Molecule 1-Methyl-5-nitroindoline Probes the Structural Change of Liquid Water with Temperature

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ABSTRACT: The molecule 1-methyl-5-nitroindoline (MNI) demonstrates to be a suitable probe to detect the change of structure of liquid water by varying temperature. The relationship of the peak wavenumber of the first absorption band of this probe with temperature clearly shows a minimum at 43 °C that indicates a change of structure in the liquid water at ambient pressure. The plot of the MNI wavenumber peak absorption against the heat capacity of liquid water shows a temperature range (35–45 °C) that resembles the crossover temperature proposed by Mallamace et al. (Faraday Discuss. 2013, 167, 95) from thermodynamic measurements (42 ± 5 °C). The crossover temperature range includes the so-called phase-transition temperature at 34.6 °C and 0.05 atm measured by Dougherty et al. (J. Phys. Chem. A 2006, 110, 7577–7580) and matches the temperature measured from solvatochromism by Catalán and Gonzalo (43 °C) (Chem. Phys. Lett., 2017, 679, 86–89) as the origin of a structural change in liquid water.

INTRODUCTION

The molecule 1-methyl-5-nitroindoline (MNI) (Scheme 1) is a molecular probe that does not change its structure when varying the temperature of liquid water, in which it is dissolved. Its solvatochromic dependence against solvent dipolarity (SdP) and solvent polarizability (SP) allows us to propose it as a suitable molecular probe to determine whether structural changes occur in the liquid water when changing temperature.

The structural changes suffered by liquid water at ambient pressure are reported for the first time in 1892 by Röntgen, when he tried to explain some of the anomalies reported for liquid water: “it may be found in the assumption that the liquid water consists of aggregate of two species of differently constituted molecules”. Röntgen proposed that thermoelectricity of molten metals could lead to the conclusion that “molecular transformations can be generated in simple liquids by temperature changes”. In 2006, with measurements of depolarization Raman, Dougherty and Howard proposed the existence of a (weak-continuous) transition of phase for liquid water at 34.6 °C and 0.057 atm. This structural change was attributed by the authors to a change from a tetrahedral coordinate water structure (ca. HOH angle of 109.47 °C) to another of locally planar water structures (HOH angle of 120 °C—hexagonal planar), which would dominate above 34.6 °C. Dougherty and Howard also drew the conclusion that “water is a structured liquid with distinct local structures that vary with temperature”. The structure of water could be interpreted in terms of different clusters that vary with temperature, which is one concept of the many models traditionally defended from theoretical calculations. The size of the clusters and the fractions of hydrogen bonds unbroken decrease with temperature. Wernet et al. concluded from X-ray absorption spectroscopy and X-ray Raman scattering that most of the liquid water molecules at ambient pressure exhibit two hydrogen-bonded configurations and on heating liquid water from 25 to 90 °C about 10% of the molecules change from tetrahedral environments to two hydrogen-bonded configurations. These models involve the rupture of the intermolecular hydrogen bonds of water. However, Fernández-Serra and Artacho challenged the latter evidence by Wernet et al. (both experiments and theory) because of first principles calculations (ab initio molecular dynamics simulations at ambient conditions). These authors defend that there is no need to invoke the breaking of hydrogen bonds with temperature; the experiments are interpreted in terms of elongated hydrogen bonds with temperature but unbroken within the whole temperature range.

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Mallamace et al. reported that the isothermal compressibility \( [K_T(T,P)] \) and the coefficient of thermal expansion \( [\alpha_p(T,P)] \) exhibit a crossover temperature at 315 ± 5 K (42 ± 5 °C). The parameter \( K_T(T,P) \) shows a minimum at 315 K; and at the same temperature and at \( [\alpha_p(315)] = 0.44 \times 10^{-3} \) K\(^{-1}\), these authors found a "singular and universal expansivity point". In agreement with Mallamace et al., Catalán and Gonzalo reported the solvatochromic behavior of some pure molecular probes in liquid water from 15 to 90 °C at ambient pressure. The results confirmed a crossover temperature at 43 °C from the SdP, solvent basicity (SB), and SP of liquid water. The pure solvent scales used by Catalán and Gonzalo prove the existence of an internal structural change in liquid water at 43 °C for the reason that they show a bilinear behavior, an evidence of a change in the dipole moment, polarizability and basicity of water at about 43 °C. This is a non-uniform change, however, weak and continuous, in the structure of liquid water. The crossover is described adequately by the molecular probes DMNAF and ttbP9 for the liquid water dipolarity, \( \alpha \), and by MNI and 5-nitroindoline (NI) for the liquid water basicity.11

A recent review by Nilsson and Petterson12 emphasizes the cause–effect relationship between the so-called anomalies of liquid water and their structural origin, all the evidence reported points out to two classes of local structural environments that are temperature-dependent.

