Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Photochemistry and Photobiology A: Chemistry 180 (2006) 1–8
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Invited paper

Time-resolved fluorescence anisotropy measurements in the study of poly(*N*-isopropyl acrylamide)-based systems☆

Choong Kooi Chee, Steve Rimmer, Ramune Rutkaite, Ian Soutar✠, Linda Swanson*

Department of Chemistry, University of Sheffield, Sheffield S3 7HF, UK

Received 16 November 2005; received in revised form 24 January 2006; accepted 25 January 2006
Available online 24 March 2006

Abstract

In this article we present recent data highlighting the power of (fluorescence) time-resolved anisotropy measurements (TRAMs) in the study of the thermoresponsive behaviour of poly(*N*-isopropylcrylamide), PNIPAM, and NIPAM-based systems. PNIPAM shows a lower critical solution temperature (LCST) at 32 °C when phase separation occurs as the macromolecule collapses into a globular structure. Manipulation of the conformational switch of PNIPAM results through changing the hydrophobic to hydrophilic balance within the polymer. However, although a degree of control of the LCST is possible through such a strategy, simple free radical copolymerisation serves to reduce the magnitude of the transition. Synthesis of graft copolymers based on NIPAM is offered as a solution to this problem: fine tuning of the thermal response results through control of the entropic term governing the thermodynamics of the process as revealed by TRAMs. Independent fluorescence labelling of both the backbone and the grafts allows the contribution of the chain dynamics from each of these sites to the thermal response to be monitored.

Keywords: Fluorescence; Time-resolved anisotropy measurements; Poly(*N*-isopropylacrylamide); Graft copolymers; Thermoresponsive; Conformational transition

1. Introduction

Over a number of years we have been interested in water-soluble polymers which exhibit "smart" behaviour (i.e. which have the ability to change their conformation in response to an external stimulus such as temperature or pH). The use of luminescence spectroscopy has featured prominently in our attempts to characterise the solution behaviour of such systems [1–8].

In general, the popularity of luminescence techniques has increased markedly over the past two decades as researchers attempt to probe the conformations of water-soluble macromolecules (see for example, [9–11] and references therein). The use of fluorescence (and phosphorescence) spectroscopy permits examination of ultra-dilute polymer solutions, allowing interrogation of purely intra-molecular effects. Time-resolved anisotropy measurements (TRAMs) is a powerful addition to the fluorescence armory available to the experimentalist, in that it allows direct probing of the polymer dynamics if suitably labelled samples are used. Consequently, such measurements can reveal the conformations of macromolecules under various solution conditions: for example, TRAMs have confirmed [3] that poly(methacrylic acid), switches conformation from an expanded coil at high pH to a hypercoiled structure at pH 4. With poly(acrylic acid), this transition is much less dramatic: the polymer adopts an expanded chain conformation at all values of pH [12]. Poly(*N*-isopropylacrylamide), PNIPAM, is another example of a polymer which can undergo a switch in conformation except that in this case the smart response is triggered thermally; at room temperature under semi dilute conditions PNIPAM forms a clear solution in aqueous media which rapidly turns cloudy on heating above 32 °C, the lower critical solution temperature (LCST) [13,14]. Since the intrinsic viscosity of PNIPAM decreases at the LCST [14], this has lead to its use as a rheology modifier in industrial applications [15]. Furthermore, at temperatures in excess of 32 °C, linear PNIPAM can solubilise organic species in its compact form while decreasing the temperature below the LCST allows release of the material into the aqueous phase [16]. The ability to expand and contract on demand has led researchers to speculate that PNIPAM could form the basis of a carrier system which may lend its use to

☆ Part of this work was presented at the International Conference on Photochemistry 2005, held 25–29 July in Cairns, Australia.

* Corresponding author. Tel.: +44 114 222 9564.
E-mail address: l.swanson@sheffield.ac.uk (L. Swanson).
✠ Deceased.

