciprocal lattice, a concept developed and expanded by Ewald in 1921. The correlation between the diffraction pattern and the arrangements of atoms is not a straightforward one. The intensity of diffraction depends on the energy of X-ray reflected by various crystal
planes. If an infinitesimally small block of a rotating crystal exposed to a radiation of intensity $I_0$ per square centimetre reflects X-rays, the total energy reflected is given by

$$E = \int \frac{I_0 \lambda^2 N^2 A}{2A} \, dv \left( \frac{e^2}{n^2 c^2} \right) L P |F|^2$$

where $e$, $m$ = charge and mass of an electron respectively

$c$ = Velocity of light

$I_0$ = Intensity of incident radiation

$\lambda$ = Wavelength of X-rays

$N$ = Number of unit cell unit volume

$dv$ = A small volume of crystal

$t$ = time of exposure

$L$, $P$ = Lorentz and polarisation factors respectively

$F$ = Structure factor.

The crystal may be regarded as a continuous distribution of electron density reaching maxima at the atomic centre and falling off asymptotically to zero and these atoms in group form the elements of the unit cell of the crystal.

In general the diffraction from each element of the unit cell will be the Fourier transform continuous throughout the reciprocal space. The value of Fourier transform, called the structure factor ($F_{hkl}$) is given by

$$F(hkl) = \sum_{j=1}^{N} \exp (2\pi i r_j \cdot \mathbf{S})$$

where $N$ is the total number of atoms and $f_j$ is the scattering factor for $j$-th atom, $r_j = X_ja + Y_jb + Z_jc$ and $S = ha^* + kb^* + lc^*$. 
Instead of considering a crystal as constituted of discrete atoms with the structure factors $f_i$, the appropriate electron density is given by the expression $P(r_j) = \frac{1}{V} \sum \sum \sum P_{hkl} \exp(-2\pi i r_j S)$ which reduces to $P(r_j) = \frac{1}{V} \sum \sum \sum P_{hkl} \cos 2\pi r_j S$ for centrosymmetric crystals.

Phase Problem:

One of the most intriguing problems in crystal structure analysis is that of the phases of $F(hkl)$, the knowledge of which is lost in recording their intensities. This is known as phase problem and irrespective of any method of structural determination by X-ray diffraction, the problem resolves into one of designing pragmatic approaches for phase determination of the diffraction spectra. In general the phase problem in X-ray crystallography may be attacked and resolved in two ways. In the first method it may be treated as a physical problem of recognizing the structure of an object directly from its X-ray Fraunhofer diffraction pattern. In the alternative approach, commonly known as direct method, the phase problem is presented in the form of a purely mathematical problem utilizing sophisticated computational facilities. Several methods used for phase determination are discussed below.

Patterson Function:

In 1935 Patterson (9) proposed an approach which did not solve the problem but helped to get information that could be obtained from structure amplitude only. He used square of the structure factors as Fourier coefficient and defined a function $P(u, v, w)$ as

$$P(u, v, w) = \sqrt{\int \int \int \hat{Q}(x, y, z) \cdot \hat{Q}(x+u, y+v, z+w) dx dy dz}.$$ 

This synthesis actually located peaks corresponding to all the interatomic
vectors where $\rho(x, y, z)$ and $\rho(x+u, y+v, z+w)$ are the electron densities at $x$, $y$, $z$ and $x+u$, $y+v$ and $z+w$. A molecule of $N$ atoms will produce $N^2$ peaks in which $N$ are located at the origin and $N^2-N$ non-origin peaks distributed throughout the cell. The height of each peak is the product of atomic numbers of the two atoms at the ends of the vectors. The peak corresponding to two atoms having higher atomic numbers than the rest will, therefore, figure prominently compared with the other in the background. In general this approach, however, is not sufficient for complex organic structures containing a number of light atoms where the vector peaks can hardly be interpreted.

