An Improved Method for Calculating the Contribution of Solvent to the X-ray Diffraction Pattern of Biological Molecules

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Solvent makes a significant contribution to the diffracted intensity of crystals and fibres of large biological molecules. Allowance for this contribution must be made in the refinement of trial structures and an improved method is described for the calculation of solvent scattering.

Crystals and fibrous assemblies of large biological molecules generally contain an appreciable proportion of mobile aqueous solvent. The solvent makes a significant contribution to the diffracted intensity, particularly at small angles of diffraction (Bragg & Perutz, 1952), and allowance must be made for this contribution when calculating structure factors intended for use in the refinement of trial structures. The effect of the solvent can be simulated by supposing that the volume occupied by the solvent is filled with an electron gas with a density equal to the mean electron density of the solvent (Wrinch, 1950). The corrected structure factor can then be calculated, using Babinet’s principle (Langridge et al., 1960), from the expression

$$F' = F - v_0 F_v,$$  \hspace{1cm} (1)

where $F_c$ is the molecular structure factor calculated in the usual way, $v$ is the volume of solvent displaced by the molecule, $q_0$ is the mean electron density of the solvent and $F_v$ is the normalized Fourier transform of a solid of uniform density with a shape corresponding to the displaced solvent. Expression (1) has been applied directly (e.g. Fraser, MacRae & Miller, 1965; O’Brien & MacEwan, 1970) but a simpler approach is to express $F_v$ as the summation of the transforms of elementary volumes around all the atoms in the molecule. The correction term can then be incorporated into the individual atomic scattering factors. If $v_i$ is the volume displaced by the $i$th atom then the modified atomic scattering factor is

$$f'(D) = f(D) - v_i q_0 F_i(D),$$  \hspace{1cm} (2)

where $D$ is a radial coordinate in reciprocal space, and $F_i$ is the normalized Fourier transform of a solid of uniform density with a shape corresponding to the volume around the atom from which solvent is excluded.

Expression (2) has been widely used to compensate for the effect of solvent with the assumptions that the excluded volume is spherical and that $v_i$ can be calculated from the van der Waals radius of the atom (e.g. Arnott & Hukins, 1973; Okuyama, Tanaka, Ashida & Kakudo, 1976). In the formula given by Fuller (1961)

$$f'(D) = f(D)\left[1 - 10r^3 \Phi(D)/nr^3\right]$$  \hspace{1cm} (3)

where $r$ is the van der Waals radius of the atom; $r_w$ is the radius of a water molecule, taken to be 2 Å; $n$ is the number of electrons in the atom and $\Phi(D)$, the normalized Fourier transform of a sphere, is given by

$$\Phi(D) = 3\left[\sin (2\pi rD) - 2\pi rD \cos (2\pi rD)\right]/(2\pi rD)^3.$$  \hspace{1cm} (4)

Fig. 1. Comparison of the atomic scattering factor for carbon (full line) with values corrected for displaced solvent according to expression (2). The assumption of a Gaussian sphere of volume equal to the observed displacement (broken line) gives the correct value for $D = 0$ and a smooth reduction in correction value with increasing $D$. The assumption of a uniform sphere (dotted line) with radius equal to the van der Waals radius gives an oscillatory correction term which in most cases overcorrects for $D = 0$. The van der Waals radius for carbon was taken as 1.7 Å (Bondi, 1964) and the corrected curves were calculated for a solvent density of 0.334 e Å$^{-3}$, which is appropriate to water at 20°C.
(b) The use of \( \Phi(D) \), based on a spherical volume, is unrealistic and introduces an oscillatory component into the corrected atomic scattering factor (Fig. 1). We suggest therefore that the correction term would be improved by using a Gaussian sphere, defined by

\[
g(r) = \exp \left[ -\left(\frac{r}{r_0}\right)^2 \right]
\]

which has a volume \( v = \frac{3}{2}r_0^3 \). The normalized Fourier transform of the Gaussian sphere is

\[
F(D) = \exp \left( -\pi v^{2/3}D^2 \right)
\]

and so expression (2) becomes

\[
f'(D) = f(D) - v_0g \exp \left( -\pi v^{2/3}D^2 \right)
\]

(c) A third problem concerns the use of van der Waals radii for the estimation of the volume of solvent displaced. The volumes so calculated from representative sets of van der Waals radii are compared in Table 1 with the actual volume of solvent displaced (Traube, 1899; Partington, 1951; Zamyatnin, 1972) and it will be seen that the value is grossly overestimated. Thus the volume of solvent displaced by a leucine residue, for example, would be estimated from column 3 in Table 1 to be 233 Å³ compared with the observed value in protein structures of 168 Å³ (Chothia, 1975) and the value of 167 Å³ estimated from Traube's values (Table 1). We therefore suggest that the atomic volume appropriate to the correction term is the observed volume of solvent displaced by the atom. This latter procedure appears to have been employed, at least in part, in the special correction terms calculated for DNA by Langridge et al. (1960).

The atomic scattering factor for carbon is compared in Fig. 1 with the factor obtained by applying expression (2) firstly with the spherical approximation and displacement volume calculated from the van der Waals radius, and secondly with the Gaussian spherical approximation and the observed displacement volume. It is difficult to assess quantitatively the improvement in the representation of solvent scattering obtained by the latter method. As some indication the following values of the \( R \) factor were obtained in connection with a linked-atom least-squares refinement of the polypeptide chain conformation in hydrated collagen. Correction of the atomic scattering factors using the spherical approximation and the van der Waals radii given by Bondi (1964) gave an \( R \) value of 0.38; correction using the spherical approximation and the observed atomic volumes gave an \( R \) value of 0.32; and the correction using the Gaussian sphere approximation and the observed atomic volumes gave an \( R \) value of 0.28.

References


Traube, J. (1899). Quoted by Partington (1951).


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Table 1. Comparison of volumes of solvent displaced by polypeptide main-chain atoms estimated from van der Waals radii compared with observed values

<table>
<thead>
<tr>
<th>Calculated volume</th>
<th>Observed volume</th>
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<tbody>
<tr>
<td>(Å³)</td>
<td>(Å³)</td>
</tr>
<tr>
<td>(d)</td>
<td>(b)</td>
</tr>
<tr>
<td>H</td>
<td>7.24</td>
</tr>
<tr>
<td>C</td>
<td>20.58</td>
</tr>
<tr>
<td>N</td>
<td>14.14</td>
</tr>
<tr>
<td>O</td>
<td>11.49</td>
</tr>
</tbody>
</table>

References: (a) Okuyama et al. (1976); (b) Arnott & Hukins (1973); (c) Bondi (1964); (d) Traube (1899), Zamyatnin (1972).