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2006.01.018

many other industrial and medical applications [15]. In view of this potential, much effort has been directed towards manipulation of the conformational switch away from 32 °C to cover a wide temperature range (e.g. 4–100 °C) including the physiological temperature of 37 °C [15]. Studies have not only been restricted to linear PNIPAM, more complex gel structures and interpenetrating networks [15,17,18] have also received attention: researchers have been keen to understand the kinetic and dynamic processes which occur during collapse of these materials both on the macroscopic and microscopic scale.

In this article, we hope to illustrate the scope and power of TRAMs through reference, in particular, to recent studies in our laboratories of the thermosresponsive behaviour of PNIPAM and our attempts to modify the temperature of the conformational switch.

2. Time-resolved anisotropy measurements (TRAMs)

Time-resolved anisotropy measurements involve the use of vertically polarized radiation in order to photoselect a distribution of chrompohores whose transition vectors (for the absorption process) are aligned parallel to that of the incident excitation. This creates a degree of anisotropy (r) or optical order within an otherwise isotropic population. Molecular motion within the duration of the excited state fluorescence lifetime results in a loss of r as the photoselected population returns to a random orientation. Monitoring this loss of r as a function of time (or the decay of r) is possible by measuring the time dependent intensity of fluorescence in the vertical ($I_{||}(t)$) and perpendicular ($I_{\perp}(t)$) planes, respectively. This allows estimation of the decay of anisotropy $r(t)$ via Eq. (1)

$$r(t) = \frac{i_{||}(t) - i_{\perp}(t)}{i_{||}(t) + 2i_{\perp}(t)} = \frac{d(t)}{s(t)} \quad (1)$$

where $d(t)$ and $s(t)$ are the difference and sum function, respectively.

Modelling $r(t)$ by an exponential function of the form of Eq. (2)

$$r(t) = r_o \exp\left(\frac{-t}{\tau_c}\right) \quad (2)$$

allows derivation of the correlation time, τ_c, which is a measure of the molecular motion extant within the chromophore population. There are several methods of analysis of time-resolved anisotropy data in recovery of relaxation information, the merits of which have been discussed, at length, elsewhere [19,20]. In this review article, we present data derived exclusively from impulse reconvolution analysis [21,22].

Introduction of trace amounts (typically 0.5 mol%) of a fluorescent label during polymer synthesis, allows motion of the fluorescent reporter to be observed by TRAMs. If it is assumed that motion of the label reflects that of the polymer then it is possible to monitor macromolecular dynamics directly. Judicious choice of label can allow various sites within the macromolecule to be monitored: for example, the chain ends, ester side group substituents and the internal segments, respectively. For the latter units, we have over the years, favoured use of acenaphthylene

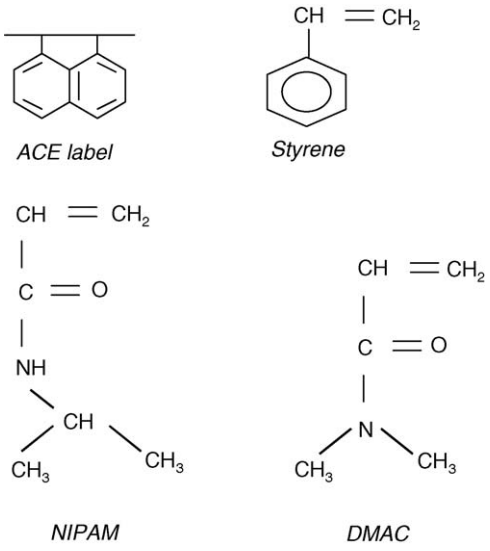


Fig. 1. Chemical structures of the label and monomers used in this study.

(ACE) (cf. Fig. 1) which can be considered an ideal label for TRAMs since it is covalently attached at two sites to the main chain such that motion independent from that of the backbone is impossible. Consequently, ACE reports exclusively on polymer segmental motion. However, since ACE is a hydrophobic monomer, it may be viewed as a species, which could itself, potentially modify the behaviour of PNIPAM. We have ensured that by maintaining the loading levels to trace amounts such perturbation is precluded: cloud point measurements (in determination of the LCST) carried out on labelled samples were in agreement with those run in the absence of a fluorescent reporter.

