



Molecular aggregation phenomena in solution: an energy dispersive X-ray diffraction study of concentrated imidazole water solutions

Lorenzo Gontrani, Ruggero Caminiti ^{*}, Luigi Bencivenni, Claudia Sadun

Dipartimento di Chimica, Istituto Nazionale per la Fisica della Materia, Università di Roma 'La Sapienza', P. le Aldo Moro 5, 00185 Roma, Italy

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Abstract

An energy dispersive X-ray diffraction study of concentrated imidazole water solutions is reported. It reveals the presence of extended solute–solute aggregates. Such results can be interpreted in terms of a branch of a pseudo-helix of imidazole dimers interacting with water molecules. The mean distance between molecules in the dimer is ~ 3.7 Å, while the mean distance between dimers is 4.75–4.95 Å depending on concentration. The model structure functions and theoretical radial distribution functions fit the experimental data. © 1999 Elsevier Science B.V. All rights reserved.

1. Introduction

Azoles constitute an interesting class of heteroaromatic molecules because of the presence of two different structural patterns, such as π electron networks and hydrogen-bond donor and acceptor groups. This fact gives them outstanding chemical properties which are at the basis of the behaviour of biological molecules where they appear as building blocks, such as nucleotides and enzymes.

The imidazole ($C_3N_2H_4$) ring, in particular, is found in the side chain of the amino acid histidine which plays a fundamental role in many proteolytic enzymes, in the structure of purine nucleobases, as well as in many other naturally occurring com-

pounds. Thus, the study of the intermolecular interactions of imidazole is particularly interesting – first in aqueous solutions which best resembles biological media. In a recent UV study, Peral et al. [1] suggested that imidazole and its methyl derivatives are extensively self-associated in water solutions, even at very low concentrations. They proposed a model based on vertical stacking of rings to account for the hypochromic effect observed. As far as we know, no structural study of such an association has yet been attempted.

The aim of this work is to achieve more detailed information by using X-ray diffraction – a technique that provides better structural insight.

Several examples of the application of angle dispersion X-ray diffraction to pure liquids and solutions of metal ions in various solvents can be found in the literature (e.g. see Refs. [2–6]). Few studies of

^{*} Corresponding author. E-mail: r.caminiti@caspur.it; fax: +39 06 490631

organic molecules in water solutions have been performed [7,8].

In the present work, the energy dispersive X-ray diffraction (EDXD) technique has been used. When compared with traditional ADXD (angular dispersion), its use has several advantages [9–12]: e.g., diffraction patterns are collected in a much shorter period of time, using only few reflection angles, but still covering q ranges equal to or larger than those obtainable by AD (see Section 2), and samples are stored in sealed cells, thus avoiding evaporation and contamination by air moisture.

2. Experimental

2.1. Sample preparation

Weighed amounts of pure crystalline imidazole (Aldrich, > 99% purity) were dissolved in distilled water to prepare two solutions of known concentration (4.5 and 7.61 M) and density. Samples for X-ray measurements were obtained filling proper teflon cells with mylar windows with $\approx 1 \text{ cm}^3$ of solution; the cells were then sealed and put into a sample holder.

2.2. X-ray diffraction: data treatment

We performed our experiments using the non-commercial energy-scanning diffractometer built in the Department of Chemistry, Rome University. Detailed descriptions of both instrument and technique can be found elsewhere [11–13].

Transmission geometry has been employed. White Bremsstrahlung component of the radiation emitted by a tungsten tube working at 45 kV and 35 mA was used. Scattered intensities for the two samples and for the empty cell were measured at seven different angles. This choice allowed us to cover a wide range of the scattering variable q , namely between 0.12 and 16 \AA^{-1} . The measuring time at each angle was set so as to obtain a minimum of 600000 counts per experimental point for $q > 5 \text{ \AA}^{-1}$ and 300000 for $q < 5 \text{ \AA}^{-1}$. The expression for q is

$$q = \frac{4\pi \sin \vartheta}{\lambda} = E \cdot 1.014 \sin \vartheta, \quad (1)$$

Table 1

Scattering parameters associated with the energy range used (14–36 keV) for each measurement angle

Scattering angle (degrees)	Q range (\AA^{-1})
26.0	6.22–16.00
10.5	2.59–6.65
5.0	1.24–3.18
3.0	0.74–1.91
1.5	0.37–0.95
1.0	0.25–0.64
0.5	0.12–0.32

when E is expressed in keV and q in \AA^{-1} ; measurement angles, used energy range and scattering parameters are reported in Table 1.

