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Fluorescence investigations of the thermally induced conformational transition of poly(*N*-isopropylacrylamide)

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Abstract

Poly(*N*-isopropylacrylamide), PNIPAM, undergoes phase separation, upon heating under semi-dilute conditions, at 32°C, the lower critical solution temperature (LCST). Upon subsequent cooling, the dispersion rapidly clears again, at the LCST, to form a single, homogeneous phase. In this paper we report that fluorescence techniques, including quenching and time-resolved anisotropy measurements (TRAMS) on ultra-dilute (10^{-3} wt%) aqueous solutions of an acenaphthylene labelled sample, provide conclusive evidence that a coil collapse mechanism is implicated in the thermoreversible behaviour: the polymer undergoes a conformational switch from an open coil below 32°C to a compact, globular structure above the LCST. TRAMS reveal that a marked reduction in the segmental mobility of the polymer occurs at the onset of the LCST. In addition, a dramatic change in the accessibility of the label to aqueous-borne quenchers is also apparent at temperatures in excess of 32°C.

PNIPAM is capable of solubilising low molar mass species in its compact form: changes in the vibrational fine structure of the emission spectrum of pyrene have been used to monitor uptake and release of the probe. Excited state lifetime measurements have also proven to be sensitive monitors of the conformational switch of PNIPAM: at temperatures greater than 32°C, τ increases to ca. 160 ns which is indicative of pyrene sequestered in a hydrophobic, protective environment. Release of the probe into the aqueous phase results in a dramatic reduction of τ to ca. 130 ns which is characteristic of pyrene dispersed in water. These data highlight the potential of NIPAM based polymers to act as carriers in controlled release applications.

Fluorescence techniques have proved capable of monitoring changes in chain mobility, the degree of coil compaction and solubilization capacity (for organic guests) of PNIPAM as it is raised through its LCST. Information of this type is not readily obtained through other techniques and fluorescence approaches should prove invaluable in future investigations of the effects of chemical modification in attempts to manipulate the LCST of NIPAM-based systems. © 2001 Published by Elsevier Science Ltd.

Keywords: Poly(*N*-isopropylacrylamide); Fluorescence; Conformational transition

1. Introduction

Polymers which exhibit "smart" behaviour (i.e. respond to external stimuli such as pH and/or temperature) have attracted much interest in both the academic and industrial communities over a number of years [1,2]. Much of this attention stems from the fact that these systems are both fundamentally interesting and industrially important. They are involved in a diverse range of technologies such as controlled-release systems, agrochemicals, adhesives, coatings, enhancers for oil recovery, foodstuffs, rheology modifiers, personal care products, super absorbents, catalysis, inks and coding systems.

The use of fluorescence spectroscopy [3,4] has been particularly prominent in investigation of smart polymers since it allows examination of *ultra*-dilute solutions, permitting examination of purely *intra*-molecular effects. Indeed, luminescence techniques have proved invaluable in confirming [5,6] that poly(methacrylic acid), PMAA, undergoes a pH-induced conformational transition from an uncoiled (at high pH) to a *hyper*coiled structure at pH 4. With poly(acrylic acid) [7] this transition is much less dramatic: the polymer coil essentially adopts an open chain conformation at all values of pH.

Poly(*N*-isopropylacrylamide) (PNIPAM) undergoes a similar conformational transition to that of PMAA, except that contraction and expansion of the coil is controlled by temperature. The onset of the coil collapse occurs at 32°C, the lower critical solution temperature (LCST) [8,9]. Upon heating above the LCST, under semi-dilute conditions ($\geq 10^{-2}$ wt%), the polymer forms a turbid solution which *rapidly* turns clear again upon cooling. The thermodynamics of the process require that specific interactions occur between the polymer and the solvent which result in *both*

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a negative enthalpy change, ΔH_m, upon mixing and a negative entropy change, ΔS_m. In addition, the relative magnitudes of ΔH_m and ΔS_m must be such as to produce a reversal of the sign of the free energy change, ΔG_m, for the mixing process, within the range of temperatures over which the solvent remains a liquid. Although this type of behaviour is not uncommon for polymer systems [9,10] and the principles involved in achieving an LCST are well established [10], limited examples are available [9,10] in the literature. The phenomenon is much less common, however, in low molar mass species: a frequently cited example of a system displaying an LCST is that of nicotine and water [11].

Interest in PNIPAM has been stimulated on several fronts: the rapid reversibility of the phase separation process has intrigued polymer scientists and much effort has been directed towards establishing the mechanism of the LCST. Fluorescence spectroscopy has featured prominently in such studies due to its sensitivity and ability to probe at the molecular level, allowing the study of both intra- and intermolecular polymer interactions. Consequently, Winnik [4], using fluorescence spectroscopy and energy transfer measurements has provided convincing evidence to support the suggestion that the LCST behaviour of the linear polymer is governed by a 2-stage mechanism [4,12]. The first step involves intramolecular coil collapse. This is followed by intermolecular aggregation between collapsed coils. It is this feature that conveys rapid reversibility on the process.