Relaxation information presented in the current article is derived through TRAMs on ACE labelled NIPAM-based samples (the structure of the NIPAM unit can also be found in Fig. 1). The excitation source used to generate the data was that from the Synchrotron Radiation Source (SRS), Daresbury, UK. A complete description of the SRS and associated detection system can be found elsewhere [19].

3. The LCST of PNIPAM: probing the mechanism

The first reports to recognise that PNIPAM displayed, what was considered at the time, unusual solution behaviour was that of Scarpa et al. in 1967 [13] and Heskins and Guillet in 1969 [14]: both groups observed that PNIPAM became less soluble upon heating forming a precipitate at 31 °C, the LCST. In 1975 Taylor and Cerankowski argued [23] that LCST behaviour in polymers should be a fairly common phenomenon being governed by the hydrophobic to hydrophilic balance within the macromolecule in question. In subsequent years, this theory has been proven experimentally [24–29] by researchers keen to modify the temperature of the LCST through copolymerization of various amounts of hydrophobic or hydrophilic monomer units with NIPAM. The thermodynamic principles of LCST behaviour dictate that specific interactions between the polymer and solvent are required which result in a negative change in both the enthalpy (ΔH_m) and entropy (ΔS_m) of mixing. However, the exact nature and

extent of these interactions remains open to much debate and speculation [30–33]. For PNIPAM at 31 °C, the magnitude of the change is such that the free energy of mixing (ΔG_m) becomes positive resulting in precipitation of the polymer. The fact that this process is both rapid and reversible has intrigued researchers and prompted studies [34,35] into determining the mechanism of the LCST.

In a series of elegant fluorescence energy transfer experiments, Winnik proposed [35] that the rapid thermorseversible behaviour of PNIPAM was due to a two stage process: initially intramolecular coil collapse occurs. This was monitored by using a donor (D) and acceptor (A) species copolymerized into the same chain and maintaining the polymer at a low concentration to observe intramolecular effects only. Under these conditions, the degree of energy transfer was observed to increase as D and A were brought within the critical transfer distance (ca. 10 nm) through intramolecular coil collapse. At higher concentrations, Winnik suggested [35] that intermolecular aggregation of the collapsed coils results, creating large scattering centres, which corresponds to the cloud point of the solution. A second series of experiments designed to observe intermolecular energy transfer required that the donor and acceptor be on separate chains and substantiated [35] this hypothesis: the degree of energy transfer increased at temperatures in excess of the LCST as the intermolecular aggregates formed.

We have subsequently confirmed [16] through TRAMs that intramoleulcar coil collapse occurs in PNIPAM as the first stage of the mechanism. In order to observe the dynamics of isolated chains, anisotropy experiments were carried out on a dilute (10^{-2} wt.% in polymer) ACE-PNIPAM sample between 5 and 65 °C. (characterisation data for this sample can be found in Table 1). Fig. 2 shows ln τ_c^{-1} plotted as a function of reciprocal temperature in "Arrhenius form" to allow better resolution of the conformational transition of ACE-PNIPAM: at low temperatures a short τ_c of ca. 5 ns results. This is indicative of rapid segmental motion extant in the water-swollen structure of the polymer below the LCST. As the temperature is increased up to the onset of the LCST, τ_c is observed to correspondingly increase displaying a linear thermal dependence. At 32 °C (which marks the LCST from cloud point measurements, cf. Table 1), the hydrogen bonding interactions between the water and amide groups

Table 1
Physical characteristics of the NIPAM-based polymers

Sample	M_n (kg mol^{-1})	Side chain (SC) in feed (mol%)	LCST[a] (°C)
ACE-PNIPAM	21	–	32
ACE-STY(16.9)-NIPAM	21	–	9
ACE-STY(16)-g-NIPAM	1554	–	37
STY(14)-g-NIPAM-ACE	1743	–	37
DMAC-g-ACE-NIPAM(5000)	251	1	35.5
DMAC-g-ACE-NIPAM(5000)	47	10	35
DMAC-g-ACE-NIPAM(5000)	37	20	34.5
ACE-DMAC-g-NIPAM(5000)	23	1	35
ACE-DMAC-g-NIPAM(5000)	31	10	35.5
ACE-DMAC-g-NIPAM(5000)	57	20	34.5

[a] Estimated from optical density (OD) measurements ($\lambda = 500$ nm) as the T of onset of the increase in OD which accompanies phase separation of the polymer.

