X-ray Diffraction Studies of Aqueous Solutions of Urea

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An X-ray diffraction method has been used to study the influence of solute concentration on molecular associations in aqueous solutions of urea. Calculations, from experimental intensities, of unsharpened electronic radial distribution functions (RDF) are presented for the range of urea concentration from 0-83 to 16-8 mol Kg⁻¹ (5 to 100°o solutions). The changes in RDF with increasing urea concentration are complex. Unequivocal interpretation of the distribution functions is not possible, but the major effect of the relatively bulky urea molecules on the water structure appears to be caused by distortion of the water matrix. At urea concentrations of 50°o and 100°o, features of the RDF not visible in the RDF's at lower concentrations indicate the presence of long-range (over a distance of about 8 Å) structural relationships possibly due to urea-urea interactions; such an interpretation would make urea-urea hydrogen-bonded associations unlikely at lower urea concentrations.

Introduction

Aqueous solutions of urea, \( \text{CO(NH}_2\text{)}_2 \), which are of particular interest owing to their influence on the conformation of certain biological macromolecules in solution, have been studied using various techniques, including nuclear magnetic resonance (Finer, Franks & Tait, 1972) and dielectric relaxation (Grant, Keefe & Shack, 1972). The results of these investigations have been interpreted to suggest that, in solutions whose concentrations are in the range 1 to 8 mol dm⁻³, the formation of hydrogen bonds between urea molecules is unlikely.

This conclusion contrasts with that of Schellmann (1955), who, on thermodynamic grounds, considered the possibility of self-association of urea molecules and suggested that the observed departure of urea solutions from ideality reflects the extension of short-range order to either solute-solute or solute-solvent complexes or both. He concluded that solute aggregation based on the formation of hydrogen bonds is the more likely, since urea solutions have a negative heat of dilution. Evidence to support this model includes the results of Stokes (1965), who, on the basis of diffusion rate experiments using radioactively labelled urea molecules, considered that dimerization of urea molecules occurs, but that further polymerization is unlikely.

The present study was undertaken with a view to comparing X-ray diffusion measurements made at five solution concentrations in the range 0-83 to 16-8 mol Kg⁻¹ in an attempt to obtain further evidence about molecular association in aqueous urea solutions. An approach was adopted based on the calculation of unsharpened radial electronic distribution functions (RDF) by Fourier inversion of diffracted X-ray intensities; results from sharpened RDF's are also referred to for pure water. The unsharpened RDF corresponds to a spherically averaged Patterson function, which may be interpreted in terms of the summation of interatomic scattering contributions associated with each atom pair in the system (Klug & Alexander, 1974). Thus, structural changes associated with changing solution concentration may be observed as departures from the distribution of interatomic spacings found for pure water. The process of sharpening (Waser & Schomaker, 1953) has the effect of removing the average profile of the single-atom distributions of electron density; since the average changes with solution concentration, a comparative study of solutions using the unsharpened RDF approach is preferred.

Experimental procedure

Angular distributions of the intensities of X-rays diffracted by the solutions were recorded with a modified Philips parafocusing diffractometer (PW1050/25) equipped with a scintillation counter detector. Cu Kx X-rays were used with wavelength \( \lambda = 1.54 \) Å. The output from the counter was taken to a single-channel pulse-height analyser and then to a scaler. A fixed-count strategy was used during data collection. When the present number of counts had been accumulated in the scaler, counting ceased and the number of counts accumulated by a second scaler was punched on paper tape. This second scaler was used to measure the number of counts received by a second scintillation counter, set up to monitor the intensity of the X-rays incident upon the specimen. Monochromatization of the diffracted X-rays, in each counting channel, was achieved by the combination of a nickel filter in front of the detector and the pulse-height analyser. After each measurement, a stepping motor advanced the detector.
to the next angular position. Two angular ranges were used with an overlap region (a) \(2\theta = 4.1 \text{ (1) 140}^\circ\) and (b) \(2\theta = 1 \text{ (0-25) 15}^\circ\). The divergence and scatter slits used for the two ranges were respectively \(\frac{1}{4}\) and \(\frac{1}{3}\).