Technological interest in PNIPAM derives from its ability to expand and contract "on demand" under thermal control. This property could lead to such systems being used as carriers with controlled release capabilities (in, e.g. drug delivery). Further potential applications for "smart" polymers include use in fibre optic sensors and as protein purification systems. Consequently, this has stimulated research into modification of PNIPAM [9,13] with a view to altering the temperature range over which the LCST occurs: manipulation of the onset temperature of the conformational switch of the polymer over a wide temperature range (including the physiological temperature of 37°C) could result in a much more extended range of industrial and medical applications than might be realised for PNIPAM, alone.

As part of a major programme targeted towards manipulation and control of the thermoreversible behaviour of NIPAM based systems, we present, in this paper, results from fluorescence spectroscopic measurements on an acenaphthylene labelled PNIPAM. A principal aim of the study was to obtain further information regarding the mechanism of the LCST phenomenon in PNIPAM and, in particular, its effect upon the intramolecular dynamics of the polymer and the nature of the conformations adopted above and below the transition temperature. In the latter respect, we wished to examine the extent to which luminescence techniques, particularly time-resolved anisotropy measurements (TRAMS) and fluorescence quenching, might provide information regarding thermoresponsive behaviour in "smart" polymers, which is inaccessible by other approaches.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAM) (Aldrich; 97%) was purified by multiple recrystallization from mixtures (60/40%) of toluene and hexane (both spectroscopic grade; Aldrich). Acenaphthylene (ACE) (Aldrich) was purified by multiple recrystallization from ethanol followed by vacuum sublimation. Methanol (Aldrich; Spectroscopic grade) was used as received. Nitromethane (Aldrich; Gold Label) was used as received. Acenaphthylene labelled poly(*N*-isopropylacrylamide) (ACE–PNIPAM) was prepared by copolymerisation of NIPAM with a trace amount (ca. 0.5 mol%) of ACE in dioxane solution (80 wt% of solvent) at 60°C using AIBN as initiator. This produces a polymer bearing a naphthyl chromophore rigidly bound to the chain backbone, through two single covalent bonds, such that motion independent of the polymer segment is not possible [6].

The polymer was purified by multiple reprecipitation from methanol into diethylether (May and Baker).

2.2. Characterization

Estimates of the molar mass of the labelled polymer were obtained by using the MALDI-TOF technique. These estimates were kindly performed by Thermobioanalysis Limited. Values of $M_n = 40$k and $M_w = 62$k were obtained for ACE–PNIPAM.

2.3. Cloud point measurements

The LCST was determined for ACE–PNIPAM, 10^{-2} wt%, in aqueous solution, from optical density measurements (Hitachi U-2010 spectrophotometer). The onset of the transition (estimated as the temperature marking the initial decrease in transmittance at 500 nm) was confirmed to be 32°C.

2.4. Instrumentation

Steady state fluorescence spectra were measured on a Perkin–Elmer LS50 spectrometer. Fluorescence lifetime data were acquired on an Edinburgh Instruments 199 time-correlated single photon counter. TRAMS were made at the synchrotron radiation source, SRS, Daresbury, UK. A complete description of the experimental set-up and a detailed discussion of analysis of anisotropy data can be found elsewhere [14].

Table 1
Bimolecular quenching constants for ACE–PNIPAM in aqueous and methanolic solutions

Sample	Temperature (°C)	$k_q(\times 10^{-9}\,M^{-1}\,s^{-1})$
ACE–PNIPAM (methanol)	25	7.8
	42	9.3
ACE–PNIPAM (H_2O)	22	7.00
	45	0.18

3. Results and discussion

3.1. Fluorescence quenching

The accessibility of a fluorescent species, F, to a low molar mass species, Q (which is capable of dynamic quenching of an excited state), can be estimated from quenching experiments.

This technique follows the general principle outlined below. The quenching process may be represented by Eq. (1)

$$F^* + Q \rightarrow F + Q^* \quad (1)$$

The efficiency with which a dynamic quencher accesses the excited state is described by the Stern–Volmer equation:

$$\tau^0/\tau = 1 + k_q\tau^0[Q] \quad (2)$$

where τ is the excited state lifetime at some concentration Q, τ^0 is that in the absence of Q and k_q is the bimolecular quenching constant. (Consequently, k_q can be considered as a measure of the "ease of access" of Q to F^*). If a fluorescently labelled polymer is used, information regarding the "openness" or compactness of the chain can be accrued from such experiments [15,16].