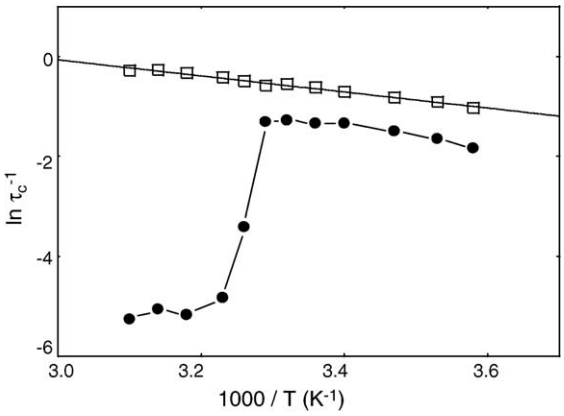


Fig. 2. Arrhenius representation of the rate of macromolecular motion, τ_c^{-1}, for ACE-PNIPAM in methanol (□) and aqueous solution (●), respectively [16].

are broken down and the hydrophobic groups (i.e. the isopropyl unit and polymer backbone) drive to collapse into a coil to reduce water contacts prevails. This results in a dramatic increase in τ_c to ca. 120 ns as a large, slow moving globular structure forms (cf. Fig. 2) (this behaviour is in agreement with that from an earlier anisotropy study using a dansyl labelled PNIPAM sample [36]). The anisotropy data recorded in aqueous media can be contrasted to that observed in methanol where no critical behaviour exists: a short τ_c (varying between 3 and 5 ns) is apparent across the entire temperature range which is indicative of a flexible open chain conformation (cf. Fig. 2). In addition, when dissolved in methanol, ACE-PNIPAM displays an Arrhenius type response in that the rate of segmental motion of the backbone increases linearly between 5 and 65 °C.

4. Manipulating the LCST of PNIPAM

4.1. Additives

Perhaps the simplest method of manipulation of the LCST of PNIPAM is through addition of solvent [37–39], salts [32,40,41] and surfactants [25–27,42] to the aqueous polymer solution. Methanol is one additive, which has received particular attention in this respect [37–39]. Although methanol is considered a good solvent for PNIPAM, when mixed with water, at a given temperature certain compositions will display what is known as co-non-solvency [37,38]: the mixture will behave as a non-solvent for PNIPAM and the polymer phase separates. We have shown through TRAMs [39] that addition of methanol lowers the onset temperature of the conformational transition of the polymer consistent with a lowering of the LCST as sensed by cloud point measurements [39]. This lowering in LCST is associated with a marked decrease in the "intensity" of the transition, as reflected in the chain dynamics: addition of methanol increases the mobility of the globular form of PNIPAM which exists above the system's LCST. Similarly, but much less dramatically, successive additions of alcohol reduces the rate of segmental motion, at temperatures below the LCST [39].

TRAMs have revealed that addition of salt can also have a marked effect on the LCST of PNIPAM. Fig. 3 again shows

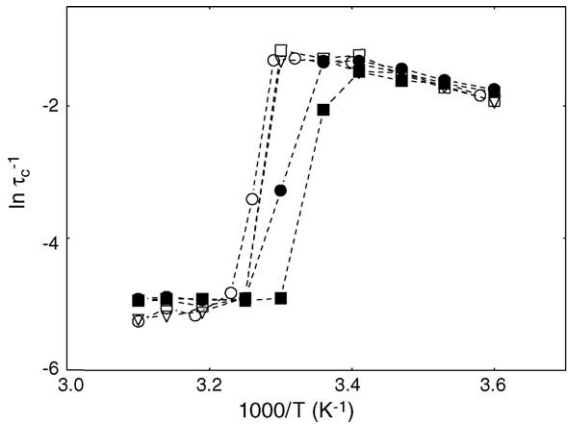


Fig. 3. Arrhenius representation of the rate of macromolecular motion, τ_c^{-1}, for ACE-PNIPAM as a function of (NaCl): 0 M (◯); 0.048 M (□); 0.06 M (▽); 0.15 M (●) and 0.5 M (■).

