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Small angle neutron scattering, total cross-sections and mass density measurements of concentrated NaCl and KCl solutions in H_2O or D_2O

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Abstract

The mass densities, total cold neutron cross sections and small angle scattering of concentrated NaCl and KCl solutions in H_2O or D_2O (^2H_2O) were measured at 20°C. The partial specific volumes of both salts increase with salt concentration and are significantly smaller in D_2O than in H_2O, showing that these salt solutions cannot be considered as isomorphous in H_2O and D_2O. As salt concentration increases for both salts, the total coherent cross sections for neutrons of the solutions also increase while the coherent small angle scattering decreases–observations that are consistent, respectively, with increasing correlations involving the ion and water components and a decrease in the particle number density and/or concentration fluctuations, in the solutions. Changes in incoherent scattering with salt concentration are essentially those expected from the solution compositions and densities.

Keywords: Densimetry; Neutron scattering; Salt solutions; NaCl, KCl; Cold neutrons

1. Introduction

Protein solvent interactions play an important role in protein folding, stability and solubility and are significantly modified by the presence of salts [1]. High concentrations of guanidine hydrochloride, for example, destabilise proteins and have been used extensively in folding studies. An example of opposite behaviour is the stabilising and precipitating action of ammonium sulphate that has been known for over a century and is applied routinely in protein purification. Proteins have adapted in interesting ways to their solvent environments. Molar concentrations of NaCl and KCl significantly modify or inhibit protein–protein and protein–nucleic acid interactions in most organisms but there exist 'halophilic' proteins that function in a physiological environment almost saturated in KCl (> 3 M) and are unstable and unfold in salt concentrations below about 2 M [2].

In order to understand the essential interactions between proteins and their environment, it is clearly of interest to have a good understanding of the

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physico-chemical properties of the environment itself. Neutrons have advantages over X-rays for studies of concentrated salt solutions because of low absorption and favourable contrast values. Studies by neutron diffraction and specific isotopic labelling of ion correlations with surrounding water molecules and each other have shown the existence of aqua ions in solutions of various salts with similar geometries to those found in crystal hydrates, i.e. salt solutions have local structure [3,4]. The neutron diffraction method is very powerful and recent experiments on the correlations between O–H, O–O and H–H pairs in water at super-critical temperatures, have shown that water, itself, and the role played by the hydrogen bond in its structure are still not completely understood [5].

In the present work, the cold (10 Å) neutron scattering from concentrated NaCl and KCl solutions in H_2O or D_2O (^2H_2O) was measured in conditions similar to those of small angle neutron scattering experiments on biological macromolecules. With a 10 Å wavelength, the scattering is not sensitive to correlations over distances shorter than 5 Å. The data were interpreted in terms of a liquid scattering factor. The neutron scattering factor curve for D_2O is shown schematically in Fig. 1 [6]. A maximum scattering vector (Q) value of 1.2 $Å^{-1}$ is theoretically accessible with a wavelength of 10 Å but the range measured in the small angle scattering experiments was $0.015 \leqslant Q \leqslant 0.080$ $Å^{-1}$, i.e. at smaller Q values than measured for the curve in Fig. 1. The peaks in the scattering factor are related to correlations between molecules in the liquid and the value at $Q = 0$ is related by thermodynamics to the compressibility of the liquid. The total neutron cross-sections of the solutions at 10 Å were calculated from transmission measurements and their mass densities measured with a high precision densimeter. All experiments were performed at 20°C.

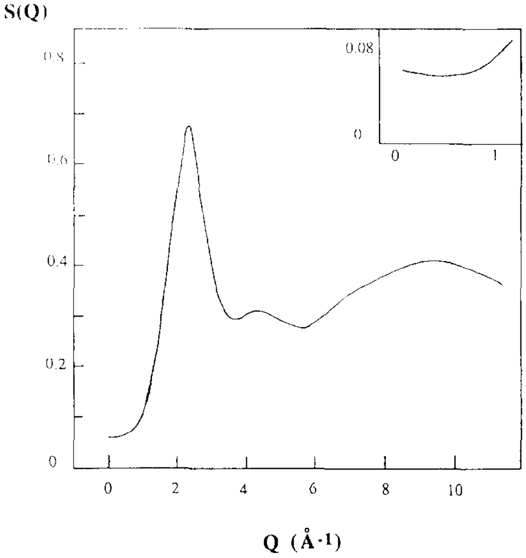

Fig. 1. Structure factor of heavy water from neutron scattering drawn schematically from Ref. [6].