The primary beam intensity $I_0(E)$ was measured directly using the same voltage, by reducing the tube current to 10 mA at zero scattering angle without the sample. Transmission of the samples was measured under the same conditions. Both quantities are needed to carry out necessary corrections to observed scattered intensities [11,14]. After correction of experimental data for escape peak suppression [11,14], the various angular data sets were combined and the re-scaled intensity, in electron units (e.u.), was normalised to a stoichiometric unit of volume containing one N atom. Such correction was performed using our program DIF1, purposely written. Detailed descriptions of procedures and formulae used can be found in Refs. [6,11]. The atomic and water concentrations, together with stoichiometric volume and density, are reported in Table 2 (in mol l^{-1} , \AA^3 and kg L^{-1} , respectively). The ‘static’ structure function $i(q)$ was constructed according to the formula

$$I(q) = I_{\text{e.u.}} - \sum_{i=1}^m x_i f_i^2. \quad (2)$$

where f_i are the atomic scattering factors, x_i are the number concentrations of i -type atoms in the stoichiometric unit, $I_{\text{e.u.}}$ is the observed intensity in electron units. Fourier transformation of $i(q)$ led to radial distribution functions (rdf)

$$D(r) = 4\pi r^2 \rho_0 + \frac{2r}{\pi} \int_0^{q_{\text{max}}} qi(q) M(q) \sin(rq) dq. \quad (3)$$

Table 2
Concentrations

C ₃ N ₂ H ₄ conc. (mol l ⁻¹)	Stoichiom. volume (Å ³)	Density (kg l ⁻¹)	O conc. (mol l ⁻¹)	N conc. (mol l ⁻¹)	C conc. (mol l ⁻¹)	H conc. (mol l ⁻¹)	H ₂ O conc. (mol l ⁻¹)
4.50	184.504	1.0247	39.86	9.00	13.50	97.72	39.86
7.61	109.102	1.0564	29.88	15.22	22.83	90.2	29.88

In this equation ρ_0 is the bulk number density of stoichiometric units and

$$M(q) = \frac{f_N^2(0)}{f_N^2(q)} \exp(-0.01q^2), \quad (4)$$

is the sharpening factor. We used the value of 16 Å⁻¹ as the upper limit of integration.

In the following pictures, we report the observed structure functions (in the form $qi(q)M(q)$ (Fig. 1, top a–c)) and the radial distribution functions (in the form $\text{Diff}(r) = D(r) - 4\pi r^2 \rho_0$ (Fig. 1, bottom a–c)) for pure water and for the two samples, measured in the same experimental conditions.

The main features emerging from a qualitative analysis of both structure functions and radial distribution functions are a marked concentration effect and the presence (in the solution curves) of evident peaks at high values of r , accounting for an increase of order in solution with respect to pure water.

This pattern is particularly evident in the $\text{Diff}(r)$ curves. As can be seen in Fig. 1, bottom a (see rdf curves obtained with ADXD by Narten and Levy for comparison and for complete discussion [3]) liquid water exhibits main peaks at 0.95 Å (O–H contacts within the molecule), 2.85 Å (O···O contacts between first-neighbour molecules), 4.65 Å (O···O contacts between second-neighbour molecules), 6.9 Å (O···O contacts of third neighbours). In imidazole 4.5 M rdf we can still notice the presence of water peaks (although reduced in intensity, the water concentration being 39.86 M in solution and 55.50 M in the pure liquid) but new ones appear, namely the molecular peaks at 1.35 and 2.15 Å (interactions between adjacent atoms in the ring and atoms across the ring of the imidazole molecule); the water peak at 4.65 Å is now shifted at 4.85 Å and a new peak at 9 Å appears. In the curve relative to the 7.61 M solution, the reduction of water concentration be-

comes more evident: water peaks are greatly lowered in intensity while peaks originating from the solute gain relative weight. We can therefore regard the peaks at 4.85 and 9 Å as due to solute–solute and solute–solvent interactions.