Heavy atom method:

The Patterson function can be used to solve a structure fairly directly when the crystal contains a relatively small number of heavy atoms of scattering factor $f_H$. The Patterson peak due to these atoms stand out against the back drop of overlapping smaller peaks corresponding to the lighter atoms of scattering factor $f_L$. If $f_H$ is too high or small compared to $f_L$, some uncertainties arise in the structure determination. The probability of $F_H$ and $C_H$ having same or opposite signs are given by

$$P = \exp \left[ - \left( \frac{|F_H| \pm |C_H|}{\sqrt{2}} \right)^2 \right]$$

where $C_H$ is the contribution of heavy atom and $\sum' = \int f^2_j$. A rule of thumb working well is given by $\sum f^2_H = \sum f^2_L$, which indicates that the average contributions of the light and heavy atoms to intensity should be equal. Woolfson (10) showed that a Fourier synthesis calculated with weighted $F'$s using the coefficient $|F_H| \times (2P+1)\times (\text{Sign of } C_H)$ gives greatest signal to noise ratio in showing up the light atoms. Sim (10a) introduced a parameter
for centric crystal and suggested a weighting scheme for non-centric crystal.

Isomorphous replacement method:

Heavy atom technique can also be successfully applied to the case where two or more crystals have same structure but contain different heavy atoms.

The intensity differences between the substituted and unsubstituted crystal act as powerful aid to phase determination. Let two crystals A and B containing heavy atoms H₁ and H₂ respectively, consist of two parts corresponding to scattering effect due to light and heavy atoms when

\[ F_A = F_L + F_{H1} \]
\[ F_B = F_L + F_{H2} \]

\( F_L \) being same for both the crystals.

From above it follows that \( F_B - F_A = F_{H2} - F_{H1} \) when the contribution of \( F_{H1} \) and \( F_{H2} \) are known for heavy atom positions, the right hand side of the above relation turns into \( \pm (|F_B|) - (|F_A|) \). The values corresponding to the four possible sign combinations can be calculated and the combination which gives a clearly superior agreement to the equation \( F_B - F_A = F_{H2} - F_{H1} \) is accepted. For non-centric crystals phases are determined with the help of phase circle diagram and the method is widely used now a days for solving macromolecular structures.

Anomalous Dispersion Method:

The phenomenon of anomalous behaviour of X-rays near the absorption edge of a scattering atom has been utilised in determining the phases of...
reflection. X-rays suffer anomalous phase shift slightly in advance of the normal phase near the absorption edge making anomalous scattering factor complex. Thus it can be represented by

\[ f = f^0 + \Delta f' + i \Delta f'' \]

where \( f^0 \) is the normal scattering factor, \( \Delta f' \) is a real correction term and \( \Delta f'' \) is the imaginary component, both the terms \( \Delta f' \) and \( f'' \) being dependent on \( \chi \). It can be shown that for non-centrosymmetric cases

\[ |F(hkl)|^2 \neq |F(h\tilde{k}l)|^2 \]

which stands in violation of Fridle's law. This gives rise to intensity difference between (hkl) and (h\tilde{k}l) which is taken advantage of for phase determination. Recently this method along with the isomorphous replacement one has been of great help in protein crystallography.

**Trial and error method:**

The methods discussed so far involve heavy atom technique. Sometimes, however, it is found impossible to obtain a heavy atom derivative, also the formation of such derivatives alter key features of the structure. The method of crystal structure analysis in such cases have to consider several factors such as the physical properties of the crystal, the nature of intensity distribution, crystal symmetry etc. The approach, which may be termed as the trial and error method, essentially consists of postulating a structure in the unique part of the cell, calculating structure factor \( F_c \) and comparing their magnitude with the observed values \( |F_o| \). A prior knowledge of habit, cleavage, refractive index, atomic size, symmetry element of the crystal etc. are prerequisites for postulating a structure. In addition pertinent information of stereochemistry and packing of the molecules
in the unit cell help in arriving at the solution of a structure.

In recent times computational facilities are utilised to explore various ways of packing for obtaining sensible intermolecular distances and structure factor agreement. When a structure thus solved is stereochemically satisfactory and chemically meaningful, a value for $R$, the disagreement factor, given by

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

is calculated out where the summation extends over all the reflexions. For appreciably small values of $R$, the postulated structure is supposed to be correct. Some idea regarding the position of a molecule can be obtained from general survey of intensities when a few very strong reflections are present.

**Fourier Transform:**

The idea that the diffraction pattern for a given distribution of scattering matter may be derived from a corresponding distribution in reciprocal space find its precise and elegant expression in terms of Fourier transform theory which may be regarded as the X-ray diffraction pattern of a crystal produced by the superposition of Fourier transform of the contents of the unit cell. The transform cancels everywhere except at the reciprocal lattice points where they add up together and, therefore, the amplitude associated with any reciprocal lattice point is proportional to the amplitude of the transform at that point. X-ray diffraction pattern of a crystal may be regarded as the Fourier transform sampled at the reciprocal lattice point and on this concept is based the Fourier transform theory as applied to X-ray diffraction. When the Fourier transform of the molecule is known, the process of determining its orientation and position in the unit cell corresponds to
finding the pattern in which the reciprocal lattice fits on the transform so that it picks up the correct intensity for X-ray reflection.