2. Materials and methods

NaCl and KCl were analytical grade salts from Carlo Erba; they were dried at 110°C before use. Laboratory double-distilled H_2O was filtered and degased; its density at 20.0°C was taken as 0.998202 g/cm^3 (International Critical Tables). D_2O (99.5%) was filtered and degased and its density at 20°C measured to be 1.10602 g/cm^3.

All solutions were prepared in aquamolality m by weighing both the salt and the solvent (m is the number of moles of salt per 55.51 moles of either H_2O or D_2O).

Partial specific volumes were calculated from density measurements on a Mettler/Paar densimeter (DMA 602) equiped with a 700 μL cell. The temperature was controlled to ±0.01°C by a circulating thermostated water bath. The density, ρ, was calculated from the vibrational period, T, of the cell tube by using the relation

$$T^2 = A\rho + B. \tag{1}$$

Measurements on water and air standards allowed the determination of the constants A and B, and to write the density as

$$\rho = \rho_{\text{water}} + \frac{1}{A}\left(T^2 - T^2_{\text{water}}\right)$$

with

$$A = \frac{T^2_{\text{water}} - T^2_{\text{air}}}{\rho_{\text{water}} - \rho_{\text{air}}}. \tag{2}$$

T_{water} and T_{air} were determined at 20°C and atmospheric pressure. ρ_{water} was obtained from the International Critical Tables, and ρ_{air} was given by the relation:

$$\rho_{t,p} = \frac{0.0012930}{1 + 0.00367\ t}\ \frac{p}{760}, \tag{3}$$

p is the atmospheric pressure in mm of mercury, t the temperature in °C. The partial specific volumes of the salts were calculated from the relation

$$\bar{V}_{\text{salt}} = \left(\frac{m_{\text{water}} + m_{\text{salt}}}{\rho_{\text{sol}}} - \frac{m_{\text{water}}}{\rho_{\text{water}}}\right)\frac{1}{m_{\text{salt}}}, \tag{4}$$

m_{water} and m_{salt} are the masses of water (H_2O or D_2O) and salt in each sample, respectively; ρ_{sol} and ρ_{water} are the measured densities of the solution and the solvent, respectively.

The fractional precision of the densimeter was tested at 20°C with $NaCl/H_2O$ solutions of absolutely known percentage of salt (4%, 10%, 12% and 20%) and densities calculated from values in the International Critical Tables. It was $\approx 5 \times 10^{-5}$.

The neutron transmission $\tau = I_T/I_0$ is related to the total cross section per unit volume, μ_T, of the sample by:

$$\mu_T = -\frac{1}{e}\ln \tau, \tag{5}$$

where e, the neutron path length in the sample is in cm, τ is dimensionless and μ_T is in cm^{-1}. With solutions containing several components, μ_T can also be written [7]:

$$\mu_T = \sum_i n_i \sigma_{T,i}, \tag{6}$$

with n_i (cm^{-3}), $\sigma_{T,i}$ (cm^2), the number density and total cross section of particles i, respectively.

$$\mu_T = \frac{N_A}{V_T}\left(55.51\ \sigma_{T,H_2O} + m\sum_{\text{ion}} \sigma_{T,\text{ion}}\right). \tag{7}$$

m is the aquamolality of the salt, V_T (cm^3) the total volume of the solution containing m moles of salt and 55.51 moles of water (55.51 is the number of moles in 1000 g of H_2O).