In this current paper, another molecular probe, the molecule MNI, is employed from −15 to 95 °C to provide evidence of the structural change of liquid water at 43 °C and of electrolyte aqueous solutions. The solvatochromism of the MNI molecule depends on the dipolarity and polarizability of the medium, and for water as a solvent, these two intermolecular interactions are crucial to understand the change of the liquid water structure with temperature.

**RESULTS AND DISCUSSION**

Recently, we have shown that liquid water exhibits a unique change of structure at about 43 °C, which is recorded because of a clear change of its SP10 and SdP9 by varying the temperature and also to a minor extent with the change of its SB.11 Only the SP, SdP, and SB scales clearly exhibit a bilinear behavior with temperature. It is important to emphasize that liquid water exhibits a large solvent acidity (SA)7,13 that decreases linearly with the increase of temperature, and it does not show any sign of structural change at about 43 °C.

It is known that the solvatochromic parameters for a given solvent (SP, SdP, SA, and SB) are assessed using suitable spectroscopic probes.9 Also, in order to measure a given solvatochromic parameter for a solvent it is necessary to use a molecular probe sensitive to the corresponding solvatochromic property and its corresponding homomorph, which allow us to discount other spurious solvatochromic contributions, which are not assigned to the solvatochromic property under study. The question raised in this work deals with determining the behavior expected for MNI in liquid water when changing the temperature the conclusions are as follows: (a) given that when temperature decreases below 43 °C the liquid water has a lower value of polarizability, see Figure 1 of ref 7, and barely changes its dipolarity, see Figure 2 of ref 7, it is clear that in this section In view of the behavior shown by the liquid water, it seems clear that this molecular probe should be very sensitive to the SP and the SdP of the liquid water in a way that would allow us to quantify how these magnitudes were varying with the temperature of water. To this end, it is important to note that we have recently shown14 that there are chromophores whose first electronic transition should be cataloged as a transition \( \pi \) (localized) \( \rightarrow \pi^* \) (delocalized). This electron transition arises from the excitation of an electron belonging to a \( \pi \) electron pair on a nitrogen atom with amine hybridization in the molecular structure and therefore strongly localized, to a \( \pi^* \) orbital delocalized along the molecular skeleton. Consequently, this \( \pi \) (localized) \( \rightarrow \pi^* \) (delocalized) electronic transition involves a strong electronic delocalization on photoexcitation, and it will be shifted bathochromically with the increase in the polarizability of the medium.15 This above-mentioned transition could also localize part of the charge at other resonant centers of a given structure and could cause variations in the dipolarity of the compound by transferring \( \pi \) charge from the amine nitrogen atom to the rest of the molecular skeleton.

The MNI can be regarded as good molecular candidate, Scheme 1, because of the rigidity of its molecular structure and the existence of a significant charge delocalization from the \( \pi \)-electron lone-pair of the nitrogen at the amino group to the nitro group substituted at a resonant position. This charge delocalization involves a significant sensitivity for MNI to the environment polarizability and dipolarity (Figure 1 and eq 1).

![Figure 1. Absorption spectra of MNI in liquid water at 20, 40, and 60 °C. The peak wavenumber for this absorption band is employed to account for its solvatochromism.](https://doi.org/10.1021/acsomega.b02722)

The solvatochromic behavior of MNI (Figure 1) has been shown to conform to eq 1:

\[
\nu/cm^{-1} = -(4837 \pm 211)SP - (2818 \pm 67)SdP + (37171 \pm 144)
\]

With \( n = 132 \), \( r = 0.984 \), and \( sd = 234 \, cm^{-1} \).

From this eq 1, the MNI solvatochromism does not depend on the SA nor on the SB; the first absorption band for MNI shifts bathochromically as both parameters SP and SdP increase their value. By analyzing the behavior expected for MNI in liquid water when changing the temperature the conclusions are as follows: (a) given that when temperature decreases below 43 °C the liquid water has a lower value of polarizability, see Figure 1 of ref 7, and barely changes its dipolarity, see Figure 2 of ref 7, it is clear that in this section
(−15 to 43 °C) as the temperature decreases, the MNI probe will shift its first absorption band hypsochromically, and (b) provided that above 43 °C water increases its polarizability, see Figure 1 of the ref 7, and very significantly decreases its dipolarity, see Figure 2 of ref 7, MNI should exhibit a much larger hypsochromism on increasing the temperature of liquid water from 43 up to 95 °C. Consequently, both temperature ranges above and below 43 °C the MNI will increase its wavenumber so that it will show the location of the temperature at which the structural change of liquid water occurs. Taking into account also the results provided by eq 1, the increase in the wavenumber of the first MNI absorption band should be higher below 43 °C, because of the change in polarization (dipolarity plus polarizability), than the increase in the wavenumber of MNI above 43 °C.