Stern–Volmer experiments were carried out on the ACE–PNIPAM polymer sample above and below its LCST using CH_3NO_2 as a quencher. In addition, quenching measurements were also made at various temperatures in methanol. The fluorescence decays were complex at each temperature and CH_3NO_2 concentration accessed in both methanol and water. A triple exponential function of the form of Eq. (3) was required to adequately describe the fluorescence decay curves, on statistical grounds

$$I(t) = A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3) \quad (3)$$

In order to treat the data (for Stern–Volmer analysis) an average lifetime $\langle\tau\rangle$ was calculated from Eq. (4)

$$\langle\tau\rangle = \frac{\sum A_i\tau_i^2}{\sum A_i\tau_I} \quad (4)$$

Linear Stern–Volmer plots were obtained for each system at each temperature accessed. The resulting bimolecular quenching constants (derived from the $\langle\tau\rangle$ data) are listed in Table 1. Examination of these k_q values for ACE–PNIPAM dissolved in methanol reveals that they are close to that which would be expected for a diffusion controlled process at each temperature. This infers that ACE–PNIPAM adopts, essentially, an open chain conformation under all conditions in methanol. In addition, a "normal" thermal response is observed in that the bimolecular quenching constant increases with increasing temperature. This is consistent with the fact that these samples do not show an LCST in methanol and consequently do not exhibit the conformational change that would accompany such behaviour.

When ACE–PNIPAM was dispersed in aqueous media, more complex quenching data were observed. At 22°C (below the LCST) a k_q value of $7.0\times10^9\,M^{-1}\,s^{-1}$ was obtained (cf. Table 1). This is similar to that obtained in methanol at a comparable temperature to viscosity ratio. This implies that the polymer adopts an expanded conformation under these conditions. Further examination of Table 1 reveals that the quenching efficiency decreases markedly when the temperature is increased to 45°C. This is consistent with collapse of the PNIPAM into a globular structure at the LCST: this would result in hindered access to the label by the quencher. The resultant k_q value is comparable to that obtained, using the same quencher, for ACE-labelled poly(methacrylic acid), PMAA, at low pH [17], in its hypercoiled conformation.

These data provide corroborative evidence in support of the proposal [4,12] that an intramolecular contraction of individual polymer chains precedes intermolecular aggregation in the mechanism governing the thermoreversible behaviour of PNIPAM. To our knowledge, this is the first instance in which purely dynamic quenching, using a water-borne quencher, has been used to demonstrate, through k_q, the compacted and hydrophobic nature of PNIPAM's interiors above the LCST. Winnik [18] has used both CH_3NO_2 and ethyl pyridinium bromide as quenchers of fluorescence from pyrene-based labels of PNIPAM. Her data are in broad agreement with the current findings in that the ease of access of the label, by the quencher, was shown to be dramatically reduced as the temperature was raised above the LCST of the system [18]. However, a direct comparison cannot be made between the current data and those reported by Winnik [18] since the earlier Stern–Volmer plots (based on fluorescence *intensity* data) were curved. In her data analysis, Winnik assumed that a fraction of the pyrenyl labels were excluded from external quenching. This approach, allied to the fact that excimer formation between pyrene species was apparent, did not allow derivation of rate constants, k_q, characteristic of the dynamic component of the quenching process, which would permit comparison with the current data. In any case, it is unlikely that the emission intensity data would prove equivalent to those based upon excited state lifetime measurements since, in our experience, the former are almost invariably affected by static quenching interactions, when CH_3NO_2 is used as quencher.

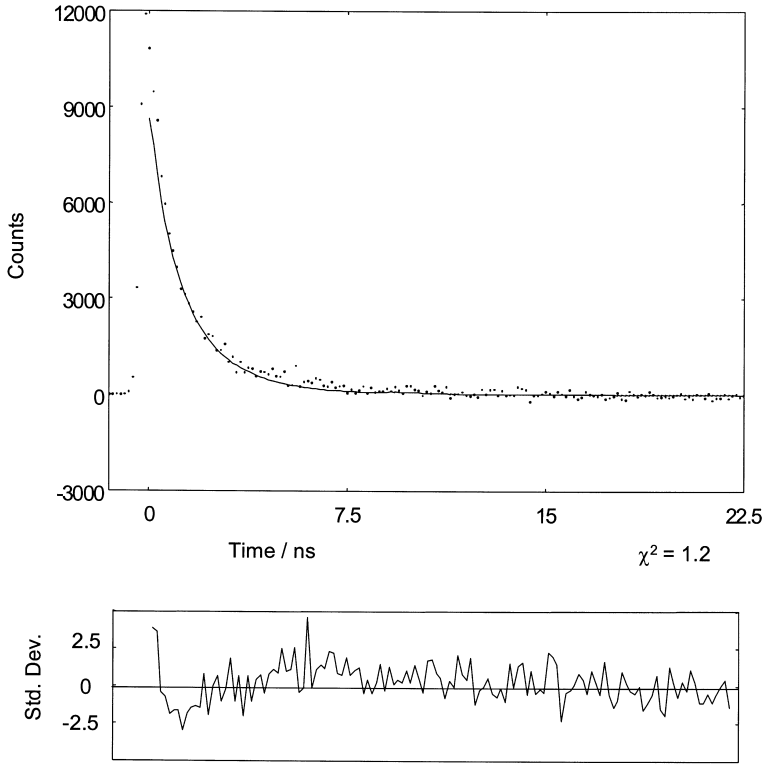

Fig. 1. Difference curve, $D(t)$, and impulse reconvolution fit for ACE–PNIPAM (10^{-3} wt%) in methanol solution at 25°C.