The total cross section $\sigma_{T,i}$ includes contributions from incoherent scattering, σ_{is}, coherent scattering, σ_{cs}, and absorption, σ_a, of salt ions and water molecules (H_2O or D_2O). Their values for the solution components are in Table 1.

Neutron scattering and transmission measurements were performed on the instrument D11 at the Institut Laue Langevin in Grenoble, with a neutron wavelength $\lambda = 10$ Å and $\Delta\lambda/\lambda$ of 8%. Sample temperature was controlled at 20 ± 0.1°C for all experiments. For transmission experiments, the sample-detector distance was 10 m (to have the transmitted beam distributed over a large detector area), with a collimation distance of 2.50 m on the incident side. For scattering experiments, the sample–detector distance was 2.80 m with other conditions unchanged. Samples were in quartz cells (Hellma) of 2.00 mm pathlength for D_2O solutions, and 1.00 mm pathlength for H_2O solutions.

The scattered intensity was corrected for the contribution of the cell and detector response and scaled by the incoherent scattering of 1.00 mm pathlength of H_2O by

$$I(Q) = \frac{I_s(Q) - T_s I_{ec}(Q)}{I_w(Q) - T_w I_{ec}(Q)}, \tag{8}$$

Table 1

Neutron scattering lengths and cross sections of the different elements in the solutions for 10 Å wavelength; 1 barn = 10^{-24} cm^2

	Spin	Coher. scattering length (10^{-15} m)	Incoher. scattering length (10^{-15} m)	$\sigma_{\text{coher.}}$ (barns)	$\sigma_{\text{incoher.}}$ (barns)	$\sigma_{\text{scat.}}$ (barns)	$\sigma_{\text{absorpt.}}$ (barns)
H_2O [a]						245	
D_2O [a]						19.5	
Na [b]	3/2	3.63	3.59	1.66	1.62	3.28	0.53
Cl [b]	3/2	9.58		11.53	5.2	16.7	33.5
K [b]	3/2	3.71		1.73	0.25	1.98	2.1

[a] From Ref. [11].

[b] From Bee [13].

$I_s(Q)$, $I_{ec}(Q)$ and $I_w(Q)$ are the scattered intensities of the sample, the empty cell and the cell of water, respectively. T_s and T_w are the transmissions of the sample and water, respectively. Q is the scattering vector amplitude, $Q = 4\pi \sin \theta/\lambda$, where 2θ is the scattering angle. The units of $I(Q)$, therefore, are relative to the scattering of 1.00 mm of H_2O in identical geometrical conditions [7].

The scattered intensity, $I(Q)$, includes incoherent and coherent components, $I(Q) = I_{inc}(Q) + I_{coh}(Q)$. With 10 Å neutrons, the incoherent scattering is isotropic whereas the coherent part will have an angular dependence arising from correlations in the solution [7]. The coherent scattered intensity $I_{coh}(Q)$ of a sample of identical point (very small compared to the wavelength) particles with a number density n is given by

$$I(n, Q) = I(0, Q)S(n, Q), \tag{9}$$

$I(0, Q)$ is a form factor that is constant for point particles. $S(n, Q)$ is an interference term that is equal to 1 at low particle density. At higher concentrations, there are correlations between the particle positions and $S(n, Q)$ will have a form similar to the curve in Fig. 1. The peaks are related to average inverse distances between the particles. For a monomolecular liquid, $S_{Q\to 0}(n, Q)$ is related to the compressibility χ of the particle distribution by:

$$\chi(n) = (1/k_B Tn)S(n, 0), \tag{10}$$

where k_B is Boltzmann's constant and T is absolute temperature. For solutions with several components, however, $S_{Q\to 0}(n, Q)$ contains terms related to the mean square fluctuations in particle number density (i.e. irrespective of the type of particle) and also to the mean square fluctuations in the relative concentrations of the different components [8].

