Refinement of the Structure

Three dimensional refinement of the structure was carried out by the method of least squares. As the errors in the observed structure factors were taken to be of Gaussian type, the function minimised in the least squares calculation was 

$$
\sum \omega (|F_0| - |F_c|)^2
$$

where the summation is over all the reflections and \( \omega \) is the weighting factor, i.e., a factor usually taken as inversely proportional to the square of the probable error of the corresponding \( |F_0|'s \). Due to limitation of the memory locations of the computer, the program employing block diagonal approximation was used in the least squares computation. Thus 3 x 3 matrices corresponding to three positional parameters for each atom and either 6 x 6 or 1 x 1 matrices respectively for anisotropic or isotropic thermal parameters were used. The overall scale factor applied to \( F_0 \) was refined by forming a 2 x 2 matrix which took care of the interaction between the scale factor and the overall isotropic temperature factors. Schomaker's correction was applied to shifts of the individual temperature factors for each atom. Thus, if \( \Delta g' \) be the overall isotropic temperature factor, change calculated from 2 x 2 matrix when interaction with \( \Delta K \) is allowed for and if \( \Delta g'' \) be the overall isotropic temperature factor change calculated from a 1 x 1 matrix then for isotropic temperature factors...
\[ B_{\text{corrected}} = B_{\text{apparent}} + \Delta B' - \Delta B'' \] (73)

and for anisotropic temperature factors

\[ \Delta B_{ij \text{corrected}} = \Delta B_{ij \text{apparent}} + (\Delta B' - \Delta B'') \frac{R_{ij}}{\lambda^2} \] (74)

The weight factor actually used in our computation was not the one defined above, as it is common experience that almost all the previous methods of weighting give the positional parameters which differ by amounts smaller than the random error in the final coordinates. In this case, unit weight was given to all reflections except the unobserved and highly intense reflections. These reflections were excluded from the initial stages of refinement. The scattering factors for Cl\(^{-}\), O, N and C atoms were taken from the international tables of X-ray crystallography (1960).

After four cycles of least squares refinement with isotropic temperature factors, the \( R \) value came down to 0.21. The isotropic refinement was stopped at this stage as the shifts in atomic parameters were found to be fairly smaller than their estimated standard deviations. At this stage the data were checked for extinction effects. It was found that no low order reflections had their \( |F_o|^2 \) much higher than the corresponding \( |F_o| \) values.
The refinement was carried on CDC 3600 computer at the Tata Institute of Fundamental Research, Bombay. The modified least squares program of Busing, Martin and Levy was used for this purpose. Using this program it is possible to perform successive cycles of refinement using the full matrix of the normal equations. The parameters that can be adjusted include several scale factors, an overall temperature factor, individual atom multipliers, atomic coordinates and individual isotropic or anisotropic temperature factors. The isotropic temperature factors for each atom obtained so far were converted to the corresponding anisotropic form using the expression,

\[ \beta_{ii} = B_i a^* a / 4; \beta_{22i} = B_i b^* b / 4; \beta_{33i} = B_i c^* c / 4 \]

\[ \beta_{12i} = B_i a^* b^* \cos \gamma^* / 4 \]

\[ \beta_{23i} = B_i b^* c^* \cos \alpha^* / 4 \]

\[ \beta_{13i} = B_i a^* c^* \cos \beta^* / 4 \]

(75)

where \( \beta_{ii} \), \( \beta_{22i} \), \( \beta_{33i} \) etc. are the anisotropic temperature factors for the \( i^{th} \) atom, \( B_i \) is the isotropic temperature factor of the \( i^{th} \) atom, and \( a^* \), \( b^* \), \( \alpha^* \), \( \beta^* \), \( \gamma^* \) are the reciprocal lattice vectors and angles of the unit cell. Full matrix anisotropic refinement was then carried out with these parameters. The observed \( F_0 \)'s were divided into nine groups corresponding to nine different layers of data collected and each of these groups was given a scale factor which was subjected to
refinement, so that the error due to interlayer scaling was minimised. All the reflections were included in this refinement. Unit weight was assigned to each reflection. The initial $R$ value was 0.21 and after two cycles of anisotropic refinement it dropped to 0.11.

Location of Hydrogen Positions

A three dimensional Fourier synthesis i.e. $(F_o - F_c)$ synthesis, was calculated to this stage with all the reflections using Zadkin's 'Fordap' program. The composite drawing of the resulting map as viewed down c-axis is shown in figure 7. The difference Fourier map showed up all the hydrogen atom peaks at the positions postulated from stereochemical considerations of the hydrogen bonding scheme. The heights of electron density peaks corresponding to hydrogen atoms varied from 0.25 $e/A^2$ to 0.45 $e/A^2$. The rest of the difference Fourier map showed nearly flat regions with a few small heights near the heavy atom positions.

