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New Advances in Neutron Diffraction Studies of Molecular Aqueous Solutions

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Neutron scattering studies have played a major role in improving our understanding of the structures not only of simple single component liquids, but increasingly of mixtures and solutions. In addition to the improved quality of structural information available from neutrons (*cf* X-rays) resulting from the Q-independent neutron scattering factor and the ability to obtain high resolution information through access to high momentum transfer Q, the use of isotopes allows information at the partial correlation function level to be obtained. Using new pulsed spallation neutron sources such as ISIS at the UK's Rutherford Appleton Laboratory, problems of recoil corrections which create difficulties when studying liquids containing light atoms – *e. g.* hydrogen – can be effectively overcome, opening up new areas of structural studies in aqueous solutions of chemical and biological importance. An example is described of recent work on the TMA Cl-water system, which has given us for the first time a detailed picture of a non-polar hydration shell, and information on both the expected ordering of the waters in this shell, and its perturbation by protein denaturants such as urea that are thought to operate through disturbing such hydration structures. Future work promises to throw much new light on the subtle balance between the competing polar and non-polar interactions that are central to biomolecule stability.

INTRODUCTION

Aqueous solutions are systems of major chemical interest. Water itself is a standard medium for chemical reactions, and contrary to the early assumptions, the solvent itself may play a significant role in such reactions. Many industrial processes use water, so its solvent properties are of importance economically. Moreover, biomolecular interactions often take place in a largely aqueous medium; an understanding of complex aqueous solutions is therefore of potential value in trying to understand biological processes.

Not surprisingly, an immense amount of data on the properties of a huge range of aqueous solutions has been collected. Particularly prolific have been *thermodynamic*

and *spectroscopic* measurements, which have helped us understand the intermolecular interactions that take place in solution. In contrast, reliable *structural* data on aqueous solutions is sparse, and is only recently becoming available to a significant degree through neutron scattering studies. Inferences concerning structure have often been drawn from thermodynamic and spectroscopic data. However, as structural conclusions drawn from such »indirect« data require the use of an interpretive model, these should in general be treated with reserve; experience has frequently shown that the structural conclusions drawn may be strongly influenced by the model used.

Direct methods of structural investigation of solutions are available which do not depend in any significant degree on an interpretive model. Extensive use of X-ray and neutron scattering has been made in studies of relatively simple liquid and glassy systems, such as liquid metals and simple alloys, metallic alloy glasses, and silicate glass. However, for reasons related to the complexity of the systems, their use until recently for studying aqueous solutions in general has been very limited.

We can understand the reason for this by considering briefly the information available from a liquid scattering experiment. Considering first a single component liquid, the information obtained from the scattering factor – the structure factor S(Q), where Q, the momentum transfer, is defined in terms of scattering angle θ and wavelength λ as $Q = 4\pi/\lambda \sin \theta/2$ – is related to the structure through a Fourier transform. The structural information obtained is the pair distribution function $g(r)$, which tells us the probability of finding an atom at a distance r from any other atom in the liquid.

For a multicomponent system such as an aqueous solution, the situation is more complex. Whereas in the single component case only a single pair correlation function $g(r)$ is needed to describe the structure, for a system containing several different kinds of atoms, we need in principle to know several *partial* pair correlations $g_{ij}(r)$, which give the probability of finding an atom of type j at a distance r from an atom of type i. Thus, for a two component system (*e. g.* water) three such functions are required (in the case of water $g_{OO}(r)$, $g_{OH}(r)$, $g_{HH}(r)$), while for an aqueous electrolyte (*e. g.* $ZnCl_2$), ten such functions are required for a full structural description.

This is clearly a tall order, and so many functions cannot be obtained from a single scattering experiment. Such an experiment would give information on the weighted sum of all the relevant pair correlation functions, each one being weighted by terms relating to the atomic concentrations and (X-ray or neutron) scattering powers of each component. In most aqueous solutions of interest, where water is the major component, the concentration terms will dominate, and the correlation function obtained will be essentially that of the water solvent. The information we really want on, say, the hydration of the solute or the distribution of the solute molecules, will be masked by the solvent signal.