The counter which monitored the incident X-ray beam was used to compensate for fluctuations in the beam intensity; these arose from such causes as variations in the temperature of the water used to cool the X-ray tube. Preliminary experiments had indicated that long-term uncertainties in the angular intensity distributions could be considerably reduced when using the incident-beam monitor (Adams, 1975). The monitor detector collected X-rays which had been scattered by the divergence slit assembly with high intensity compared with the intensity scattered by the specimen. Consequently the statistical fluctuations in the monitor intensity were negligibly small. The solutions were contained on the diffractometer in a cell which was made by milling a cavity in a perspex block. The cavity was covered by a 0.001 inch polyethylene sheet. The cell was filled from external reservoirs. The temperature of the liquid was controlled by passing water, at a constant temperature, through a stainless-steel pipe which passed through the cavity, below the liquid surface. The cavity had a rectangular cross section of \(22 \times 15\) mm and a depth of 10 mm; this allowed the detection of X-rays diffracted through an angle as small as \(2\theta = 1\)°. The depth was great enough to prevent the detection of X-rays diffracted from the bottom of the cell when measurements were made at high angles. After the cell had been filled, the heights of the sample reservoirs were adjusted to minimize the pressure difference across the polyethylene cover; a flat sample surface minimizes departures from the conditions for the parafocusing geometry. Experimental requirements influencing the design and construction of the cell are described elsewhere (Adams, 1975).

For each sample 32000 counts were accumulated at each angle by means of two measurements of the intensity distribution in each of which 16000 counts were accumulated at every angle. The statistical error in counting was thus about \(\frac{1}{4}\)% and \(\frac{1}{2}\)\%

Experimental runs on each sample were repeated at least once. A sample temperature of \(40.0 \pm 0.5\)°C was maintained. In order to correct for stray scattering, cell-cover scattering, air scattering etc., the measurements were repeated with the cell empty, 1000 counts being accumulated at each angle. These background runs showed a number of sharp peaks in the diffraction pattern due to scattering by the partially crystalline polyethylene cover. Similar peaks were also present in the diffraction patterns of the samples but were completely removed on subtraction of the background, leaving the slowly varying intensity distribution characteristic of liquids.

Urea solutions were prepared from AnalR grade 99.7\% pure urea in glass-distilled water, immediately prior to the intensity measurements. Solution concentrations and measured bulk densities are given in Table 1.

### Table 1. Physical properties of the samples

<table>
<thead>
<tr>
<th>Specimen concentration (Mol kg(^{-1}))</th>
<th>Density (mg mm(^{-3}))</th>
<th>Linear absorption coefficient ((mm^{-1}))</th>
<th>Mean electron density ((\text{A}^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-00 (water)</td>
<td>0.99</td>
<td>1.026</td>
<td>0.334</td>
</tr>
<tr>
<td>0.83</td>
<td>1.01</td>
<td>1.023</td>
<td>0.338</td>
</tr>
<tr>
<td>2.49</td>
<td>1.03</td>
<td>1.020</td>
<td>0.341</td>
</tr>
<tr>
<td>4.20</td>
<td>1.05</td>
<td>1.020</td>
<td>0.348</td>
</tr>
<tr>
<td>8.4</td>
<td>1.09</td>
<td>1.019</td>
<td>0.360</td>
</tr>
<tr>
<td>16/8</td>
<td>1.15</td>
<td>1.019</td>
<td>0.375</td>
</tr>
</tbody>
</table>

**Data processing and calculations**

Reduced intensity functions were calculated with the expression

\[
i(s) = \alpha I_{m}(s) - I_{\text{coh}}(s) - I_{\text{inc}}(s),
\]

where \(s = 4\pi \sin \theta /\lambda\) and \(2\theta\) is the angle of scattering. \(I_{m}(s)\) is the measured intensity distribution from which the background scattering has been subtracted after correction for polarization, multiple scattering and absorption, both in the cover and the sample; \(I_{\text{coh}}(s)\) is the coherent intensity from the specimen due to intra-atomic interference, which is independent of relative atomic positions; \(I_{\text{inc}}(s)\) is the incoherent Compton scattering from the specimen, which is also independent of the structure; \(\alpha\) is a scaling factor used to place the measured intensities on an absolute scale to allow the subtraction of the structure-independent components. \(I_{\text{coh}}(s)\) and \(I_{\text{inc}}(s)\) were calculated from analytic functions fitted to theoretical values for atomic scattering factors (Cromer & Waber, 1965) and Compton scattering intensities (Balyuzi, 1975) for the various elements in the samples with the equations

\[
I_{\text{coh}}(s) = \sum_{i=1}^{M} x_i f^2_i(s), \quad I_{\text{inc}}(s) = R \sum_{i=1}^{M} x_i f^{inc}_i(s).
\]