3.2. TRAMS

Time-resolved (fluorescence) anisotropy measurements, TRAMS, upon suitably labelled samples, allow estimation of the degree of macromolecular segmental mobility within a polymer. Briefly, the experiment involves the use of vertically polarized excitation radiation. Detection of the 2 time-dependent orthogonal components of the intensity of fluorescence emitted from the label, in planes parallel and perpendicular to that of the polarized excitation, $I_{\|}(t)$ and $I_{\perp}(t)$, respectively, is achieved by use of a rotatable polarizer [14]. $I_{\|}(t)$ and $I_{\perp}(t)$ are related to the anisotropy $r(t)$ via Eq. (5);

$$r(t) = \frac{i_{\|}(t) - i_{\perp}(t)}{i_{\|}(t) + 2i_{\perp}(t)} = \frac{d(t)}{s(t)} \tag{5}$$

where $d(t)$ is the time-dependent "difference function", $s(t)$, the "sum function" and $i(t)$ represents emission intensity data which have been "deconvoluted" to eliminate the perturbing influence of the time-dependent excitation pulse.

For a simple, single relaxation process, involving reorientation of a "pseudo-spherical" rotor, the time-dependent anisotropy $r(t)$ can be described by a single exponential function of the form of Eq. (6)

$$r(t) = r_0 \exp(-t/\tau_c) \tag{6}$$

where r_0 is the intrinsic anisotropy and τ_c, is the correlation time characteristic of the motion under study.

There are several methods of analysis that can be used to recover relaxation information from anisotropy data. The merits of the various approaches have been discussed at length elsewhere [14]. For the current decay sets, we have favoured the impulse reconvolution (IR) technique [19] that is particularly useful [14] when the timescale of the motion under investigation is close to that of the duration of the excitation pulse.

When dispersed in methanol (10^{-3} wt%) at 25°C, the relaxation behaviour of ACE–PNIPAM was adequately described by Eq. (6) (cf. Fig. 1). (Fig. 1 shows an IR fit to the *observed* difference curve, $D(t)$). The low value of χ^2 (χ^2 should be close to unity for a good fit) and the random distribution of residuals provide statistical confidence in the quality of fit. The resultant estimate for τ_c of ca. 1.9 ns indicates that PNIPAM, in methanol at 25°C, has a reduced segmental mobility when compared to that of poly(dimethylacrylamide), PDMAC, under similar conditions [20]. (Presumably this reflects, in part, the effect of the bulky *n*-isopropyl group in PNIPAM, which hinders segmental backbone motion).

The complexity in anisotropy behaviour increases markedly when ACE–PNIPAM is examined in aqueous media: Fig. 2a shows an example of an IR fit to the difference function, $D(t)$, of ACE–PNIPAM at 25°C, assuming a single exponential model of the form of Eq. (6) for $r(t)$. Although the value of χ^2 is close to unity, a non-random distribution of the residuals is clearly evident, particularly at early times within the decay. This implies that the anisotropy behaviour is not strictly described by Eq. (6). The improvement in