A way round the problem is provided by neutrons. The central point in favour of neutrons is that the neutron scattering power of a nucleus depends *not* – as in X-rays – on the number of electrons, or its atomic number (*i. e.* its chemical nature) but on the nature of the scattering *nucleus*. Thus, two isotopes of the same element may scatter differently, although the two isotopically-distinct solutions on which two parallel scattering experiments can be made are chemically similar. Using such isotope substitutions it is therefore possible to perform two (or more) neutron scattering experiments, in which the weighting factors of those pair correlation functions that involve the substituted nucleus are changed. Subtracting the two data sets results in all correlation functions *apart from those involving the substituted nucleus* cancelling out.

The interested reader is referred elsewhere for the formal statement of this isotope substitution difference technique,[1,2] which was developed originally in application to electrolyte solutions by Enderby and coworkers.[1] The method in effect allows us to »sit on« an atom of interest in an aqueous solution, and measure a partial correlation function from this atom to its surroundings. For example, using molecules containing ^{14}N and ^{15}N, we can sit on the nitrogen atom in a solution of acetamide, and measure the distribution of distances from the nitrogen to the surrounding atoms (largely the water molecules). We thus obtain information on the hydration of the molecule from the vantage point of the isotopically substituted atom on the acetamide molecule.

We can, using H/D substitution, take the technique even further in one of two ways. First, noting that the neutron scattering powers of H and D are not only different, but of different sign (–0.37 and +0.67, respectively), by suitably mixing H and D on solvent water molecules, we can vary the contribution of the hydrogen to the correlation function seen (in the acetamide case) from the nitrogen. Consequently, we can identify those regions of the pair correlation function that arise from water hydrogens – only those pair distances related to the hydrogen atoms will be affected by the hydrogen substitution – and thus obtain information on water *orientations* in the acetamide hydration region. Secondly, by performing measurements on *three* solutions in which the H_2O:D_2O ratios are different (*e. g.* 100:0; 50:50; 0:100) we can, as demonstrated by Soper and Silver[3] obtain detailed structural (including orientational) information on the water structure itself in an aqueous solution of arbitrary complexity. Thus we can observe how water structure may be perturbed by, for example, the nearby presence of non polar groups, or the addition of molecules thought from other techniques to be »structure breakers«.

THE TETRAMETHYLAMMONIUM SYSTEM

As an illustration of the power of the technique, we summarise below the results obtained so far on a system which has been studied extensively by many other techniques. Rather than repeat here the experimental details and detailed arguments which are presented elsewhere,[2] we limit ourselves to stating the results, and discussing briefly some interesting possible conclusions relating to the so-called hydrophobic interaction often thought central to biomolecular stability and interactions. The system exampled is the tetramethylammonium ion [$N(CH_3)_4^+$] in aqueous solution as chloride over a range of concentrations from 0.5 m (100 water molecules per solute) to 4.0 m (12 1/2 water molecules per solute). Although TMA is charged, the surface presented to the water molecules is dominated by methyl groups. As these groups will to some extent shield the charge, it is not clear whether polar or apolar hydration would be expected. Thermodynamic data on aqueous solutions of larger tetraalkylammonium ions[4] suggest the existence of »water structure enforced ion pairing«,[5] while spectroscopic data imply that the water structure is »enhanced« close to the tetrabutylammonium ion.[6] However, thermodynamic data suggest TMA is less apolar and more ionic than the higher alkylammonium ions, so it is not clear from these indirect methods how the TMA system should hydrate.

The ultimate aim of this continuing investigation – which is a collaborative study with Dr Jacky Tumer of Birkbeck College, London, and Dr Alan Soper of the Rutherford Appleton Laboratory – is to obtain a full, structural characterisation of the solution to assist the interpretation of other experiments and theoretical work on this and related systems. We would like to understand the cation and anion hydration as well

as the ion-ion correlations, and in particular to see if the TMA hydration is of apolar or cationic character (in the former case we might expect something akin to a clathrate cage structure, while the latter would show water hydrogens pointing away from the TMA, as has been observed around many positive ions in solution[1]). Other points of interest include the balance of the charge on the cation and apolar driving forces in TMA interactions, the influence of the anion on the cation hydration, and the possible existence of solvent-enforced ion pairing. In addition, if an apolar hydration structure *is* observed, it would be interesting to probe the effects on the hydration shell of adding a »structure breaking« molecule such as urea, which is thought to act as a protein denaturant by breaking down apolar hydration structures.