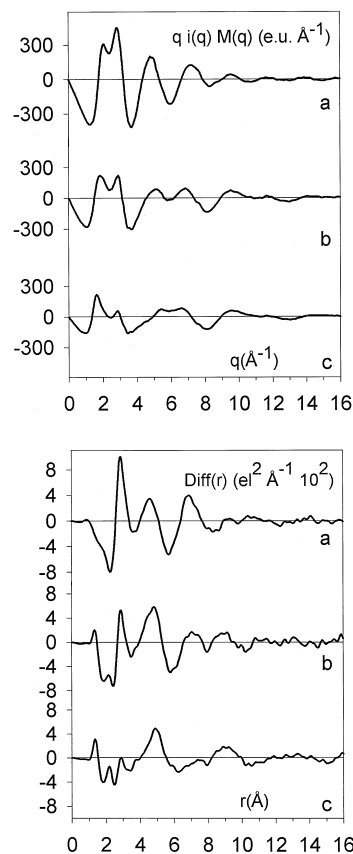


Fig. 1. Top: experimental structure functions in the form $qi(q)M(q)$: (a) pure water, (b) imidazole 4.5 M, (c) imidazole 7.61 M. Bottom: experimental radial distribution functions in the form $\text{Diff}(r) = D(r) - 4\pi r^2 \rho_0$: (a) pure water, (b) imidazole 4.5 M, (c) imidazole 7.61 M.

3. Results and discussion

The features discussed above were used to build a structural model the structure function and radial distribution function of which best resembled the experimental data. Theoretical peaks were calculated by Fourier transformation of a model structure function obtained by the Debye equation for pairs of interactions:

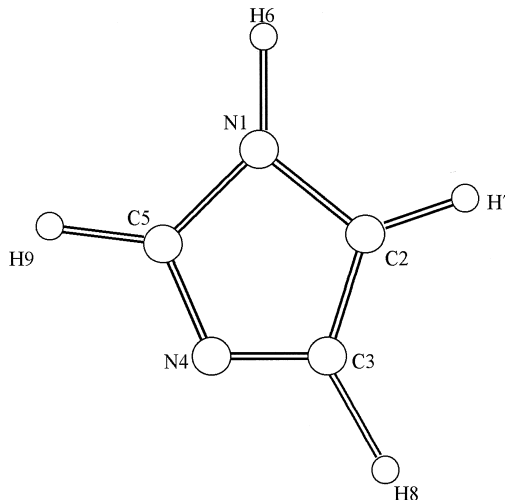
$$i_{mn}(q) = \sum f_m f_n \frac{\sin(r_{mn}q)}{r_{mn}q} \exp\left(-\frac{1}{2}\sigma_{mn}^2 q^2\right), \quad (5)$$

using the same sharpening factor, the same q_{\max} value as for experimental data and assuming σ_{mn} to be the root-mean-square (r.m.s.) variation in the interatomic distance. Instead of using a σ_{mn} value for each distance r_{mn} , we reduced the number of parameters by assigning the same σ value to distances falling within predefined ranges. σ values and predefined ranges used are reported in Table 3.

The Fourier transform process requires extrapolation to zero of the model structure function to account for long distance interactions (uniform distribution–‘continuum’). We decided simply to replace model data below 1.325 \AA^{-1} with experimental ones because the proposed equations for the continuum have no direct physical meaning.

The interpreting model has been constructed in the following way. In the first step, an ab-initio calculation was performed so as to optimise the geometry of the single imidazole molecule. Different levels of theory were used, including Møller–Plesset perturbation methods (MP2) and density functional methods (B3-LYP), with various basis sets, namely D95 + +(d,p), 6-311 + G(d,p) and 6-311 + + G(d,p). Such methods are all implemented in the

GAUSSIAN94 package [15]. The geometries obtained employing such sets of functions do not differ significantly from each other; in all cases the molecule is planar. We therefore decided to use the results of B3-LYP/6-311 + + G(d,p) calculations, since 6-311 + + G(d,p) is the largest set. Bond distances and angles are reported below.



Distances (\AA)	Angles (degrees)
N1–C2 1.380	C2–N1–C5 107.2
N1–C5 1.367	C2–N1–H6 126.3
N1–H6 1.008	N1–C2–C3 105.1
C2–C3 1.371	N1–C2–H7 122.3
C2–H7 1.077	C5–N1–H6 126.5
C3–N4 1.377	N1–C5–N4 111.5
C3–H8 1.079	N1–C5–H9 122.5
N4–C5 1.313	C3–C2–H7 132.5
C5–H9 1.079	C2–C3–N4 110.6
	C2–C3–H8 127.9
	N4–C3–H8 127.9
	C3–N4–C5 127.9
	N4–C5–H9 127.9

Table 3
Final values of the adjusted r.m.s. σ for the model used

σ value (\AA)	Distance range (\AA)
0.01	$0.0 < r \leq 1.1$
0.02	$1.1 < r \leq 1.6$
0.05	$1.6 < r \leq 2.7$
0.11	$2.7 < r \leq 3.8$
0.20	$3.8 < r \leq 8.0$
0.25	$r > 8.0$

The calculated geometry was then tested against experimental data by comparing the first part of experimental $D(r)$ curves (of both solutions) with theoretical peak shapes. The latter were constructed by adding to molecular contributions also the short distance interactions present in water (O–H and O \cdots H) [16]. The resulting curves are shown in

Fig. 2a,b. Agreement between experimental data and theoretical peaks is good.

