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The influence of water structure on the rotational depolarization of fluorescence

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Abstract

A method based on the rotational depolarization of fluorescence of dye molecules acting as luminescent sonds is proposed to study the temperature changes in the structure of water. A visible discrepancy of the experimental points from the linear Perrin formula was found for erythrosine B in water. From the emission anisotropy and the mean fluorescence lifetime, the effective volume V of erythrosine B in water was determined. It was shown that the temperature changes in the effective volume can be associated with those in the water structure.

Keywords: Rotational depolarization; Effective volume; Water structure

1. Introduction

The properties of water have been systematically investigated for over 100 years [1,2]. However, it is only in the last three decades that certain progress has been made in this field [3,4]. It has been found that the structure of water near a water–solid interface often appears to be significantly different from that in bulk. The thermal structural changes of vicinal water are readily discernible contrary to those of bulk water for which these changes are less pronounced and have not been so firmly demonstrated [5,6]. Structural changes of bulk water strongly influence processes of biological and medical importance. Apart from the methods already used to describe these changes, each new method can prove useful, since the character of these changes has not been fully rationalized until now. In this paper we propose to apply a new method to describe the temperature changes in the structure of normal water.

2. Experimental details

To investigate the temperature-dependent structural changes of water in bulk we used the method of rotational depolarization of fluorescence. As is well known, the rotational depolarization of the dye fluorescence in water is usually very high. In view of this fact the molecule of erythrosine B, with the mean fluorescence lifetime $\tau_{fl} = 77$ ps [7], was selected. Such a short lifetime ensures that a relatively high emission anisotropy can be obtained. An aqueous solution of erythrosine B at $C = 10^{-6}$ M was investigated in a 1 cm cuvette. The erythrosine fluorescence originates above all from bulk water. The contribution of fluorescence from the dye adhering to the cuvette wall and present in the layer of vicinal water does not exceed 0.01% and can be totally neglected. This estimation results from the assumed thickness of vicinal water not exceeding 6 nm [8] and from the vicinal dye concentration higher by two orders of magnitude than that in bulk [9]. An extremely low concentration of the dye allowed the radiative and non-radiative energy transfer as well as the dye aggregation to be neglected.

The fluorescence anisotropy was measured by the single-photon-counting technique with an accuracy of 0.002 using the very sensitive apparatus described previously [10,11]. The temperature was controlled with an accuracy of 0.1 K. Fluorescence was recorded upon the front-face excitation and observation of the sample. The angle between the excitation beam and the observation axis was about 30°.

The values of the water viscosity $\eta(T)$ for each temperature between the melting and boiling points were calculated from the power law in the form [12]

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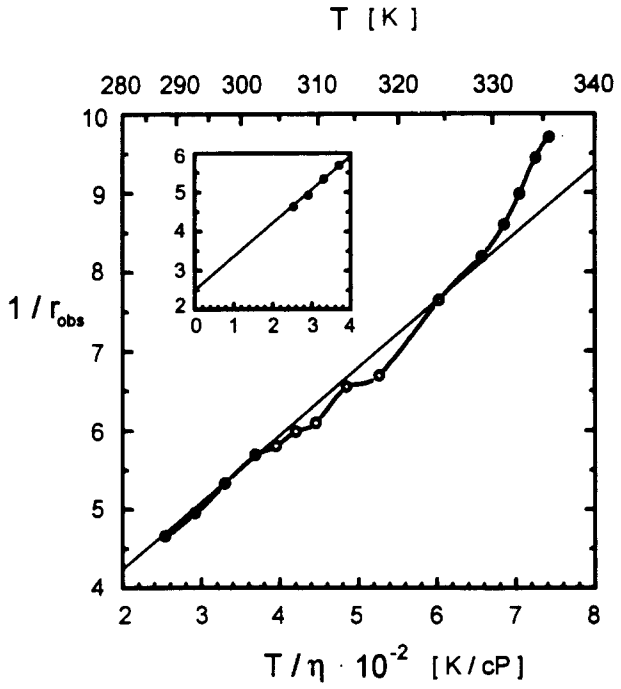

Fig. 1. Dependence of $1/r_{obs}$ of erythrosine B in water on T/η: —, line given by the Perrin formula Eq. (2); ○, ●, experimental points. As seen in the inset the value of r_0 equal to 0.4 was obtained as a result of linear extrapolation of four lowest $1/r_{obs}$ values.

$$\eta(T) = \frac{\eta_0}{(T/T_s - 1)^{1+T_s/T_b}} \quad (1)$$

where T_s and T_b are temperatures of singularity and boiling respectively. The values of $T_s = 227$ K and $\eta_0 = 0.138$ cP determined as best-fit parameters of Eq. (1) with the water viscosity data taken from [13] were used.