To obtain information relevant to these questions, the following sets of isotope substitution experiments have been performed, at various concentrations between 0.5 and 4 m.

1. Nitrogen substitution on the TMA molecule in D_2O. This yields information on the nitrogen to water correlations, and hence the TMA hydration.
2. Nitrogen substitution on the TMA molecule in an H_2O/D_2O mixture (in this case 30% H_2O). As indicated above, this changes the size of the contribution of the nitrogen-hydrogen distances to the nitrogen-centred correlation function, and hence gives information on the solvent orientation, which should allow us to distinguish between apolar and cationic hydration mechanisms.
3. H/D substitution on the *solvent*, to extract information on the water orientational structure close to the exposed methyl groups. If an apolar hydration structure is observed, then conventional wisdom of the hydrophobic interaction suggests this hydration water should in some way be »more structured« than in the bulk. Extraction of $g_{III}(r)$ and $g_{OII}(r)$ using this technique should allow us to pick up any such ordering.
4. H/D substitution on the TMA solute will give information on the TMA-TMA correlation, and hence on any solvent-enforced aggregation effects that might occur if hydrophobic interactions operate significantly.
5. Repetition of experiments (1) and (3) but in a 2 m solution urea, may indicate whether or not urea has the expected effect of breaking in some way the hydration structure of TMA.
6. Chloride ion substitution will allow us to check if the Cl^- hydration is normal, *i.e.* similar to that found using similar techniques on stronger chloride-containing electrolytes.[8]

Figure 1 gives the results obtained from the first two nitrogen substitution experiments, and shows the nitrogen-centred pair distribution functions at three concentrations. Considering first the 1.91 m concentration in D_2O, we see a first peak at 1.47 Å, which corresponds to the intra-molecular N–C distance within the TMA moiety, as expected. This is followed by a deep negative peak at 2.10 Å, and again corresponds to an intramolecular distance, this time from the central nitrogen to the methyl hydrogens. The peak is negative because of the negative scattering power of the hydrogen. Within the expected errors (~10%), the magnitudes of these two peaks are consistent with 4 carbon atoms at 1.47 Å and 12 hydrogens at 2.10 Å. The fact that these values are correct gives us confidence also in our results; if the molecular structure is reproduced correctly, we can have confidence also in the data when we look at the hydration region further out. [Alternatively, for a flexible molecule, this isotope substitution technique may also be used to study molecular structure in – not necessarily aqueous – solution].