The results shown in Figure 2, which collect the peak wavenumber data for the first absorption band of MNI measured between −15 and 95 °C, clearly show that within this temperature range water only changes its structure around 43 °C. These results clearly validate the above-mentioned information and contribute to confirm the excellent solvatochromic behavior of the MNI probe.

The heat capacity (Figure 3) (cf. ref 16) is also proposed in biochemistry17 to exemplify changes in the structure of proteins with temperature, cf. temperature-induced conformational phase transitions in proteins. Figure 4 shows the dependence of the MNI probe in water at different temperatures with the corresponding heat capacity of the water at the same temperatures. Both the MNI solvatochromism and the heat capacity must exhibit a parallel behavior as it is seen in Figure 4. Two main trends are observed: on the one hand, two linear dependencies almost parallel between 0 and 35 °C and between 45 and 94.4 °C, that is, as the heat capacity increases, the maximum absorption of the MNI moves to the blue, at higher wavenumbers; a second tendency adds a different behavior between 35 and 45 °C, in which as the heat capacity increases the maximum of the probe moves to the red, this locus of temperature 35–45 °C can be interpreted as a crossover or transition stage between two water structures, a smooth and continuous transition, a non-uniform change in the structure of the water. It must be borne in mind that the solvatochromism of MNI depends mainly on the polarizability (−4837 SP) compared with dipolarity (−2818 SdP), besides the polarizability of the water in that section increases, with a point of change at 43 °C, and the dipolarity of the water decreases with a point of inflection also at 43 °C. The slight increase in polarizability between 35 and 45 °C compensates the decrease in SdP, and as a result, the MNI peak–wavenumber decreases as the heat capacity increases, to the converse of what happens from 0 to 35 °C and from 45 to 94.4 °C.

**Figure 2.** Relationship of the first absorption band of the MNI probe in liquid water vs temperature. The MNI solvatochromism shows a minimum at 43 °C. The red line denotes the fit of the data experimental values against temperature to a polynomial of order 3, $\tilde{\nu}_{abs} (\text{cm}^{-1}) = 22,308.7 - 6.2T + 0.0897T^2 - 0.000297T^3$.

**Figure 3.** Heat capacity of liquid water with temperature at ambient pressure.

**Figure 4.** Wavenumber of the peak absorption of the MNI molecule in liquid water vs the heat capacity at constant pressure of liquid water, at the same temperatures. The temperature range 35–45 °C demonstrates to be a crossover in which the peak absorption wavenumber decreases as the heat capacity increases, to the converse of what happens from 0 to 35 °C and from 45 to 94.4 °C.
Structure of water and electrolyte aqueous solutions. As mentioned above, a change of the structure of liquid water was first reported by Röntgen in 1892. In 2006, Dougherty and Howard tentatively reported the feasible explanation for the change, from a tetrahedral (hexagonal) structure to a planar (hexagonal) structure at ca. 35 °C. When electrolytes dissolved in water, they will affect its molecular structure, and therefore, the change of structure with temperature will change as well as the solvatochromism of MNI. The probe MNI has been dissolved in liquid water at different concentrations of KOH and HCl (Figure 5), in order to check the feasible variations in its solvatochromism and in the structure of water and therefore to be compared with the results already reported for the corresponding values of heat capacity (Figure 6, cf. ref 16). As discussed above, the heat capacity measurements with temperature correlate well with the solvatochromism of the MNI probe for pure liquid water. Alexandrov reported that as the molality of KOH increases to ca. 0.5 M, non-linear behavior in pure liquid water becomes a linear relationship for the heat capacity against temperature. This represents a progressive change in the response of the heat capacity with temperature at the minimum of the curve decreases when concentration increases. The temperature at the minimum of the heat capacity dependence reaches a temperature equal to 0 °C at a solution molality of 0.455 mol/kg.

Figure 6 shows data values for the heat capacity of both KOH water solutions and HCl water solutions. At 2% (ca. 0.5 M) of KOH the linear behavior is achieved; at 4% of KOH the linear behavior is kept up, and the heat capacity values are smaller with increasing the concentration of KOH. The same occurs for the HCl water solutions, Figure 6, but at 2% HCl concentration the linear relationship is not so clear, there is still a minimum, though it can be due to an experimental error.

