For a more quantitative analysis, analytical expressions are available to complement the graphical representations [see (*4*) and (*5*) for definitions and details]. The algebraic equations allow one to extract measures of the affinity of receptors for ligands (*6–9*). In almost all circumstances, it is possible, by a simple graphical procedure (*10*), to evaluate the first stoichiometric binding constant (*4*, *5*). With more effort, the amount depending on the quantity and accuracy of the binding data, one can evaluate succeeding stoichiometric binding constants (*6–9*). In general it is not possible to obtain site-binding constants (*5*, *8*) unless some model of the interactions between receptor sites is assumed or has been established as realistic. Such a model introduces additional ad hoc assumptions.

How are we going to obtain the total number of receptor sites? We cannot find this parameter unless the experimenter is willing to make, and the system is amenable to the collection of, the necessary extensive measurements. Unless reliable binding data can be obtained at ligand concentrations that place the points unequivocally above the inflection point of Fig. 1a, any estimate of the position of the plateau will be uncertain. It will be even less reliable from a presumed intercept on a Scatchard graph.

We would all do well to recall a perceptive admonition made by T. H. Huxley over a century ago:

> Mathematics may be compared to a mill of exquisite workmanship, which grinds you stuff of any degree of fineness; but, nevertheless, what you get out depends on what you put in; and as the grandest mill in the world will not extract wheat-flour from peascods, so pages of formulae [or graphs] will not get a definite result out of loose data.

IRVING M. KLOTZ
Department of Chemistry,
Northwestern University,
Evanston, Illinois 60201

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Direct Determination of Ionic Solvation from Neutron Diffraction

Abstract. *Much information on ionic solvation in electrolyte solutions has been inferred from macroscopic thermodynamic and transport properties and from spectroscopy. These ion-water interactions can now be probed directly and unambiguously by neutron diffraction. Such measurements have been done with neodymium trichloride solutions in heavy water that are identical in every respect except the isotopic state of the neodymium ions; these experiments yield in a straightforward manner the distribution of oxygen and deuterium atoms from the water molecules in the first hydration sphere of the neodymium ion. Each ion is surrounded by 8.6 oxygen atoms at a distance of 2.48 angstroms and 16.7 deuterium atoms at 3.13 angstroms indicating a well-defined first hydration sphere of water molecules, the deuterium atoms pointing away from the cation.*

In order to understand and predict the properties of electrolyte solutions it is necessary to know what the entity called an ion is—that is, whether it is the bare ion, or whether it carries with it water molecules sufficiently firmly bound to be regarded as part of the ion and, if so, how many such water molecules are involved. A traditional approach has been to infer these hydration numbers from measurements of bulk thermodynamic and transport properties that are predominantly determined by ion-water interactions. Other techniques, such as nuclear magnetic resonance and Raman and infrared spectroscopy, have also been used in this way. In many instances the numbers thus obtained vary greatly with the experimental approach and with the method used to analyze and interpret the data (*1*).

It has now become clear that these difficulties in probing the ion-water interactions directly at the molecular level can be overcome by diffraction studies. Defining ion-water interactions as the positional and orientational correlations between ion-water pairs in solution, we have a measurable quantity, the diffraction pattern, from which these correlations can be determined. Ionic solutions were among the first liquids to which x-ray diffraction was applied, and a large number of studies have been reported over the years (*2*). However, the interpretation of a single diffraction pattern is always difficult, often ambiguous, and never unique. This ambiguity of interpretation can be eliminated by measuring the neutron diffraction patterns from several ionic solutions that are identical in all respects except the isotopic state of one of the ions. The algebraic difference between any two sets of measurements then yields the ion-water correlations (*2*). In order to obtain sufficiently good statistics for the differences, very large numbers of neutrons have to be counted. Hence, the method is at present limited to the study of relatively concentrated solutions (> 1 molal), and the results may not be representative for the dilute regime.