\(R\) is the Breit–Dirac recoil factor, \(x_i\) is the mole fraction of atoms of type \(i\) and \(M\) is the total number of atom types. The scaling factor \(\alpha\) was calculated in two ways, (a) by a high-angle intensity matching procedure (Klug & Alexander, 1974) and (b) with the method of Krogh-Moe (1956) and Norman (1957). Similar values for \(\alpha\) were obtained from the two methods, the differences normally being about 4\% with a maximum difference value of 9\%; usually the value from (a) was used and any error was reduced by an intensity-function refinement procedure to be described below. The absolute intensity scale to which the experimental intensities were normalized was established by calculating the independent intensity for a structural unit, in which the number of atoms of a given type was the mole fraction of atoms of that type.

Absorption corrections were made with the expressions given by Milberg (1958) for weakly absorbing samples. A further correction was made for the absorption of X-rays in the cell cover. The linear absorption coefficients needed for these corrections were calculated with the expression
where \( \rho_s \) is the specimen density and \( w_i \) and \( (\mu/\rho_i) \) are respectively the weight fraction and the mass absorption coefficient for atoms of type \( i \) (see Table 1). Corrections for multiple scattering were applied by the method of Dwiggins & Park (1971).

From the experimental reduced intensity functions, unsharpened and sharpened electronic radial distribution functions \( g(r) \) and \( g'(r) \) were calculated from the expressions

\[
g(r) = 4\pi\int_0^{s_{\text{max}}} s i(s) \sin rsds,
\]

and

\[
g'(r) = 2\int_0^{s_{\text{max}}} s i(s)M(s) \sin rsds,
\]

respectively. Here \( s_{\text{max}} \) is the upper limit in reciprocal space to which the diffracted intensities were measured, \( \varrho \) is the mean electron density of the sample (see Table 1) and \( M(s) \) is a modification function (Waser & Schomaker, 1953) taken as \( \left( \sum_{i=1}^{M} f_i(s) \right)^{-2} \).

A method of systematic refinement, based on that of Kaplow, Strong & Averbach (1965), was used to minimize spurious features in the reduced intensity and distribution functions which arise from causes such as inaccurate scaling factors and absorption corrections. This is an iterative procedure for removing peaks in the small-\( r \) region of the RDF by forcing the experimental function to a straight line of slope \( -4\pi\varrho \) for those values of \( r \) for which \( r < r_{\text{min}} \), where \( r_{\text{min}} \), which was taken as 0.7 Å, is less than the shortest possible interatomic distance in the sample. Successive Fourier inversions were performed with the quadrature method of Filon (1928).

The effect of the process of refinement is shown in Fig. 1 by a comparison of the calculated RDF for water before and after refinement. Considerable alteration to the RDF occurs for \( r < \sim 1.5 \) Å, and smaller effects are found throughout the curve, e.g. the ripples across the peak centred at about 4.5 Å found in the raw data are virtually removed on refinement. The reproducibility of both the measurements and the data processing is shown by comparing the refined curve of Fig. 1 with Fig. 2(a), which, with intensity data from an independent experiment, is also a refined unsharpened electronic RDF for water. There are differences at small \( r \), especially in the depth of the minimum at \( r \sim 1.5 \) Å. Further, there is a shoulder on the RDF of Fig. 2(a) at about 1.2 Å and also ripples on the peak at \( \sim 4.5 \) Å, neither of which occur in Fig. 1. Physically, differences between the curves arise as a result of factors such as the change in diffractometer alignment between the two runs and deviations of the cell surfaces from plane parallel. The major differences occur in those regions most affected by the process of refinement, but the principal features of the RDF for water are clear in both curves in relation to first and higher near-neighbour separations. It is of interest that Narten, Danford & Levy (1967) present their results for the RDF for water only for \( r \geq 2 \) Å.

**Results**

Refined unsharpened RDF's for water and for five concentrations of urea (0.83 to 16.8 mol Kg\(^{-1}\): 5 to 100% solutions in terms of g urea in 100 g water) are shown in Fig. 2(a) to (f).