3. Results and discussion

The densities of NaCl and KCl solutions as a function of salt concentration in H_2O or D_2O are in Figs. 2a and 2b. Present data, collected at 20°C, are in good agreement with the results of Dessauges et al. [9] for NaCl solutions at 15°C and 30°C. The plots show a difference in density of about 10% between H_2O and D_2O for both salts, with a departure from ideality when the salt concentration increases. This difference is due only in part to the isotopic mass effect when deuterium replaces hydrogen. The partial specific volumes of each salt (calculated from Eq. (4)) are in Fig. 3. They are systematically lower in D_2O than in H_2O, for both NaCl and KCl, an effect that cannot be explained by the difference in the isotopic mass of the water. The relative difference is smaller for KCl that has the larger cation. It has already been observed by Conway and Laliberté [10] for small aquamolalities (up to 0.2 molal) of NaCl and interpreted in terms of higher electrostriction in D_2O, leading to a more negative partial specific volume for the cation; i.e. D_2O molecules are on average closer to the ion than H_2O molecules. It is seen from Fig. 3 that the effect

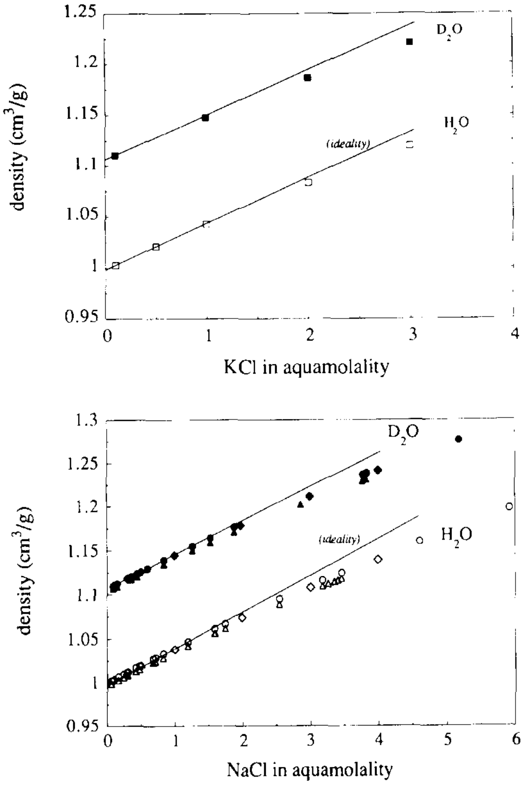

Fig. 2. (a) Experimental densities (◇) of NaCl solutions at various aquamolalities at 20°C, compared with densities at 15°C (○) and 30°C (△) from Dessauges et al. [9], in H_2O (empty symbols) and in D_2O (full symbols). The straight line represents ideality of solutions. (b) Experimental densities of KCl solutions in H_2O (□) and in D_2O (■) at various aquamolalities, at 20°C.

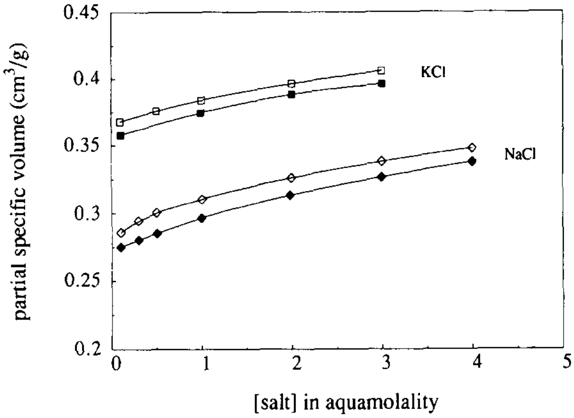

Fig. 3. Partial specific volumes of NaCl/H_2O (◇), NaCl/D_2O (◆), KCl/H_2O (□) and KCl/D_2O (■), at various aquamolalities, at 20°C.

persists to very high salt concentrations. Salt solutions in H_2O or D_2O are not isomorphous, therefore, so that contrast variation experiments that make this assumption should be interpreted with care.

Departure from ideality observed at high salt concentrations reflects correlations in the solution structure due to the presence of the salt ions that should also affect the scattering properties of the solution.