All the hydrogen atoms were located from the difference Fourier map and were included in the subsequent refinement of the structure. The temperature factors assigned to the hydrogen atoms were the isotropic temperature factors of the heavier atom to which they were bonded. The structure factors were now calculated with the contributions of hydrogen atoms included. The $R$-value at this stage was found to be 0.095. After one cycle least squares refinement using anisotropic temperature factors
Fig. 7. Morpholine biguanide HCl: Composite drawing of the three dimensional difference Fourier projected down 0 axis. The contours are drawn at the intervals of 0.1 e/A³ starting from 0.1 e/A³.
for non-hydrogen atoms and isotropic temperature factors for hydrogen atoms, it came down to 0.087. With unobserved reflections included, $R$ becomes 0.094. Further refinement was stopped at this stage as the shifts in parameters were found to be much smaller than their e.s.d.'s and also the previous cycle had not shown a significant fall in the $R$-value.

The standard errors (e.s.d.'s) of all the atoms were calculated. The standard error $\sigma$ of a parameter $f(p_i, p_j)$ is given by,

$$
\sigma^2 = \sum_{i=1}^{n} \sum_{j=1}^{n} C_{ij} \left( \frac{\delta f}{\delta p_i} \right) \left( \frac{\delta f}{\delta p_j} \right) V_{ij}
$$  \hfill (76)

where,

$$
C_{ij} = 1, \text{ if } i = j
$$  \hfill (77)

$$
C_{ij} = 2, \text{ if } i \neq j
$$

$V_{ij}$ is an element of the variance covariance matrix which describes the errors of atomic parameters

$$
V_{ij} = \left[ \omega (OBS - CALC)^2 / m - n \right] b_{ij}
$$  \hfill (78)

where $b_{ij}$ is an element of the inverse matrix of the normal equations of the last least squares refinement and constant within bracket is the weighted sum of the squares of residuals divided by the number of degrees of freedom. The errors in the unit cell parameters were not calculated and hence could not be
included in the calculation of errors in atomic parameters.

The estimated standard deviation of a bond distance A-B was calculated using the expression,

\[ \sigma^2(A-B) = \cos^2 \theta (\sigma^2 \chi_A + \sigma^2 \chi_B) \]
\[ + \cos^2 \phi (\sigma^2 y_A + \sigma^2 y_B) \]
\[ + \cos^2 \chi (\sigma^2 \epsilon_A + \sigma^2 \epsilon_B) \]

where,

\[ \cos \theta = \left[ (AB)^2 + (\Delta x)^2 - (\Delta y)^2 - (\Delta z)^2 \right] / 2 (AB)(\Delta x) \]
\[ \cos \phi = (\Delta y)/(AB) \]
\[ \cos \chi = \left[ (AB)^2 + (\Delta z)^2 - (\Delta y)^2 - (\Delta x)^2 \right] / 2 (AB)(\Delta z) \]

The estimated standard deviation of a bond angle ABC was computed using the expressions,

\[ \sigma^2 \theta = \left[ \sigma^2 A / (AB)^2 \right] + \sigma^2 B \left[ 1 / (AB)^2 - 2 \cos \theta / (AB)(BC) \right. \]
\[ \left. + 1 / (BC)^2 + \left[ \sigma^2 c / (BC)^2 \right] \right] \]

where,

\[ \sigma^2 A = (\sigma^2 x_A + \sigma^2 y_A + \sigma^2 z_A) A / 3 \]
\[ \sigma^2 B = (\sigma^2 x_B + \sigma^2 y_B + \sigma^2 z_B) B / 3 \]
\[ \sigma^2 c = (\sigma^2 x_c + \sigma^2 y_c + \sigma^2 z_c) c / 3 \]
Different steps in the solution of the structure and its refinement are sequentially given below in a tabular form:

<table>
<thead>
<tr>
<th>Successive steps</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Chlorine atom positions from $(010)$ and $(001)$ Patterson projections</td>
<td>Not successful</td>
</tr>
<tr>
<td>(ii) Location of chlorine atoms from 3-D Patterson synthesis</td>
<td></td>
</tr>
<tr>
<td>(iii) Approximate structure from three dimensional weighted Fourier</td>
<td>$R = 0.23$</td>
</tr>
<tr>
<td>(iv) Full matrix least squares refinement with interlayer scaling and isotropic temperature factors for individual atoms (4 cycles)</td>
<td>$R = 0.32$ $0.28$ $0.25$ $0.22$ $0.21$</td>
</tr>
<tr>
<td>(v) Full matrix anisotropic refinement (2 cycles)</td>
<td>$R = 0.21$ $0.14$ $0.11$</td>
</tr>
<tr>
<td>(vi) Three dimensional difference Fourier and location of hydrogen atoms</td>
<td>$R = 0.095$</td>
</tr>
<tr>
<td>(vii) Full matrix refinement with anisotropic temperature factors for non-hydrogen atoms and isotropic temperature factors for hydrogen atoms.</td>
<td>$R = 0.087$ $R = 0.094$</td>
</tr>
</tbody>
</table>

With the unobserved reflections included
Fig. 8. Morpholine biguanide HCl : Composite drawing of the three dimensional Fourier projected down 0 = 0. The contours are drawn at the intervals of 1 e/A³ starting from 1 e/A³ in case of C, N and O and at the intervals of 2 e/A³ starting from 2 e/A³ in case of chlorine.