3. Results and discussion

In the simplest case of spherical molecules the rotational depolarization can be described by the Perrin [14] formula

$$\frac{1}{r_{obs}} = \frac{1}{r_0}\left(1 + \frac{\tau_{fl} kT}{V\eta}\right) \quad (2)$$

where $r_{obs} = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$ is the observed emission anisotropy [15], and $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensity components, parallel and perpendicular to the electrical vector of the exciting light respectively; r_0 denotes the limiting fluorescence anisotropy, τ_{fl} is the mean fluorescence lifetime in the excited state of the dye molecule, k is the Boltzmann constant, η is the solvent viscosity and V is the volume of the dye molecule with its solvent shell (the effective volume).

The results of the measurements are presented in Fig. 1. Linear dependence expected from Eq. (2) was not observed as clearly seen from the results shown. However, two temperature ranges 288–302 K and 328–335 K can be seen in which two linear dependences with different slopes appear. Rather roughly defined inflections at about 308 and 313 K can also be seen.

We would like to emphasize that the maximal error of the measurements herein reported, represented by the circle's radius, is much lower than the majority of deviations of the experimental points from linearity. For these discrepancies, temperature variations in the volume V as well as those in τ_{fl} may be responsible. Visser and van Hoek [7] have found that the fluorescence lifetime of erythrosine B in water is somewhat temperature dependent between 273 and 313 K. To obtain detailed information, the measurements of the erythrosine B fluorescence lifetime in water were carried out using the frequency-domain fluorometry method [16,17]. The modulation frequency was 2.96 GHz. Fluorescence was excited at $\lambda_{ex} = 514$ nm and observed at $\lambda_{obs} = 540$ nm. The results are listed in Table 1.

It is seen that the changes in τ_{fl} are evident and their character is quite unexpected. An explanation of this fact is not easy [18–20]. By taking into account the measured values of $\tau_{fl}(T)$, one can determine the effective volume V of erythrosine B using Eq. (1). In Fig. 2 the temperature changes in this volume are shown.

Table 1
Fluorescence mean decay times for erythrosine B in water measured for $\lambda_{ex} = 514$ nm and $\lambda_{obs} = 540$ nm, where the sample temperature was monotonically increased starting from the freezing point, τ_p is the lifetime calculated from the phase shift measurement, τ_m is the lifetime obtained from the modulation measurement, τ_{fl} is the average value of τ_p and τ_m, and ϕ is the phase shift and m denotes the modulation

T (K)	τ_p (ps)	$\Delta\tau_p$ (ps)	τ_m (ps)	$\Delta\tau_m$ (ps)	ϕ (°)	m	τ_{fl} (ps)	$\Delta\tau_{fl}$ (ps)
282.4	72	2	69	1	53.3	0.613	70.5	1.5
287.7	71	2	70	1	52.7	0.609	70.5	1.5
291.1	77	2	73	1	54.9	0.593	75	1.5
294.0	75	2	76	1	54.5	0.579	75.5	1.5
298.0	77	2	76	1	55.1	0.577	76.5	1.5
303.9	78	2	80	1	55.4	0.559	79	1.5
308.7	82	2	82	1	56.8	0.548	82	1.5
314.1	82	2	83	2	56.9	0.545	82.5	2
320.2	89	2	87	2	58.7	0.525	88	2
325.4	89	3	89	2	58.8	0.517	89	2.5
330.2	88	3	90	1	58.7	0.514	89	2
339.2	94	3	98	2	59.9	0.483	96	2.5