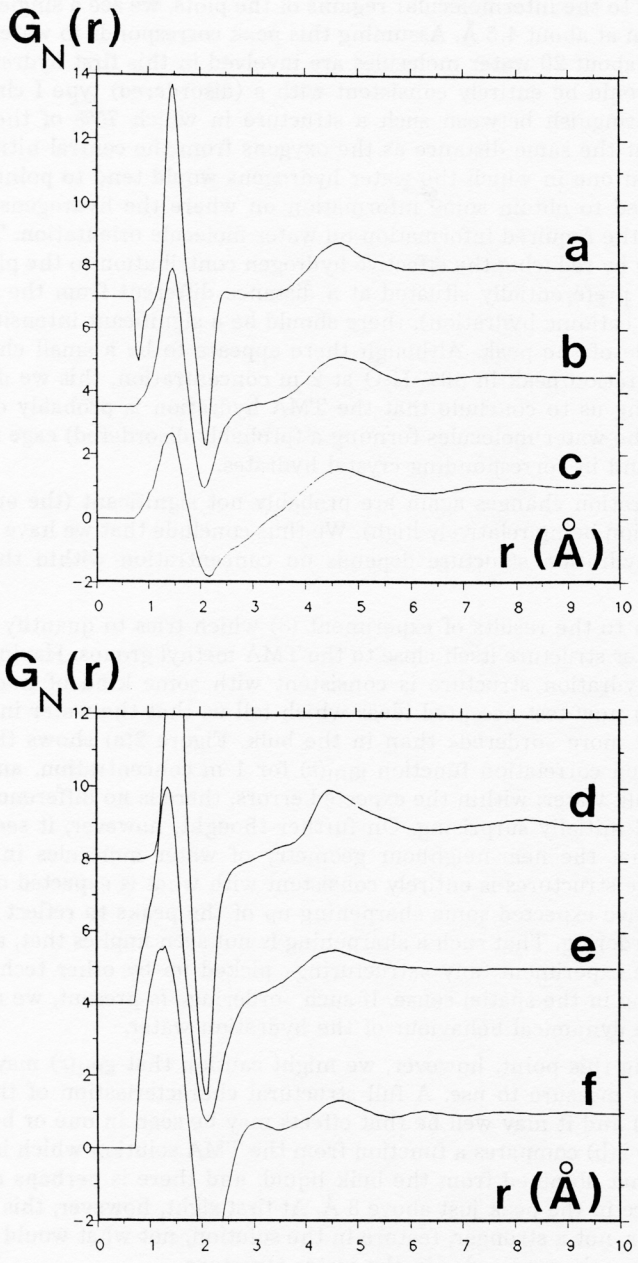

Figure 1. Partial pair correlation functions from nitrogen-isotope substitution: (a) 1.91 m, (b) 1.00 m and (c) 0.50 m TMACl in D_2O; (d) 2.00 m, (e) 1.00 m and (f) 0.50 m TMACl in 30% H_2O. Successive plots have been translated by increments of 3.5 units (a)–(c) and 4.0 units (d)–(f) on the y axis for clarity.

Turning now to the intermolecular regions of the plots, we see a single broad peak with its maximum at about 4.5 Å. Assuming this peak corresponds to water molecules, its area suggests about 20 water molecules are involved in this first hydration shell, a number which would be entirely consistent with a (disordered) type I clathrate cage structure. To distinguish between such a structure in which 75% of the hydrogens would be at about the same distance as the oxygens from the central nitrogen, and a cationic hydration one in which the water hydrogens would tend to point away from the TMA, we need to obtain some information on where the hydrogens are in this peak, and hence the required information on water molecule orientation. This is done in experiment (2) by reducing the effective hydrogen contribution to the plotted $G_N(r)$: if hydrogens are preferentially situated at a distance different from the oxygens (as they would be in cationic hydration), there should be a significant intensity reduction to the high r side of the peak. Although there appears to be a small change in the shape of the hydration peak in 30% H_2O at 2 m concentration, this we do not think significant, leading us to conclude that the TMA hydration is probably of non-polar character, with the water molecules forming a (probably disordered) cage structure similar to that found in corresponding crystal hydrates.

The concentration changes again are probably not significant (the errors on the 0.5 m concentration being relatively high). We thus conclude that we have no evidence that the TMA hydration structure depends on concentration within the range explored.

We now turn to the results of experiment (3) which tries to quantify any perturbation in the water structure itself close to the TMA methyl groups. Having concluded that the TMA hydration structure is consistent with some kind of non-polar cage structure, we can now test accepted ideas which tell us that the water in such structures is probably more »ordered« than in the bulk. Figure 2(a) shows the extracted hydrogen-hydrogen correlation function $g_{HH}(r)$ for 1 m concentration, and compares it with that in bulk water: within the expected errors, there is no difference. This conclusion we found initially surprising. On further thought, however, it seems entirely reasonable, in that the near-neighbour geometry of water molecules in pentagonal dodecahedral cage structures is entirely consistent with what is expected of water. We might perhaps have expected some sharpening up of the peaks to reflect some increased structural ordering. That such a sharpening is not seen implies that, again within the errors of the experiment, any »structuring« picked up by other techniques does *not* imply ordering in the spatial sense. If such »ordering« *is* present, we may need to look for it in the dynamical behaviour of the hydration water.