The method, however, is suitable for planar molecules and its application was more or less limited till the production of transform by simple physical procedure was devised by Lipson and Taylor (11) who derived an analogy between the diffraction of X-rays by atoms and that of light by holes. The projection of unit cell of a structure on to a plane can be represented by a set of holes on an opaque mask. The diffraction pattern due to a set of holes called optical transform can be derived by optical diffractometer. For rapid comparison, however, through optical diffractometry it is found useful when there are some representation of the relative intensities of the reflections on the reciprocal lattice. The graphic representation is obtained by drawing at each point a spot whose size represents the structure factor and the best result is obtained when unitary structure factors are used. If the unit cell of the crystal contains two heavy atoms, they will produce fringes analogous to Young's fringes which are alternately positive and negative from the centre outwards.

From this fringe system, structure factors can be assigned phases. Another important component is the regular hexagon of benzene ring, the simplest transform of which the most prominent features are six strong peaks at a distance of $0.8 \AA$ from the origin. When this is recognised on the weighted reciprocal lattice, the orientation of the benzene ring in the crystal is immediately characterized.

**Direct method:**

On the plausible assumption that the intensities contain all the necessary informations, it is reasonable to accept that the structure should
also be obtainable from the idea of the intensities. Harker and Jasper (12) first utilized this concept and led to work out their inequality method in course of structural investigations of decaborane.

The authors showed that the inequality relationship existed between the structures and is capable of giving phase informations. The main advantage of the method being that it is completely independent of any previous knowledge about the structure. The approach rests on two very simple ideas, namely, that any structural complex comprises of atoms and electron density can never be negative. From the above ideas Harker and Kaspr deduced inequality relationships involving amplitudes whose indices are related in specified ways. Utilizing Cauchy's inequality relationship, the resultant inequality follows as

$$\sum_{\rho}^{2} \leq 1$$

and

$$\sum_{\rho} \rho = \frac{F_{\rho}}{\sum_{j=1}^{n} f_{j}}$$

For a centrosymmetric case the relationship assumes the form

$$\sum_{\rho}^{2} = (1 + \sum_{2n})$$. The above inequality relationship holds for structure factor having all indices zero. To ascertain the signs of the other, it is applied to the sum of two reflections $h$ and $h'$ and the resulting expression becomes

$$|\sum_{\rho} + \sum_{\rho'}|^{2} \leq (1 + \sum_{\rho} + \rho') (1 + \sum_{\rho'} + \rho')$$

Sayre (13) devised a new set of relationship based on the fact that for a crystal having equal atomic structure, $\rho(x)$, $\rho(x)^{2}$ are very much similar differing only in peaks which are sharper in the latter than the former. If $f$ be the scattering factor of atoms and $g$ be the same for the squared atom, the following relation known as Sayre's relation is obtained.
\[
C_h = \frac{2}{\sqrt{V}} \sum F_{h'} F_{h-h'}
\]

Where \( \Theta_h = \frac{f}{(g \nu)} \)

If the product \( F_h, F_{h-h} \) be large enough to affect the summation, \( F_h \) may develop a sign similar to that of the product. The summation may be expressed as

\[
S(h) S(h') S(h-h') \simeq 1
\]

This is called the triple product sign relationship. When the structure factors are too small to decide inequality information, a probability relationship is calculated which was simultaneously put forward by Sayre, Cochran (14) and Zachariasen (15). The same relationship by Cochran and Woolfson (16) is given by

\[
P_+ (h, k) = \frac{1}{2} + \frac{1}{2} \tanh \left\{ \frac{\epsilon^2}{2|U_h U_k U_{h-k}|} \right\}
\]

Where \( \epsilon_m = \sum_{j=1}^{n} r_j \)

Zachariasen successfully applied this approach to solve the structure of HBO\textsubscript{2} and used letter symbols to which arbitrary signs were assigned. Hauptman and Karle (17) applied direct method, may be called a statistical approach, for phase determination. In this context, the term normalised structure factor \( E_h \) may be defined as

\[
E_h = \frac{U_h}{\langle |U|^2 \rangle^{1/2}}
\]

Where \( \langle |U|^2 \rangle^{1/2} \) is the root mean square value of unitary structure factor. Thus \( \langle E^2 \rangle = 1 \) for any space group and the distribution of \( E \)'s for both
centric and non-centric crystal is independent of the structural complexity. The typical formulae were deduced considering Miller indices as fixed and the atomic coordinates uniformly distributed.