From the solvatochromism of the MNI molecule in Figure 5, similar conclusions with Figure 6 can be drawn. In KOH 0.6 M the minimum disappears, a linear relationship is shown, and it tentatively represents only one liquid water structure for all the temperature range investigated. Even a tiny concentration of KOH (10⁻⁴ M) perturbs the solvatochromic curve with temperature, the maximum wavenumber is shifted to the red, a minimum is still present, at about 46.8 °C (obtained by the fit of the experimental data to a polynomial of order 3, 22.278.5 — 6.19028 T + 0.091777T² — 3.65378 × 10⁻⁴T³). The curve becomes flatter at 0.422 M, with a minimum at 46.2 °C—an apparent minimum is shown at ca. 40 °C, probably due to an experimental error in the measurements. At 0.6 M of KOH, no minimum can be found in the solvatochromism of MNI with temperature, in good correlation with the heat capacity measurements; this is a sign of a unique liquid structure at 0.6 M KOH water solutions. For the HCl water solutions, some differences are pointed out: first, a tiny amount of HCl does not perturb significantly the structure of water, ca. from 10⁻⁴ to 0.01 M, the curves as close to that in pure water. This could be feasible because the acidic-species contribution (H⁺) is embedded adequately into the liquid molecular structure; some slight modifications are happening, however, as the minimum is red-shifted from 44.6 in liquid water to 44.9 and 46.2 °C for the HCl solutions 10⁻⁴ and 10⁻² M, respectively. Finally, the water solution for HCl 0.6 M shows a minimum at 41.1 °C (Figure 5). The heat capacity for the 2% concentration of HCl also shows a kind of minimum at ca. 50 °C (Figure 6). A concentration 1 M of HCl (ca. 4% HCl) might have depicted a linear relationship for the MNI solvatochromism, without any minimum, in agreement with the heat capacity in Figure 6. To the reverse, MNI is not able to probe at 1 M concentration of HCl, the absorption spectra show an isosbestic point at 382 nm when changing the temperature, and those changes are not reversible with temperature. A feasible explanation lies in the fact that most probably, the −N−CH₃ site gets protonated, and tentatively the methyl group could be eliminated. For instance, N,N-dimethylacetamide exhibits a pK(NH₃⁺) of −0.28; actually, it is reported that for the −NH₃⁺ → −NH₂ equilibrium, pK(NH₃⁺) for acetamide and urea in water solutions increases with temperature, and as a matter of fact, it gets closer to 0.0. Therefore, for 1 M HCl in water solution, about 50% of the MNI molecules get protonated at the nitrogen site.

CONCLUSIONS

The MNI probe describes adequately the origin of the structural change of liquid water at 43 °C, and by plotting its solvatochromism against the corresponding heat capacity...
values for liquid water against temperature, a crossover temperature ranging from 35 to 45 °C is found.

In addition, the MNI solvatochromism accounts for the changes in liquid water described by heat capacity values. At KOH 0.6 M water solution, the MNI solvatochromic relationship with temperature shows no minimum in good agreement with the corresponding heat capacity values. Furthermore, even a small quantity of KOH affects the liquid water structure. To the reverse, an amount of HCl acid from 0.0001 to 0.01 M added to water hardly changes the structure of liquid water, the amount of H+ cation and Cl− anion gets embedded in the liquid water without any severe change in the solvatochromism of MNI, and from that, the structure of water does not change significantly.

## EXPERIMENTAL SECTION

### Materials

Ultrapure water of Milli-Q quality is used. The molecule MNI is synthesized as described elsewhere and purified using silica-gel chromatography (eluent: hexane/dichloromethane/ethylacetate, 55:30:15). KOH water solutions were prepared from potassium hydroxide from Aldrich. HCl water solutions were prepared from hydrochloric acid fuming 37% from Fluka.

### Characterization

All the UV–vis absorption spectra for MNI in pure water solutions were recorded on a Cary 5 spectrophotometer from −15 to 94.4 °C. The concentration used was 1.19 × 10−6 M, and the extinction coefficient in liquid water at 451 nm and at 20 °C is log ε = 4.23. No aggregation effects are monitored. Figure 2 of this paper shows a good resolution of the peak absorbance against temperature from −15 to 94.4 °C. All the measurements were recorded with the Cary 5 spectrometer (using quartz cells with an optical path-length of 10 cm) that exhibits for this range of data values (22 183 cm−1 to 22 183 cm−1) a standard deviation of 0.008 nm for a series of 10 measures at each wavelength peak. Those absorption spectra for MNI in electrolyte water solutions were recorded using a Shimadzu 2100 spectrophotometer from 5 to 88 °C (by using quartz cells with an optical path-length of 1 cm). A thermostat Fisons-Haake D8 GH was used for controlling temperature within ±0.1 °C.

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J.C. and J.C.V. contributed equally. The manuscript was written through contributions of both authors. Both authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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