Before leaving this point, however, we might caution that $g_{HH}(r)$ may not be the most appropriate measure to use. A full structural characterisation of the water requires also $g_{OO}(r)$ and it may well be that effects may be seen in one or both of these functions. Figure 2(b) compares a function from the TMA solution which is dominated by $g_{OH}(r)$ with that obtained from the bulk liquid, and there is perhaps a suggestion of some difference in the peak just above 3 Å. At first sight, however, this comparison suggests a weaker, not a stronger, feature in the solution, not what would be expected were there of any enhanced order in the water structure.

While discussing water structure perturbations, it is perhaps appropriate here to consider the effects on the TMA hydration shell of adding 2 m urea to the TMACl solutions, *i. e.* experiment (5) above. Again, recalling the literature which suggests protein denaturants such as urea operate by disrupting hydration structures (by implication

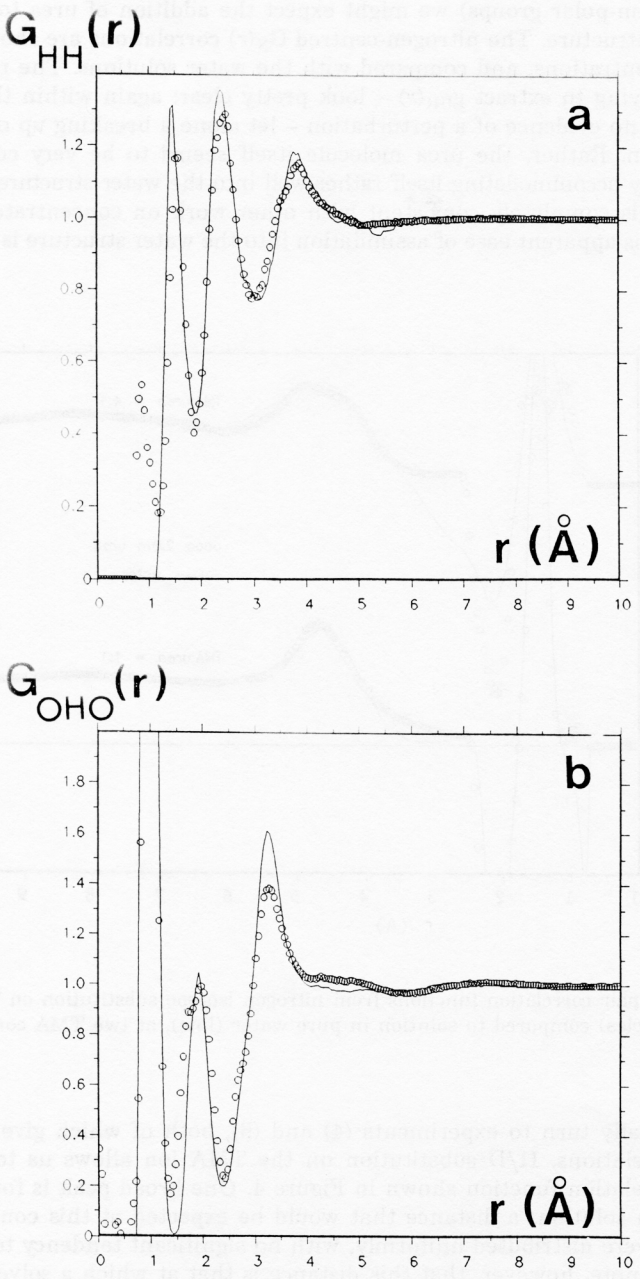

Figure 2. Partial pair correlation functions from H/D substitutions in the water for 1.00 m TMACl(O) compared with the result from pure water (—): (a) HH correlation function; (b) OH + OO correlation function after subtraction of a calculated intramolecular function for the TMA ion.

those around non-polar groups) we might expect the addition of urea to perturb our presumed cage structure. The nitrogen-centred $G_N(r)$ correlations are shown in Figure 3, for two concentrations, and compared with the water solutions. The results here – even without trying to extract $g_{IIII}(r)$ – look pretty clear: again within the errors expected, there is no evidence of a perturbation – let alone a breaking up of – the TMA hydration region. Rather, the urea molecule itself seems to be very comfortable in water, apparently accommodating itself rather well into the water structure. This tentative conclusion is completely consistent with other work on concentrated urea solutions,[7] where this apparent ease of assimilation into the water structure is also evident.