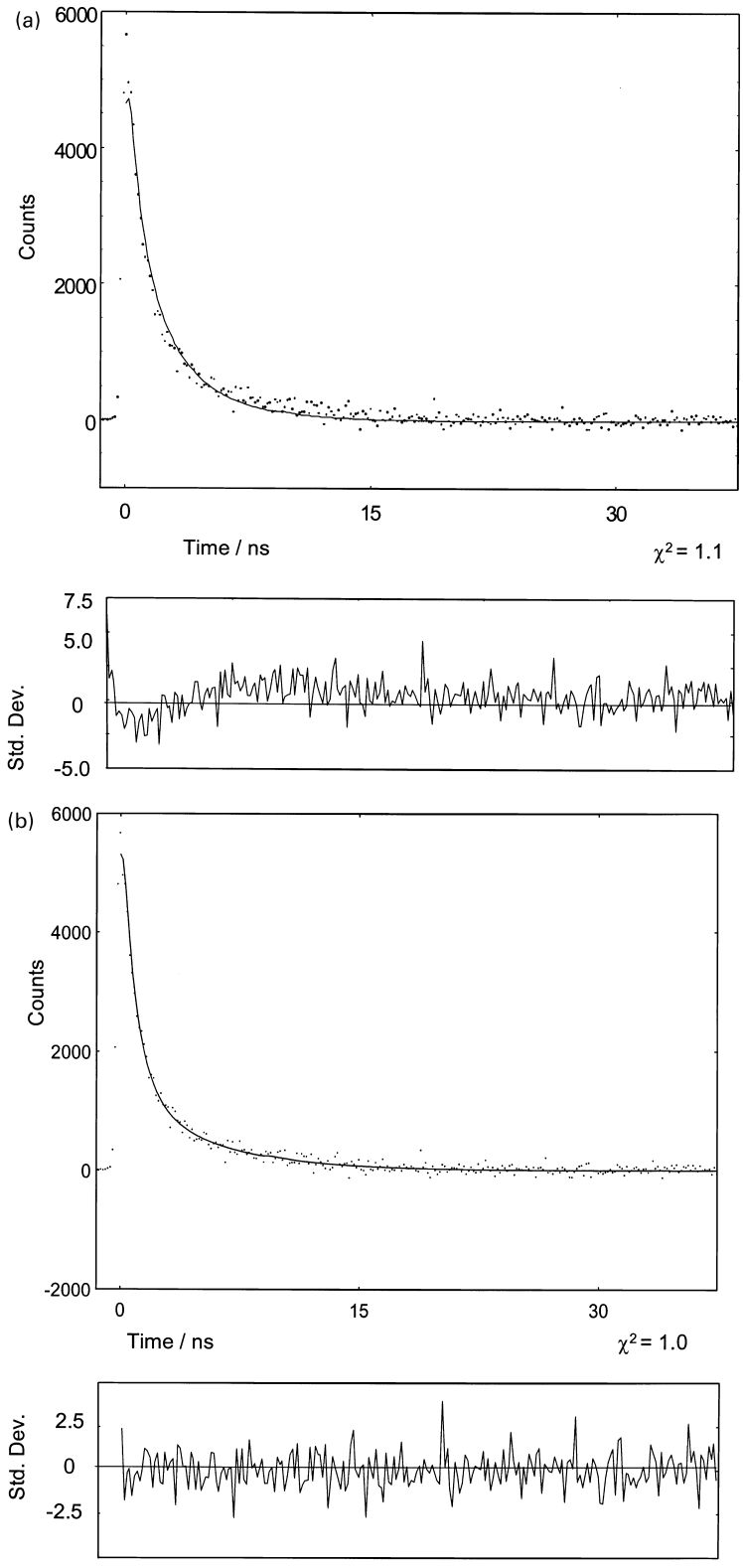

Fig. 2. (a) Difference curve, $D(t)$, and impulse reconvolution fit [using a single exponential model for $r(t)$] for ACE–PNIPAM (10^{-3} wt%) in aqueous solution at 25°C. (b) as for (a) but using a double exponential function.

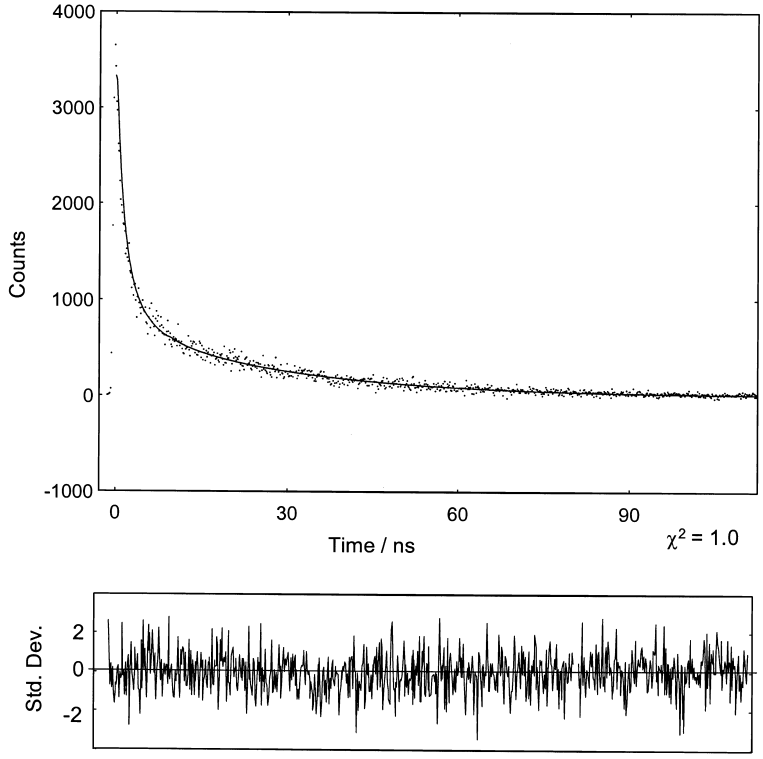

Fig. 3. Difference curve, $D(t)$, and IR fit [using a double exponential model for $r(t)$] for ACE–PNIPAM (10^{-3} wt%) in aqueous solution at 37°C.

fitting statistics, which results upon use of a double exponential model (as described by Eq. (7)) is clearly visible in Fig. 2b

$$r(t) = r_1 \exp(-t/\tau_{c1}) + r_2 \exp(-t/\tau_{c2}) \quad (7)$$

Correlation times of ca. 1 and 7 ns, respectively, result from this form of analysis.