We measured the neutron diffraction patterns of four neodymium trichloride solutions in D_2O (Table 1). The experi-

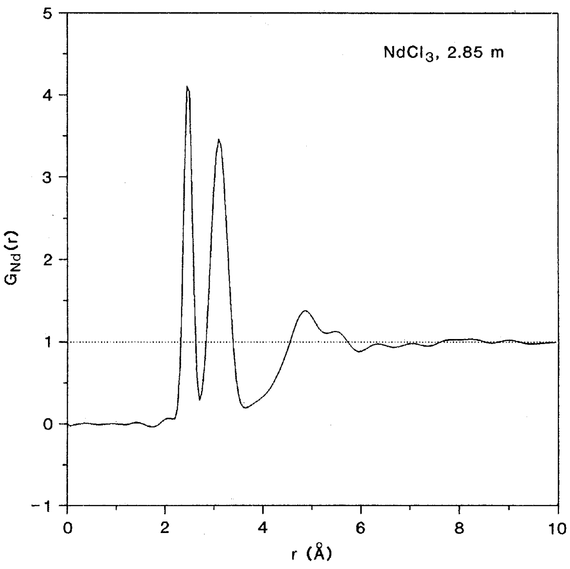

Fig. 1. Radial distribution of water molecules around the Nd^{3+} ion in a 2.85 molal solution of $NdCl_3$ in D_2O. The peaks at 2.48 and 3.13 Å correspond to 8.6 oxygen and 16.7 deuterium atoms from 8.5 ± 0.2 water molecules in the first hydration sphere.

ments were carried out at the High Flux Isotope Reactor. We discuss here only the main results of our study (*3*). The neutron cross sections of the $^{144}NdCl_3$ solution (having the smallest scattering factor; see Table 1) were subtracted from those of the other three solutions to yield three difference curves, from which a structure function was extracted, namely

$$H_{\mathrm{Nd}}(k) = 0.279 h_{\mathrm{NdO}}(k) + 0.642 h_{\mathrm{NdD}}(k) + 0.079 h_{\mathrm{NdCl}}(k) \qquad (1)$$

where $k = (4\pi/\lambda)\sin\theta$, $\lambda = 0.89$ Å being the neutron wavelength and 2θ the scattering angle.

The numerical coefficients in Eq. 1 depend only on the neutron scattering factors and the concentrations of the nuclides in the solutions (*2*). The functions $h_{ij}(k)$ are the Fourier transforms of the atom pair correlation functions $h_{ij}(r) = g_{ij}(r) - 1$, where the atom pair distribution functions $g_{ij}(r)$ measure the probability of finding a j atom at a radial distance r from an i atom in the solution. Fourier transformation of the function $H_{\mathrm{Nd}}(k)$ derived from experiment yields an average radial distribution function, namely

$$G_{\mathrm{Nd}}(r) = 0.279 g_{\mathrm{NdO}}(r) + 0.642 g_{\mathrm{NdD}}(r) + 0.079 g_{\mathrm{NdCl}}(r) \qquad (2)$$

which contains the desired information on the ion-water interactions in the functions $g_{\mathrm{NdO}}(r)$ and $g_{\mathrm{NdD}}(r)$. The function $G_{\mathrm{Nd}}(r)$ is shown in Fig. 1.

The maxima in the function $G_{\mathrm{Nd}}(r)$ derived from experiment correspond to the most frequent Nd–O, Nd–D, and Nd–Cl distances in the solution. The first two pronounced maxima at 2.48 and 3.13 Å must be ascribed, respectively, to Nd–O and Nd–D interactions. This is because the areas under these peaks, which are related to the number of atoms at these relative positions, have a ratio of ~ 1:2. The area under the peak at 2.48 Å corresponds to 8.6 oxygen atoms, and the area under the peak at 3.13 Å to 16.7 deuterium atoms around a neodymium ion. Taking the average (8.6 + 16.7/2)/2 as the best estimate of the number of water molecules around the ion, we obtain a coordination number of 8.5 ± 0.2. These numbers, together with the sharpness of the first two peaks in Fig. 1, show that the Nd^{3+} ion has a very well-defined first hydration sphere with the deuterium atoms pointing away from the ion at a tilt angle of 55°, as shown in Fig. 2. This picture is, of course, an average one; at any instant an average Nd^{3+} ion "sees" the water molecules in its primary hydration sphere as indicated in Fig. 2. The residence time of these molecules is finite but may be as long as 10^{-6} second (*4*).