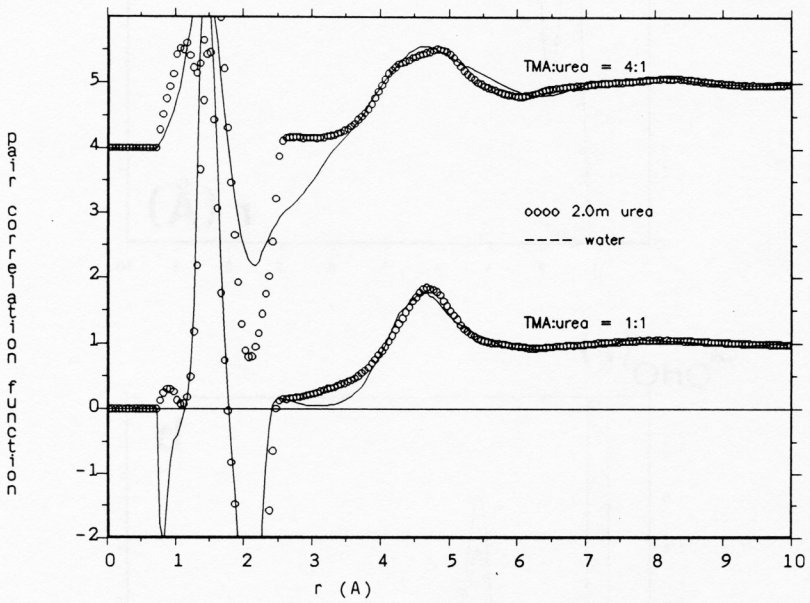

Figure 3. Partial pair correlation functions from nitrogen isotope substitution on TMA in 2.0 m urea solution (circles) compared to solution in pure water (line), at two TMA concentrations.

We now finally turn to experiments (4) and (6), both of which give information on ion-ion correlations. H/D substitution on the TMA ion allows us to extract the TMA-TMA correlation function shown in Figure 4. One broad peak is found at about 8.2 Å for a 4 m solution, a distance that would be expected at this concentration if the TMA ions were distributed uniformly, with no significant tendency to cluster. We should perhaps note, however, that this distance is that at which a solvent-separated pair of TMA ions would be found, with neighbouring water cages a sharing faces in common. Similar experiments at lower concentrations are needed before wider conclusions can be drawn about any solvent-involved driving forces that may tend to bring together two TMA ions.

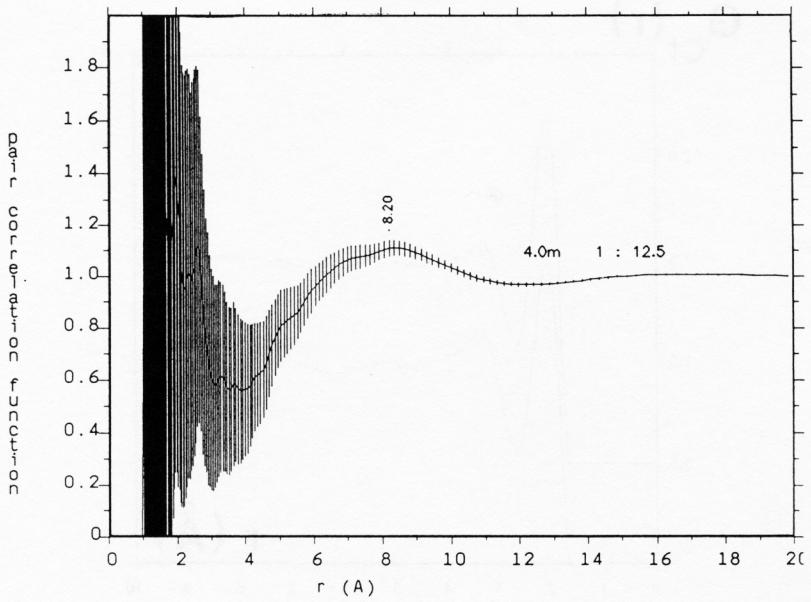

Figure 4. TMA-TMA pair correlation function in a 4.00 m solution in D_2O, obtained by H/D substitution on TMA. The vertical lines represent error estimates.