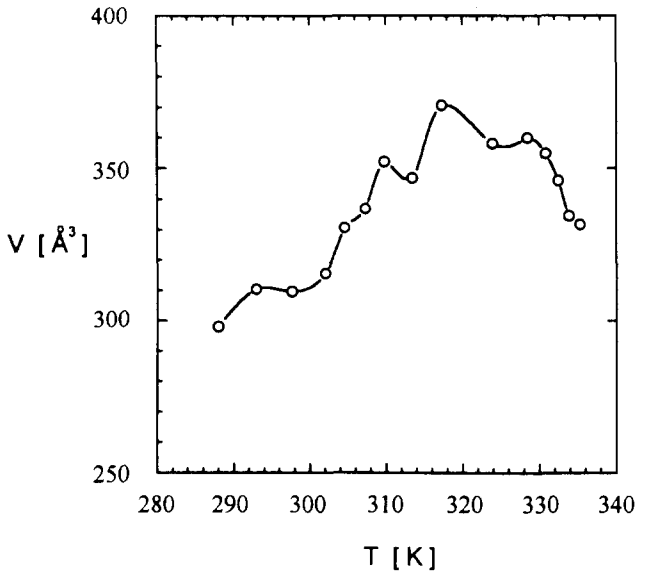

Fig. 2. Temperature changes in the effective volume V of erythrosine B in water determined from Eq. (2) for interpolated values of the lifetime $\tau_n(T)$ listed in Table 1.

In the transient region 302–310 K a visible increase in the effective volume V followed by a rapid decrease from $T = 328$ K was found. These changes in the effective volume can be associated with that in water structure taking place at 310 K stepwise. The non-monotonic character of volume temperature changes strongly supports this statement. Also the smooth temperature dependent course of V alters at 328 K and becomes sharp. This can also be associated with the change in water structure.

A certain effect on the discrepancy of three last experimental points from linearity at lowest viscosities (Fig. 1) may also result from disregarding the inertial effects in the Perrin [21] formula. The maximal value of the effective volume V amounts to 371 Å^3 and is close to the theoretical value $V_{th} = 374$ Å^3 computed from atomic increments [22]. Although the theoretical value may be treated only as a rough approximation, nevertheless the coincidence of V with V_{th} does not appear to be fortuitous, because the emission anisotropy r_{obs} and the lifetime τ_n, were measured with high accuracy. An approximate character of the Perrin formula obtained within the framework of the hydrodynamic Stokes–Einstein–Debye model (SED) for molecular reorientational relaxation should also be kept in mind. In this model the solvent is treated as a continuum defined only by its viscosity and temperature, but the size and shape of the solutes are not considered. Several rotational reorientation studies have stressed the limitations of the model (see [23] and references therein). However, for the studies of reorientational relaxation in systems with a given solute dissolved in a single solvent as a function of temperature, the SED model generally agrees with the experimental results [24,25]. To such category of solutions belongs the system under consideration.

It should be noted that the deviation of the emission anisotropy values from the linear dependence predicted by the Perrin formula appears, if the measurements are carried out for increasing temperatures starting from the freezing point. The emission anisotropy measurements performed for the decreasing temperature did not show the effect discussed. This is consistent with the results reported in [26], where among others the temperature dependence of the derivative $(\partial n/\partial T)_p$ of water refractive index under a constant pressure was investigated. These workers found two different temperature dependences of $(\partial n/\partial T)_p$ on T: (i) for samples heated to boiling before the measurements; (ii) for samples frozen before the measurements. In this latter case a visible minimum of $(\partial n/\partial T)_p$ at 308 K was observed. The temperature dependence of $(\partial n/\partial T)_p$ as well as the electronic polarizability α were investigated earlier by Frontasev and Schreiber [27] for several wavelengths. These workers observed the stepwise changes in $(\partial n/\partial T)_p$ and irregular changes in α at 308 and 328 K. On the basis of the temperature measurements of the intensity of water IR absorption peak in the vicinity of 2100 cm^{-1} it has been shown that this intensity has a well-defined inflection point at 305 K [28]. Very recently, some interesting results concerning the changes in water structure with the temperature have been obtained using the correlation method of scattered-light spectroscopy [29]. The monotonic character of the dependence of the correlation time as well as the scatterer volume on temperature have been reported. For temperature up to 305 ± 2 K an increase in the volume of water clusters and, at $T = 308 \pm 2$ K, a sharp drop in the correlation time was found. The results presented as well as the reviewed results of other workers (see also [30–32]) prove that in the range 303–310 K many anomalies of water properties may be observed. Certain spread in temperatures at which these anomalies take place is undoubtedly connected not only with the method employed but also with different water structure near aqueous interfaces and near impurities embedded in water.

The method offered in this paper using luminescent molecules as probes seems promising. It is characterized by high accuracy, which allows us to account for the water structure in bulk.

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