For $P'I$, these formulae are

\[
\sum_{1} = S(E_{2h}) \propto S(E_{h}^{2-1}) \\
\sum_{2} = S(E_{h}) \propto S \left\{ \sum_{h-h'} \frac{E_{h-h'}}{2\pi} \right\} \\
\sum_{3} = S(E_{h}) \propto S \left\{ \sum_{h-h'} \left( \frac{E_{h-h'}}{2\pi} - 1 \right) \right\} \\
\sum_{4} = S(E_{2h}) \propto S \left\{ \left( E_{h}^{2-1} - 1 \right) \left( E_{h+h}^{2-1} - 1 \right) \right\}
\]

It is evident that TPSR i.e.

\[ S(h) S(h') S(h-h') = 1 \text{ in the special case} \]

\(S(2h) S(h) S(h) = 1\) can give indication that if $E_{h}$ and $E_{2h}$ are both large $S(2h)$ is +ve.

The various methods for the efficient application of TPSR and different tests of their figure of merit were devised during early sixties. Karle and Karle (18) proposed a figure of merit based on Sayre's relation

\[ R_{K} = \frac{\sum_{h} |E_{h}| - K |\langle E_{h}^{k'} E_{h-h'}^{k'} \rangle_{h'}}{\sum_{h} |E_{h}|} \]

where $K$ is an empirically determined smooth function of $\sin \frac{\Theta}{\lambda}$ which gives least value of $R_{K}$.

The relationship widely used nowadays for structure determination of non-centrosymmetric crystal applying direct method is given by
Refinement of the Structure:

Refinement of a crystal structure is usually undertaken when a plausible and stereochemically satisfactory model of the structure has been obtained. In the refinement process the set of atomic parameters most consistent with the observed intensities are determined, latter being free from systematic error like extinction, absorption etc. and subject only to random error of measurement. The agreement between observed and calculated structure factors mentioned earlier gives the measure of correctness of the structure. Different methods of structure refinement are discussed below.

Fourier method:

The main advantage of the method is that it provides information which is not given in the input. Fourier synthesis computed with observed amplitudes and calculated phases manifest the strong peaks at the position of input along with some other weak ones which may correspond to correct peaks. For noncentric crystals, successive Fourier syntheses are calculated till no sign change of $F_c$ occurs.

A combined method of a few cycles of least squares refinement with the calculation of Fourier syntheses enables one to determine the correct structure.

Difference Synthesis:

The $(F_0-F_c)$ or $(F_o-F_c)$ synthesis (first developed by Cochran (19)) differ from the previous one by the fact that in this approach $(F_0-F_c)$ instead
of $F_0$ is taken as the amplitude. When the proposed model matches exactly with the actual crystal structure, the difference map is characterised by flat topography at the final stage of refinement. Since series termination errors are substantially identical, more accurate atomic coordinates can be derived from the difference map. If an atom requires a small correction $\xi$ for its correct location, $\xi$ may be obtained from curvature and slope of the atomic position using the relation

$$\xi = \Delta r = \frac{\delta \left( F_0 - F_c \right)}{\delta r}$$

where $\Delta r$ is the distance measured in the direction in which $(F_0 - F_c)$ was measured, and $\delta r$ represents the true electron density at the atomic centre. The hydrogen atoms can be located with the help of $(F_0 - F_c)$ synthesis.

Differential synthesis:

Booth (20) showed that correct atomic position could be derived from a knowledge of the first derivative or the slope of electron density function at the assumed atomic position and the second derivative or curvature at the atomic centres which are obtained by expanding the slope $\frac{\delta^2}{\delta x^2}$, $\frac{\delta^2}{\delta y^2}$, and $\frac{\delta^2}{\delta z^2}$ as Taylor series. Neglecting the second and higher order powers at the point of maximum density,

$$\left. \frac{\delta^2}{\delta x^2} = \frac{\delta}{\delta y} = \frac{\delta}{\delta z} \right|_{\text{max}} = 0$$