There is, however, further information on this possible TMA aggregation at lower concentrations available from considering the $G_N(r)$ (Figure 1). Were two TMA ions to begin to approach, water molecules in their hydration shells would begin to be displaced by methyl hydrogens from the approaching TMA. Again, recalling the negative scattering power of hydrogen, this would show itself as an apparent reduction in the 4.5 Å peak in the $G_N(r)$, an effect which is not observed. We conclude therefore that over this concentration range there is no observable tendency for TMA to aggregate.

Figure 5 shows the chloride-centred $G_{Cl}(r)$ correlation 2 m and 1 m concentrations. The first peak corresponds to nearest-neighbour atoms to the chloride ion, which here are expected to be deuterium atoms from hydrating waters. The coordination numbers extracted from these peaks are about 5 for both concentrations, a figure consistent with the 5–6 expected for a free chloride ion from related experiments on a variety of chloride-containing solutions.[8] Thus, this result of itself gives no evidence for either the contact or solvent-separated $N(CH_3)_4^+$ Cl^- ion pair suggested in other experiments.[5] However, if we look at the second peak at 3.3 Å, it is relatively lower and less well defined in the 2 m case. This may indicate a decrease in the order in the Cl^- hydration shell at this high concentration where the TMA and Cl^- ions would be close. Alternatively, remembering that the hydrogens on the methyl groups contribute negatively to the scattering, this change in the 3.3 Å peak may be evidence that the methyl groups of the TMA ion approach as close as 3.2 Å. This could be possible if the Cl^- ion began to be incorporated into the TMA hydration shell, or if the TMA and Cl^- formed an »ion pair« with a rather long N···Cl distance of about 5.3 Å, as has been suggested in a recent simulation study.[9]

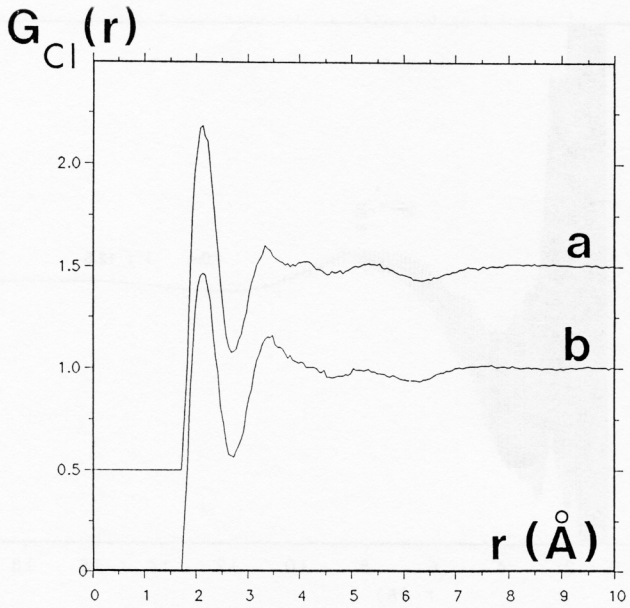

Figure 5. Partial pair correlation functions from chlorine-isotope substitution: (a) 2.00 m and (b) 1.00 m TMACl in D_2O. Plot (a) has been translated by 0.5 units on the y axis.