The quality of the agreement found allowed us to subtract structure function contributions originating from intramolecular distances, using ab-initio molecular parameters previously calculated.

In the second step, we made the assumption that the perturbation induced by the presence of the solute had a limited influence on bulk water structure, which was mainly preserved, and therefore we subtracted the weighted amount (39.86/55.5 and 29.88/55.5 for 4.5 and 7.61 M solution, respectively) of the experimental water structure function from the total $qi(q)M(q)$ curves. In so doing, we were able to focus our attention to all new interactions present, namely long-range solute–solute and solute–solvent interactions. The difference curves (solid) shown in Fig. 3a,b (rdf) contain all interactions which are neither present in pure water nor in the imidazole molecule. Only molecule–molecule and molecule–water (except $N \cdots OH$ and $NH \cdots O$ contacts which would fall in the same range of water H-bonds) are left.

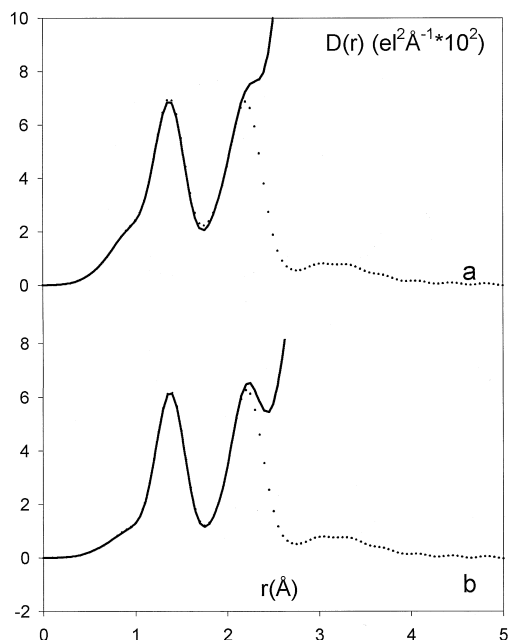


Fig. 2. Experimental radial distribution functions $D(r)$ (solid) vs. theoretical molecular peak shapes (dotted): (a) imidazole 4.5 M; (b) imidazole 7.61 M.

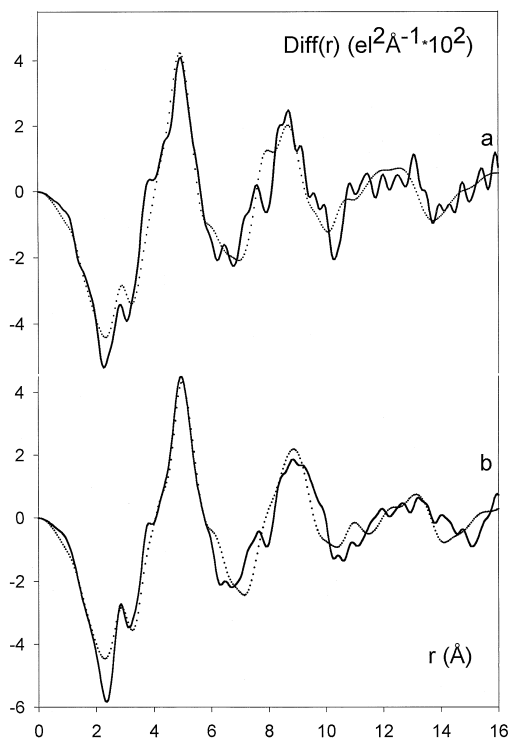


Fig. 3. Difference curve for radial distribution function $Diff(r)$ (solid) vs. model $Diff(r)$ (dotted) (see text): (a) imidazole 4.5 M, (b) imidazole 7.61 M.

Several arrangements were tried, including sets of planar dimers and linear polymers, but all of them led to peaks too intense and in the wrong positions. Subsequently, a set of five stacked imidazole molecules was built. A step-by-step variation of relative orientations and inclinations was carried out. The best geometry obtained was then submitted to least-squares refinement. We fitted the model structure function to the experimental curve, imposing no constraints to molecular motion in space. (LSHS Fortran program [17], modified on purpose, was used.)