Similarly, a single exponential function was a poor descriptor of the decay of anisotropy from ACE–PNIPAM in aqueous media at 37°C: a χ^2 value of ca. 1.9 coupled with a non-random distribution of residuals resulted from this form of analysis. Fig. 3 shows an IR fit to $D(t)$ using a double exponential model for $r(t)$ for ACE–PNIPAM above the LCST. The random distribution of residuals indicates that this model provides an adequate description of the fluorescence, on statistical grounds. The values of τ_c which result from this form of analysis are ca. 3 and 450 ns, respectively. Clearly, a dramatic reduction in the segmental mobility of the polymer occurs above the LCST, which is consistent with collapse of the chains into compact globular structures.

It is not clear at this time why $r(t)$ requires a function of the form of Eq. (7) for adequate description. Such complex anisotropy behaviour has been observed previously in water-soluble systems [6,7] and presumably reflects the heterogeneous range of environments afforded to the ACE label, particularly in the compact form of PNIPAM: it is conceivable that large, slow moving globules may be linked by much looser, more flexible units producing a structure akin to that of a string of pearls. Indeed, such a model has been proposed [3] previously for PMAA in its hypercoiled form. Alternatively, it could be that the complexity of the anisotropy behaviour merely reflects the existence of a broad distribution of correlation times, which cannot be associated with distinct rotating entities.

Despite the uncertainty regarding assignment of an appropriate dynamic model for this system, valuable relaxation information can still be derived for PNIPAM (under the various experimental conditions) by adopting a simplistic approach as far as data analysis is concerned [21]. Modelling of the anisotropy decays by simple, single exponential functions, of the form of Eq. (6), *is* capable of broadly characterizing the polymer dynamics and thereby revealing unique information regarding the mobility of single, isolated chains. This analysis procedure was adopted to give an *average* rate of macromolecular motion, τ_c^{-1}. The thermal dependence of τ_c^{-1} for ACE–PNIPAM, in both methanol and aqueous solutions, respectively, are shown, in Arrhenius form, in Fig. 4.

ACE–PNIPAM exhibits normal Arrhenius behaviour in methanol over the temperature range accessed in these experiments. The resultant activation energy, E_a, was estimated as ca. 13.4 ± 0.5 kJ mol^{-1}. This value is very similar to those obtained for poly(dimethylacrylamide) PDMAC (11.4 kJ mol^{-1}), PMAA (14.3 kJ mol^{-1}) and poly(acrylic acid) PAA (11.4 kJ mol^{-1}), in dilute methanolic solution [7]. A common approach to resolving the separate contributions of motion of the polymer segment over a potential

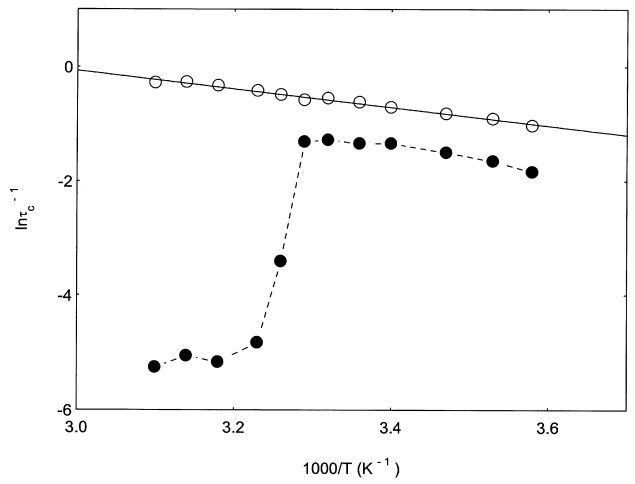

Fig. 4. Arrhenius representation of the rate of macromolecular motion, τ_c^{-1}, for ACE–NIPAM in methanol (○), and aqueous solution (●), respectively.

energy barrier, E_s, and solvent flow, E_η, to the overall activation energy, E_a, involves invoking the adaptation by Helfand et al. [22,23] of Kramers' [24] treatment of the passage of a particle over a potential energy barrier. Within this framework, and in the high friction limit, (which might be expected to apply to macromolecular segmental relaxation in solvents of moderate viscosity) τ_c would be given by

$$\tau_c = B\eta \exp(E_s/RT) \tag{8}$$

where B is a constant provided, as in this case [25], that the temperature dependence of the solvent viscosity, η, is Arrhenius in form, over the relevant temperature range. This, in turn, would relate E_s to E_η via

$$E_s = E_a - E_\eta \tag{9}$$

Given that, for methanol, $E_\eta = 11\ \text{kJ mol}^{-1}$ and E_a was estimated from values of viscosity over the temperature range 175–323 K taken from Ref. [25], it is clear that the solvent exerts a dominant influence upon the relaxation characteristics of each of these polymers. This is an interesting observation and a detailed study of the effects of solvent-polymer solute interaction is currently in progress (which will complement the investigations of Waldow et al. [26], Adolf et al. [27] and Zhu et al. [28,29] for example).