The total cross sections per volume of NaCl and KCl solutions in H_2O or D_2O for 10 Å neutrons were calculated from transmission measurements (Eq. (5)) and plotted in Figs. 4a and 4b, in which they are compared to calculations based on the solution densities and the cross-sections of their components (Eqs. (6) and (7)). The total cross-section values taken in the calculation (242 barns for H_2O, and 18.7 barns for D_2O) were determined experimentally from the transmissions of the pure samples. They are in good agreement with values in Ref. [11] and those measured by May et al. [7] (245 barns for H_2O and 19.5 barns for D_2O). The calculated cross section in H_2O decreases in value with increasing salt, reflecting the smaller number density of H nuclei, since the scattering is dominated by their incoherent cross section. In D_2O, on the other hand, the calculated cross-section remains approximately constant because of compensatory effects between the decrease in deuterium oxide number density and the increase in salt number density, the cross sections of these components being similar. The experimental total cross sections, however, increased significantly with increasing salt concentration when compared to the calculation.

The total cross section is made up of absorption, incoherent and coherent scattering terms. In H_2O, the predominant part is incoherent scattering because of the very large incoherent cross section of H and its negative coherent scattering length that gives the molecule a coherent cross section close to zero. In D_2O, on the other hand, the coherent cross section is about three times the incoherent one. For both, the absorption term in the cross section is negligible.

The scattered intensities were constant within errors for H_2O or D_2O solutions in the scattering

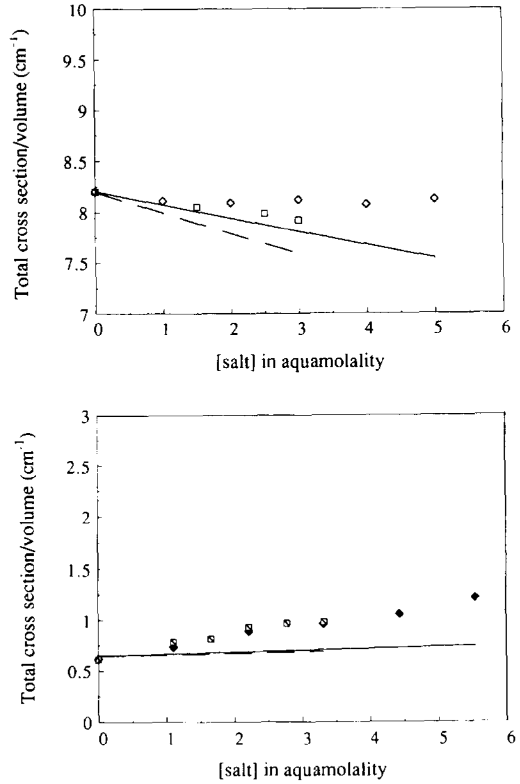

Fig. 4. (a) Experimental total cross sections per volume of NaCl/H_2O (◇) and KCl/H_2O (□) solutions as a function of salt aquamolality. The lines correspond to calculated total cross sections per volume for NaCl/H_2O (———) and for KCl/H_2O (– – –) obtained from Eq. (7). (b) Experimental total cross sections per volume of NaCl/D_2O (◆) and KCl/D_2O (□) solutions as a function of salt aquamolality. The line corresponds to calculated total cross sections per volume for NaCl/D_2O (———) and for KCl/D_2O (– – –) obtained from Eq. (7).