If $\Delta x$, $\Delta y$, $\Delta z$ represent small deviations from the assumed positions of $xyz$, the correction for exact position is the solution of the equations.

$$\frac{\delta^2}{\delta x^2} \Delta x + \frac{\delta^2}{\delta x \delta y} \Delta y + \frac{\delta^2}{\delta x \delta z} \Delta z + \frac{\delta}{\delta x} \Delta x = 0$$

For spherically symmetric electron density and orthogonal axes, the correction
term is given by
\[ \Delta \chi = \left( \frac{\partial^2 \mathcal{F}}{\partial \chi^2} \right) \text{etc.} \]

Much computational effort may be spared by following Booth's (21) suggestion of evaluating the curvature from the symmetric Gaussian form of the electron density distribution of an atom such as
\[ \rho(r) = Z \left( \frac{\rho_0}{r} \right)^3 e^{-p r^2} \]
where \( \rho(r) \) is the electron density at a distance \( r \) from the centre of the atom, \( Z \) is the atomic number and \( p \) is a constant differing in different atoms.

In all the three procedures described above, \( F_0 \) and differential synthesis give results not free from serious termination effect. In \( \triangle F \) synthesis, accounts for the anisotropic temperature effect while \( F_0 \) synthesis do not take into account scale change or temperature factor.

**Least square refinement:**

It follows from the theory of error that if the error in the measured quantity obey the normal or Gaussian law, the best parameters for \( n \) number of observations are those which result in a minimization of the quantity
\[ D = \sum_{n=1}^{n} W_{\chi} \left( f_{\text{or}} - f_{\text{cr}} \right)^2 \]
where \( W_{\chi} \) is the weight to be assigned to an observation, \( f_{\text{or}} \) is one of the \( n \) observed values of the function, \( f_{\text{cr}} \) is the corresponding calculated value.

In case of crystal structure analysis, it is
\[ R = \sum W_{h} \left( |F_0| - |F_c| \right)^2 \]
\[ = \sum W_{h} \Delta^2(hkl) \]
where $\Delta (hkl)$ is the difference between the observed and calculated structure factors and the summation extends over all the reflections. Let $u_1, u_2, \ldots, u_n$ be the number of parameters occurring in the $|F_c|$ value to be determined. These parameters comprising of isotropic and anisotropic vibration parameters, site occupation factors, atomic coordinates, scale factors etc. When $R$ is minimum

$$\frac{\partial R}{\partial u_j} = 0 \quad (j = 1, \ldots, n)$$

i.e. $\sum W(hkl) \Delta (hkl) \frac{\partial |F_c|}{\partial u_j} = 0$

This leads to the following $n$ equations

$$\sum W(hkl) (|F_o| - |F_c|) \frac{\partial |F_c|}{\partial u_j} = 0 \quad (j = 1, \ldots, n)$$

Now expanding $F_c$ by Taylor series and neglecting the second and higher order terms we finally obtain

$$\sum W(hkl) \frac{\partial |F_c|}{\partial u_i} \frac{\partial |F_c|}{\partial u_1} \Delta u_i + \sum W(hkl) \frac{\partial |F_c|}{\partial u_1} \frac{\partial |F_c|}{\partial u_n} \Delta u_n$$

$$= \sum W(hkl) (|F_o| - |F_c|) \frac{\partial |F_c|}{\partial u_1} \Delta u_1$$

These equations can be solved to find the values of $\Delta u_i$'s. Thus using the new approximate corrections to the parameters of $F_c$, the calculation is repeated until there is no significant change in the parameters between successive cycles.
The coefficient of the normal equation can be represented by matrix notations. The diagonal elements of this coefficient matrix are sums of squares which are always positive and the accumulative sum is large. The off diagonal elements, however, are sums of products, which may be either positive or negative and their sum therefore, reasonably smaller than that of the diagonal element. When all the off diagonal terms are small relative to the diagonal ones, they are neglected and only diagonal terms are calculated. This is known as block diagonal least square refinement. Least square refinement of full matrix leads to more rapid convergence than block diagonal methods. In fact one cycle of full matrix refinement is equivalent to three cycles of block diagonal least square refinement and yields a more accurate estimate of standard error.

Refinement by least square method has certain advantages over other methods of refinement. It is free from serious termination error and it is possible to refine temperature and scale factor by this method which, however, is not possible with the Fourier refinement method. Moreover, a suitable weighting scheme can be applied during the process of refinement to avoid unreliable Fo's.