Further examination of Fig. 4 reveals that, in aqueous solution, ACE–PNIPAM shows non-Arrhenius behaviour: a large discontinuity is apparent at 32°C, the temperature which marks the onset of phase separation in more concentrated solutions. Several additional features are apparent in consideration of this plot and are worthy of note:

(a) at temperatures lower than ca. 32°C, the dynamic behaviour of ACE–PNIPAM is much as expected: the rate of macromolecular motion increases with temperature.
(b) the onset of the LCST for ACE–PNIPAM is accompanied by a marked decrease in τ_c^{-1}. (Clearly, the thermoreversible coil to globule transition of PNIPAM results in a dramatic reduction in the macromolecule's segmental mobility. Indeed, the magnitude of this change in macromolecular mobility is similar to that observed for the pH dependent conformational change of PMAA [6]).

The current TRAMS data provide irrefutable, supporting evidence for the dual–mode mechanism of phase separation for the PNIPAM/water system proposed by Winnik [4] on the basis of intramolecular fluorescence energy transfer experiments: the first stage involves intramolecular coil collapse, intermolecular aggregation of the collapsed coils occurring subsequently, as a distinct second step. It is this mechanism and resultant absence of extensive interchain entanglements, which conveys rapid reversibility upon the thermally induced phase transition.

3.3. Pyrene probe experiments

The fluorescence emission spectrum of pyrene is sensitive to the polarity of the environment in which it is dispersed [30,31]. In particular, the ratio of the fluorescence intensities of two vibronic bands, termed III and I, respectively, can serve as a sensor of hydrophobicity. Consequently, this property has been used by polymer chemists interested in the conformational behaviour of water soluble polymers. PMAA, for example, in the *hyper* coiled structure formed at low pH, is capable of solubilizing low molar mass material [32]. A III/I intensity ratio of 1.0 has been reported [15,16,33] for pyrene under these conditions, which decreases to 0.55 at high pH. PAA, on the other hand is not capable of accommodating organic guests within its coils at any pH. As a result, pyrene dispersed in PAA solutions exhibits a constant III/I ratio (0.55) regardless of pH [34].

In the current work, pyrene (10^{-6} M) was dispersed in ACE–PNIPAM (10^{-2} wt%). Examples of the fluorescence emission spectra, recorded at 25 and 37°C, respectively, are shown in Fig. 5. Clearly, the vibrational fine structure of the probe is sensitive to the change in conformation, which occurs at the LCST: the III/I ratio increases from 0.55 (characteristic of that of pyrene in water) to 0.7 (indicative of a more hydrophobic environment). Although this value is less than that observed for dispersions of the probe in dilute solutions of PMAA [15,16,33] at low pH, it is of the same order as that of certain MAA copolymer samples containing styrene [15] or acenaphthylene [35] and copolymers of AA with methylmethacrylate [34], styrene [34] or vinylnaphthalene [36].

Examination of the excitation spectra recorded at 25 and 37°C, respectively, reveal much less marked changes in profile: the only discernible difference is that a slight red shift occurs in the spectrum at higher temperature. Similar behaviour has been reported [37] in systems in which excimer formation involving 'preassociated' pyrenes occurs and

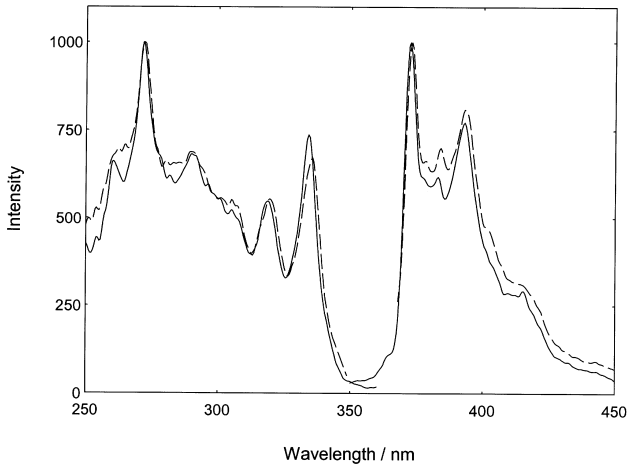

Fig. 5. Steady state fluorescence excitation and emission spectra of pyrene (10^{-6} M) dispersed in PNIPAM (10^{-2} wt%) at 25°C (—) and 37°C (- - -), respectively. ($\lambda_{ex} = 345$ nm; $\lambda_{em} = 380$ nm).