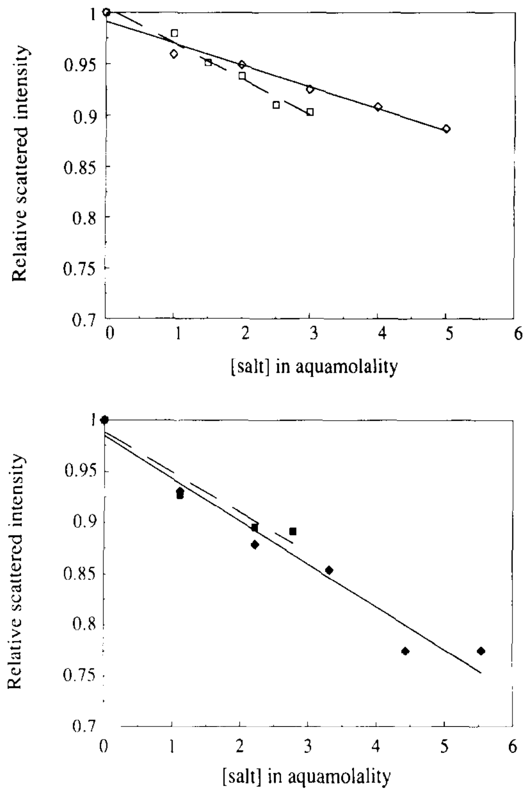

Fig. 5. (a) Relative scattered intensities (see Section 2) of NaCl/H_2O (◇) and KCl/H_2O (□) as a function of salt aquamolality. (b) Relative scattered intensities (see Section 2) of NaCl/D_2O (◆) and KCl/D_2O (■) as a function of salt aquamolality.

vector range measured ($0.015 \leqslant Q \leqslant 0.080$ Å^{-1}) and were taken to be essentially identical to $I(Q=0)$. They are plotted in Figs. 5a and 5b, for the different conditions. In H_2O, the intensity decreases with increasing salt concentration for both NaCl and KCl with a slope similar to that of the line calculated for the total cross section in Fig. 4a. The incoherent scattering follows what would be expected from the density of the solution and the total cross sections of its components. Qualitatively, therefore, the increase in total cross section with salt concentration in Fig. 4a should be attributed to coherent scattering at Q vectors outside the measured range but within the 1.2 Å^{-1} limit for scattering by a 10 Å wavelength (Fig. 1). In D_2O, the scattering is mainly coherent and can be interpreted by using Fig. 1 and Eq. (10). It also drops with increasing salt concentration, but, contrary to the H_2O scattering, this effect cannot be accounted for by the density and cross-section calculation in Fig. 4b, which is approximately constant with salt concentration in the D_2O case. It is likely, therefore, that the fall in scattered intensity at small angles as the salt concentration increased corresponds to a decrease in $S(0)$, i.e. a decrease in number density and/or concentration fluctuations in the solutions [8]. A corresponding increase in the coherent scattering at larger angles is suggested from the increase in the experimental total cross section with salt concentration (Fig. 4b). This could be due to increased correlations, i.e. a higher main peak in the $S(Q)$ curve (Fig. 1) or to a shift in the position of the peak to smaller Q values, which would raise its contribution to the Q range observed with the 10 Å neutrons. Such a shift was observed in LiCl solutions below the glass transition. The main peak in 5 molal LiCl is at 1.9 Å^{-1} compared to 2.0 Å^{-1} for pure D_2O [12].

The neutron diffraction work on various salts of Enderby et al. has demonstrated the existence of aqua ions in aqueous solutions with geometries similar to that in crystal hydrates [3]. At high salt concentrations, the hydration numbers in these ions approaches 6. In a 4 molal solution of NaCl, for example, 48 of the 55.51 moles of water are within aqua ions and correlations between them will be unavoidable, leading to more 'structure' and lower fluctuations in the solutions. Despite the fact that the main correlations in the salt solutions correspond to Q values well beyond the cold neutron cutoff, they do have measurable effects at small Q values and can account, qualitatively at least, for the observed transmission and small angle scattering of 10 Å neutrons by these solutions.

4. Conclusion

Salt type and concentration as well as isotope effects were observed by mass density and neutron scattering measurements on NaCl and KCl solutions in H_2O or D_2O. Apart from the well known differences between Na^+ and K^+ ions, smaller partial specific volumes found for both salts at all concentrations in D_2O were indicative that the H_2O and D_2O solutions of each salt are not isomorphous. At high concentrations of both salts, deviations from ideality in the density measurements parallel increases in the coherent scattering cross-sections of the solutions while their scattering at very small angles decreases, suggesting enhanced correlations between the solution components and smaller fluctuations.

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