In order to describe better the cluster spatial arrangement, we chose to refer all structural parameters to the molecular centres of mass. The results were interpreted in terms of a branch of a pseudo-helix in which stacked dimers interact with each other, giving a short–long–short–long distance progression. The angle formed by the lines linking adjacent centres of mass is about 150° . Within the

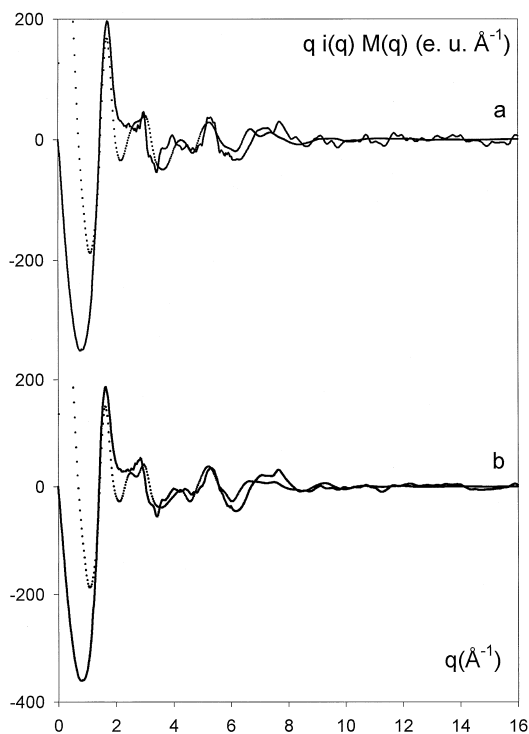


Fig. 4. Difference curve for structure function $qi(q)M(q)$ (solid) vs. model structure function (dotted) (see text): (a) imidazole 4.5 M, (b) imidazole 7.61 M.

dimer, the distance between centres of mass is 3.75 Å, molecules are rotated about 55° and tilted by about 35°.

The distance between dimers is 4.95 Å for the 7.61 M sample and 4.75 Å for the 4.5 M solution.

The previously described model is intrinsically defective in the sense that solute–solvent interactions are not at all taken into account. We tried to avoid this discontinuity by allowing imidazole molecules to interact with water molecules, at most one for each H-bond site (N and N–H). $N \cdots O$ and $NH \cdots O$ distances were set at 2.78 and 2.85 Å, according to previous structural studies on H-bonding found in the literature and to test ab-initio calculations on molecule hydrates, which we performed at the same level of theory used for the single molecule and which will be reported elsewhere.

Theoretical $qi(q)M(q)$ and radial distribution functions were then compared with experimental difference curves in which the correctly weighted struc-

ture function of water had been subtracted. Among all the models tested, the best candidate seems to be one in which one water molecule ‘links’ two imidazole molecules in the dimer, by interacting with N(1) and NH(2) and two other water molecules occupy the vacant H-bond sites, N(2) and NH(1). We used χ^2 values, calculated for the couple theoretical–experimental structure functions (Fig. 4a,b) to evaluate agreement. A picture of the model is reported in Fig. 5. Structure functions and radial distribution functions for this model are shown (Fig. 4a,b and 3a,b, respectively), together with difference curves.

A noteworthy feature is that the model is consistent with the assumption that the bulk water structure is largely retained. In fact, all H_2O molecules included in the cluster are at least 5 Å away, thus allowing water bridges to link them with no modification of solvent structure.

In conclusion, this study shows that concentrated imidazole water solutions have peculiar and unexpected properties. In their X-ray diffraction $Diff(r)$ functions intense peaks are found which can be attributed to direct interactions between molecules, which cluster to form quite extended structures.

These ‘clusters’ can be thought of as pseudo-helices containing dimers in which molecules, on average, are 3.7 Å apart. The mean distance between

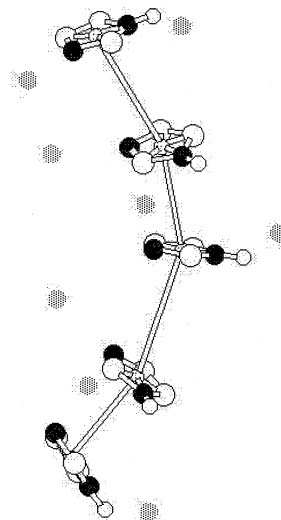


Fig. 5. Picture of the model; for clarity H atoms (except those of NH) are not shown and a dummy atom occupies the centre of mass of each imidazole molecule (see text).

dimers is 4.75–4.95 Å. Water surrounds the cluster by occupying H-bond sites of imidazole molecules; its bulk structure is not significantly modified.

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