Table 1. Neutron-scattering factors (f) of the Nd nuclei in the four 2.85 molal solutions studied by neutron diffraction.

Isotope	Abundance (%)	f (10^{-12} cm)
^{144}Nd	97.51	0.31
$^{\mathrm{nat}}$Nd*		0.72
^{142}Nd	97.55	0.78
^{146}Nd	97.63	0.87

*Natural abundance.

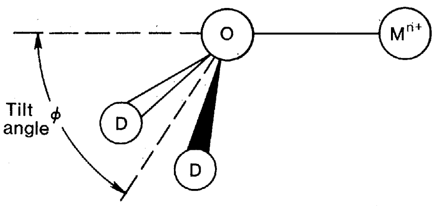

Fig. 2. Arrangement of a water molecule in the first hydration sphere of a cation, M^{n+}, in solution. There are 8.5 such molecules around Nd^{3+}, and the tilt angle is 55°.

We emphasize that these distances and coordination numbers should be regarded as direct measurements, no assumptions or models being used in the analysis of the neutron data. This method avoids the difficulties encountered in other experimental techniques (*5*); the primary hydration sphere is precisely defined in terms of the number of water molecules included and their average distance from the cation. The 8.5 water molecules are sufficiently firmly bound to be regarded as part of the Nd^{3+} ion in statistical mechanical calculations of the properties of these solutions (*6*).

A. H. NARTEN
R. L. HAHN

Chemistry Division,
Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

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Chemoprevention of Neonatal Jaundice: Potency of Tin-Protoporphyrin in an Animal Model

Abstract. *The substantial increases of hepatic, splenic, and renal heme oxygenase levels that occur shortly after birth in neonatal rats were prevented by a single administration of tin-protoporphyrin (10 micromoles per kilogram of body weight). With this treatment serum bilirubin levels declined within 24 hours to near-normal adult levels and remained low throughout the postnatal period. Zinc-protoporphyrin at doses up to 50-fold greater than the effective dose of tin-protoporphyrin did not prevent the immediate increases in tissue heme oxygenase activities and in serum bilirubin levels that occur postnatally. Studies in vitro with microsomal heme oxygenase in human spleen indicate that tin-protoporphyrin is a potent competitive inhibitor of the oxidation of heme to bile pigment in this tissue.*

Bilirubin is a potential central nervous system toxin for infants in the period immediately after birth when the blood-brain barrier is still permeable to many substances (*1*). In the human newborn, large amounts of this bile pigment are produced as a result of lysis of fetal red cells and enhanced degradation of the heme (iron-protoporphyrin) moiety of the fetal hemoglobin molecule. Since the capacity of the newborn liver to detoxify bilirubin by glucuronide conjugate formation is not fully developed, the unconjugated bile pigment accumulates in the bloodstream during the first week or more of neonatal life. Quite high plasma levels of bilirubin may occur during this period and the term "physiological" or neonatal jaundice has been applied to this phenomenon in humans (*2*).

Neonatal jaundice is common, and many clinical events such as prematurity, infection, and hypoxia can exaggerate the degree of hyperbilirubinemia to such an extent that serious risk of the development of subtle or overt neurotoxicity may occur (*2*). Treatments for excessive hyperbilirubinemia include phototherapy to degrade bilirubin to more hydrophilic isomers (*3*), drugs to induce