The significant maxima in the RDF for pure water are centred at 2.95 Å, 4.5 Å (with peak at about 4.1 Å) and 7 Å, corresponding to the well-defined first neighbour distance and higher neighbour separations. To compare these results with the corresponding sharpened data of Narten, Danford & Levy (1967) for water at 4°C, sharpened RDF's were produced for the two sets of results of Figs. 1 and 2(a). Three effects were introduced on sharpening: reduction in width of the peaks of the RDF, displacement of the first neighbour separation to \( \sim 0.03 \) Å, and the superposition of minor ripples on the peaks centred at 4.5 Å and 7 Å. The agreement with the results of Narten et al. is good, if account is taken of the larger value of \( s_{\text{max}} \) for Mo K\( \alpha \) radiation as used by them.

Fig. 2 shows that the principal effects on increasing the urea concentration are (i) alterations to the ~3 Å peak corresponding to first neighbour interactions in water, (ii) progressive fusing together, as the urea concentration increases, of the peaks corresponding for water to the first and second neighbour separations, (iii) the progressive decrease in prominence with increasing urea concentration, and alteration in profile...
for urea concentrations of 50% and above, of the peak centred at 7 Å.

The effect of the reducing mole concentration of water relative to urea must be considered in respect of points (i) and (iii) and the positions of the peaks of the distribution functions for different concentrations in connexion with point (ii). The positions and heights of the first three maxima derived from the results of Fig. 2 are shown in Table 2; the reproducibility in peak height from run to run was better than 10%. The changes in peak position indicate the result of the overlapping of distributions of atomic separations with features changing in relative weight. The peak positions serve to identify the peaks and the positions where the peak heights have been measured; the movements of the peaks carry little significance.

The progressive fall in peak height of the ~7 Å peak with increasing urea concentration, within experimental error, corresponds to the decreasing water mole fraction regarded as free water, for the 5, 15 and 25% solutions. For each concentration of urea a multiplying factor $K$ is given in Table 2, which may be used to multiply the given peak heights to produce values which, if the water in the solutions were free water, would produce a water RDF on the same scale as Fig. 2(a). For the urea solutions of 50 and 100% concentration, the half width of the ~7 Å peak is greater than the values for lower concentrations and the peak value has moved to higher spacing. For the 100% urea solution, the peak value is reduced in magnitude by about 50% below the value corresponding to the mole fraction of water present.

The peak of the distribution corresponding in pure water to the second neighbour distance, after an initial small increase for 5% urea solutions, remains remarkably stable in terms of height, position, and peak profile towards large radius, over the entire range of increasing urea concentration.

**Discussion**

The changes in the RDF for water on addition of urea are complex. At low concentrations of urea there is a large reduction in the height of the ~3 Å peak, which corresponds to O...O hydrogen bonds in water–water association, and may also have contributions from urea–water and urea–urea hydrogen-bonding interactions. For the 5% urea solution, which corresponds to 67 water molecules per urea molecule, the first neighbour peak is reduced to 78% of the peak height for water. This reduction is four times as large as can be explained in terms of the reduced water mole fraction of the solution in relation to pure water, even making the assumption that no interactions involving urea contribute to the first-neighbour peak. As the urea concentration increases above 5% the further decrease in height of the ~3 Å peak is relatively small, the reducing number of O...O hydrogen bonds for water due to both the reducing water mole fraction and the effect of the urea being partly counterbalanced by increasingly important interactions involving urea; the two peaks at ~3 and ~4.5 Å progressively merge with increasing urea concentration, confusing the interpretation of height measurements of the first-neighbour peak.

<table>
<thead>
<tr>
<th>Concentration (mol kg⁻¹)*</th>
<th>Nominal position 3.0 Å Height position (e² Å⁻²) (Å)</th>
<th>Nominal position 4.1 Å Height position (e² Å⁻²) (Å)</th>
<th>Nominal position 7.0 to 8.0 Å Height (e² Å⁻²)</th>
<th>Multiplying factor $K$ (see text)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 (water) (0)</td>
<td>5.9 2.95</td>
<td>2.65 4.1</td>
<td>2.75 1.75</td>
<td>1.05</td>
</tr>
<tr>
<td>0.83 (5)</td>
<td>4.6 3.00</td>
<td>3.30 4.1</td>
<td>3.65 3.75</td>
<td>1.14</td>
</tr>
<tr>
<td>2.49 (15)</td>
<td>4.1 3.00</td>
<td>3.60 4.1</td>
<td>3.75 4.75</td>
<td>1.23</td>
</tr>
<tr>
<td>4.2 (25)</td>
<td>3.6 3.90</td>
<td>3.60 3.95</td>
<td>3.65 3.75</td>
<td>1.46</td>
</tr>
<tr>
<td>8.4 (50)</td>
<td>Not resolved</td>
<td>3.50 3.90</td>
<td>3.75 3.75</td>
<td>1.91</td>
</tr>
<tr>
<td>16.8 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The percent concentration (g urea in 100 g water) is given in parentheses.
The experimental data allow one extreme model for the molecular association in aqueous solutions to be immediately discarded. This is a model where independent water–water and urea–urea interactions occur. The observed changes in RDF with urea concentration are not consistent with the addition of two component RDF’s of constant shape and varying relative weighting.