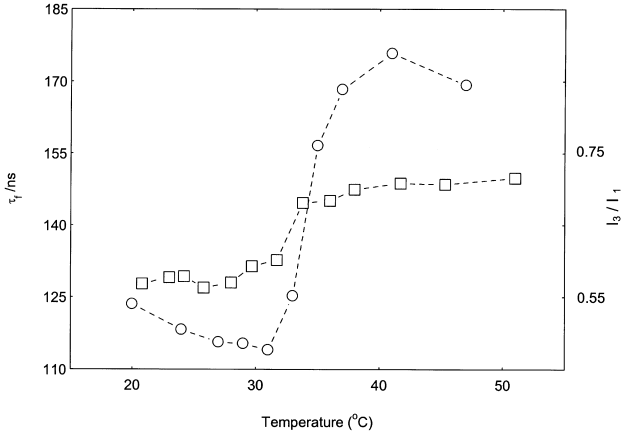

Fig. 6. III/I intensity ratios (□) and fluorescence lifetime, τ (○) as a function of temperature, respectively, for pyrene (10^{-6} M) dispersed in ACE–PNIPAM [All samples contained 10^{-2} wt% in polymer].

also when probes are solubilized within the hydrophobic cavities of certain water-soluble polymers [15,34]. In the latter instance, the spectral shifts were considered to be indicative of pyrene molecules residing in a solvating hydrophobically-rich medium. Since there is no evidence of excimer formation in the current work, it seems likely that the spectral shifts reflect the fact that above the LCST, the polymer chain collapses, solubilising the probes into hydrophobic domains in the process.

The III/I intensity ratios from the emission spectra were measured at various temperatures and the data plotted in Fig. 6. With reference to Fig. 6 it is clear that the polarity of the microenvironment sensed by the dispersed probe, depends upon the temperature of the system. The dependence of I_3/I_1 upon temperature acts as a sensor of the collapse of the polymer chain; from low temperature where the probe resides in the aqueous phase to the hydrophobic domains formed within the compact coils above the LCST. Furthermore, a clear discontinuity in the I_3/I_1 vs. temperature profile is apparent at ca. 32°C. This is close to that at which phase separation occurs as determined by cloud point measurements (see Section 2) and presumably marks the onset of the LCST.

The excited state lifetime, τ_f of pyrene is also sensitive to its environment [38] and has been used as a gauge of hydrophobicity in water-dispersible systems [15,34,39]. Fluorescence decays were measured for pyrene (10^{-6} M) dispersed within ACE–PNIPAM at various temperatures.

The transient fluorescences of the probe were complex at each temperature accessed requiring a minimum of three exponential terms of the form of Eq. (3) for adequate statistical description. In this respect the temporal characteristics of the fluorescence from the dispersed pyrene are similar to those observed for the ACE labelled PNIPAM system. (This is not surprising. *At least* 2 distinct population distributions could be envisaged for the probe: one would be associated with pyrenes residing in the aqueous phase while the second could have its origins in the protected environment of the hyrophobic, collapsed coil. However, it is equally conceivable that a gradation of environments would be accessed by the probes, resulting in more complex excited state behaviour, which could find adequate description by use of, for example, a triple exponential function). In this respect, the transient pyrene fluorescences suffer the same limitations in terms of their kinetic interpretation as those discussed in the context of the labelled polymer (see earlier). Consequently, the same "solution" has been adopted here, in characterizing the duration of the existence of the excited state population: an average τ_f, was calculated, using Eq. (4), for each dispersion and plotted as a function of temperature in Fig. 6.

With reference to Fig. 6 it is clear that the lifetime of pyrene dispersed in the PNIPAM solution at temperatures less than 32°C is identical (within experimental error) to that in water (130 ± 5 ns). As the temperature is increased the conformational transition of PNIPAM is clearly apparent: the lifetime of pyrene increases as the polymer coil collapses to form a globule at the LCST. (Presumably this reflects encapsulation of the probe into the hydrophobic confines of the domains formed upon collapse of the chains. In this way the lifetime data mirror the trends of the I_3/I_1 ratios (see earlier). Indeed, the pyrene lifetime data seem to offer a more reliable measure of hydrophobicity than steady state spectroscopy of the probe).

4. Conclusions

1. Fluorescence techniques have proven useful in probing the thermoresponsive behaviour of PNIPAM. The data, which can be obtained, are inaccessible by other methods. Consequently, photophysical approaches should prove invaluable in future studies of chemically-modified NIPAM-based systems.

2. Luminescence spectroscopy has revealed that PNIPAM, when dissolved in methanol, adopts a flexible open coil conformation at all temperatures. In contrast, more complex dynamic behaviour is observed in aqueous solution: time-resolved anisotropy and fluorescence quenching measurements, using an ACE label, have confirmed that PNIPAM undergoes a conformational change as the first stage of the mechanism governing the thermoreversible phase separation behaviour of this polymer.
3. PNIPAM is capable of soubilizing organic species such as pyrene at temperatures above its LCST. These data illustrate the potential of NIPAM-based polymers for use as carriers in controlled release applications.

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