SUMMARY

The TMACl-water system is not only incredibly rich in the amount of thermodynamic and spectroscopic data available, and in the mixture of possible driving forces relevant to its behaviour. It is also rich in its potential for study neutron scattering. Both the cation and anion can be isotopically substituted, allowing access to the hydration structures of both ions, which – because of the spherical symmetries – can be relatively simply interpreted. In addition, the H/D substitution possible on the TMA gives a further weapon in probing cation-cation correlations, while the large negative scattering power of the methyl hydrogens could – through reducing the heights of the ion-centred pair correlation functions – give evidence of both cation-cation and cation-cation pairing. The lack of exchangeable hydrogens on the cation also facilitates measurement of solvent hydrogen-hydrogen correlations, which can be used to probe possible solvent perturbation around non-polar groups, and any »structure breaking« effects of denaturants.

Although work on this system is far from complete, we can already draw several tentative conclusions. First, TMA hydrates as an apolar molecule. The charge density on the ion seems to be insufficient to influence the water dipoles, and rather than a cationic hydration structure in which the hydrogens would point away from the ion, a disordered – probably clathrate-like – cage structure is found to be consistent with the data. There appears to be no significant change in hydration with concentration, although at high concentration there may be a tendency to cation-cation pairing, perhaps with (as found in *e.g.* TMAOH crystals) the anion becoming incorporated into

the hydration cage of the cation. Initially surprisingly, seen from the water's viewpoint, being part of the cation's hydration »cage« does not involve its structure being perturbed significantly from that in the bulk: structural »ordering« of water expected from years of literature speculation is just not observed. There is no evidence at any concentration up to 4 m for any tendency for TMA ions to aggregate, as might be expected if »hydrophobic« effects were operating significantly. Finally, adding 2 m urea – a protein denaturant thought to act through perturbing hydration structures, particularly around non-polar groups – does not significantly affect the hydration shell. Rather, urea seems to fit well into the water network, a conclusion consistent with other neutron studies of urea-water solutions.

We have concentrated here on the TMACl-water system as it illustrates the kind of experiments that can now be performed using current neutron sources, and the kind of detailed information that can be extracted without having to invoke models in interpreting the results. This system is, however, only one of many solutions of molecules containing charged, polar and apolar groups that can be tackled using these techniques. Other examples of work either performed or in progress includes aqueous solutions of amides such as formamide, acetamide and urea,[7] charged molecules such as methylamine (as hydrochloride), polar molecules like DMSO, and important bifunctional ones such as methanol. The field of interaction in solution is so wide that this represents only a very small fraction of the interesting problems that can be tackled using neutrons. The problems such experiments can help to solve are some of the most fundamental in both chemistry and biology.

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SAŽETAK

Novi napredak u studiju neutronske difrakcije vodenih otopina

J. L. Finney

Proučavanja neutronske difrakcije odigrala su glavnu ulogu u poboljšanju tumačenja strukture ne samo jednostavnih jednokomponentnih tekućina, nego sve više i smjesa i otopina. Osim poboljšane kvalitete strukturnih informacija dobivenih iz neutrona (u usporedbi s rendgenskim zrakama), koje rezultiraju iz faktora neutronskog raspršenja neovisnih o Q i sposobnosti dobivanja informacija s visokim razlučivanjem putem pristupa visokom momentu prijenosa Q, korištenje izotopa omogućava dostupnost informacija na razini djelomične korelacijske funkcije. Upotrebom novih pulsnih izvora neutrona s kalanjem, kao što su oni na ISIS u Rutherfordovu Appleton-laboratoriju u Velikoj Britaniji, mogu se djelotvorno nadvladati problemi korelacije uz-

mačnog odbijanja, koji uzrokuju poteškoće kada se proučavaju tekućine koje sadrže lake atome (npr. vodik), otvarajući novo područje strukturnih istraživanja u vodenim otopinama kemijskih i bioloških sustava. Opisan je primjer novijeg rada na sustavu TMACl–voda, koji je po prvi puta pružio detaljnu sliku nepolarnih hidratacijskih ljuski, te informacije o očekivanom rasporedu vode u ovoj ljuski i o smetnjama od strane denaturanata proteina, kao što je urea, za koje se smatra da remete takve hidratacijske strukture. Budući rad obećava da će rasvijetliti suptilnu ravnotežu polarnih i nepolarnih interakcija koje su u kompeticiji, te su središnji problem biomolekulske stabilnosti.