Narten et al. (1967) define the extent of order in water in terms of the radial extent of significant maxima and minima on a sharpened RDF before the onset of the continuum. The present results show that for urea concentration up to 25%, the peak centred at ~7 Å for water is little affected by the presence of urea, when allowance is made for the water mole fraction: at higher concentrations, interactions involving urea are significant in the region of this peak. There is no clear difference in the total radial range of order with change of urea concentration. The order extends over a range of about 10 Å for water at 4 °C (Narten et al., 1967).

The large reduction in the height of the first-neighbour peak, below that corresponding to the mole fraction of water present in, for example, 5%, urea solution, must be almost entirely at the expense of reduced numbers of O–O hydrogen bond interactions between water molecules, since the additional numbers of O–O and N–O links involving possible urea–water and urea–urea interactions with similar spacings (indistinguishable because of the effect of overlapping of the ~3 Å and the ~4.5 Å peaks) will be relatively small. The reduction in nearest-neighbour hydrogen bonding in water is presumably due, in major part, to the geometrical distortion of the hydrogen-bonded water matrix by the relatively bulky molecules of urea, which a priori may be single molecules or hydrogen-bonded associations. The ratio of the heights of the ~3 Å and ~7 Å peaks, which is about 0.36 for water, increases to a constant value of about 0.46 for each of the 5, 15 and 25%, urea solutions for which the ~3 Å peak can be clearly distinguished. In addition the height of the ~7 Å peak remains virtually constant in accordance with the water mole fraction for this concentration range. Urea–water relationships are consequently required that affect the first neighbour, r ~ 3 Å, interactions, but have relatively little effect on the longer range interactions, r ~ 7 Å, until the urea concentration exceeds 25%.

In some way the relative importance of interactions at separations corresponding to the second-neighbour peak for water centred at ~4.5 Å is preserved over the entire range of urea concentration. However, we hesitate to speculate further on the interpretation of the RDF, especially as Narten & Levy (1969) have pointed out that even the first coordination sphere for water is complex, i.e. the first-neighbour peak, which is reasonably discrete, cannot be described by a single Gaussian distance distribution.

Returning to the question of models for the urea–water system, the principal disrupting effect of urea on the water matrix at low concentration seems to be geometrical. At higher urea concentrations, in the range 50 to 100%, changes in the RDF for r ~ 7–8 Å show that the effect of the urea extends beyond short-range interactions – that is, beyond predominantly nearest-neighbour interactions – to longer ranges, although the total extent of ordered structure, as indicated by significant features of the RDF, seems to be independent of the urea concentration. The results do not give a direct indication as to whether the urea molecules are distributed singly throughout the water matrix, or in, for example, hydrogen-bonded dimers. The changes in the RDF with increasing urea concentration are systematic for urea concentrations from 5 to 25%, with a change in behaviour emerging at higher concentrations especially concerned with longer-range interactions. This could be interpreted to indicate that the principal interactions involving urea at low concentration are N–O and O–O urea–water hydrogen bonds, while, at high concentration, interactions occur involving the longer-range urea–urea vectors of a hydrogen-bonded urea association. On this basis, the two models of either unassociated or associated urea molecules in aqueous solution would have validity according to the urea concentration. This possibility is not inconsistent with the results of Finer et al. (1974) and Grant et al. (1972) since the present results extend the range of concentration considered by these workers; in the region of overlapping concentration the results would be equivalent, apart from the relatively small departure shown by the X-ray results for the 50% solution from an extension of the behaviour of the solutions of lower concentration.

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References