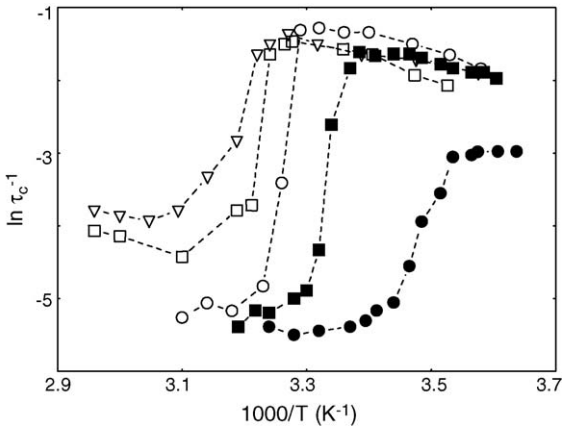


Fig. 4. Temperature dependencies of the segmental mobilities of ACE-PNIPAM (◯) ACE-STY(8.9)-NIPAM (■), ACE-STY(16.9)-NIPAM (●), ACE-DMAC(10)-NIPAM (□) and ACE-DMAC(20)-NIPAM (▽), respectively.

an "Arrhenius representation" of the relaxation data consequent from an ACE-PNIPAM sample upon addition of various concentrations of NaCl and the effect on the thermal response of the system. Consideration of the data shown in Fig. 3 reveals that in contrast to that observed upon addition of methanol [39], salt has little impact on the magnitude of the transition of PNIPAM. These data infer that in the presence of electrolyte, the uncoiled state is as flexible and expanded as that in the absence of salt. Similarly, above the LCST, NaCl has a minimal effect on the degree of compactness of the globular conformation (cf. Fig. 3). However, the addition of salt does have an impact on the onset of the conformational transition: increasing the concentration of electrolyte in the system decreases the LCST. Such a depression in the LCST has been observed previously in both linear PNIPAM and in gels [32,40,41,43] and has been attributed to structural changes in the bulk aqueous phase and/or hydration shell surrounding the polymer [32].

4.2. Random copolymerisation

We have recently modified PNIPAM [28,29] and synthesized microgels [7] based upon PNIPAM, so that we can control the conformational switch of the polymer over a wide temperature range. Simple free radical copolymerisation serves [28,29] to change the hydrophobic to hydrophilic balance within the polymer. As revealed in Fig. 4, this results in a shift of the LCST to lower temperature when a hydrophobic monomer such as styrene is used [28] or raises it, if a hydrophilic species (such as dimethylacrylamide (DMAC)) is incorporated [29] (cf. Fig. 1 for the structures of these monomers). These observations are in agreement with theoretical predictions [23]. Consequently tunablity of the conformational switch can be achieved between 4 and 100 °C. Further examination of Fig. 4 reveals that the degree of modification has an impact on the segmental dynamics of the resulting copolymer above the conformational transition if DMAC is used and below the LCST in the case of styrene. One major consequence of increasing the hydrophobic content is the formation of intramolecular styrene aggregates below the conformational transition (presumably this is a consequence of the system's drive to minimize hydrophob water contacts). This results in a partially coiled state which contracts further above the LCST as revealed in the anisotropy data in Fig. 4. With DMAC modification, on the other hand, increased levels of modifier has an impact on the chain conformation above the LCST: increasing the degree of hydrophilicity results in a more water swollen expanded state above the critical temperature. Consequently, the transition of the DMAC modified samples is marked by a switch from an expanded state below the LCST to a partially collapsed water swollen structure above (cf. Fig. 4). These effects serve to reduce the intensity of the LCST upon increasing degrees of modification which is in agreement with independent observations from DSC [44] measurements. An unfortunate consequence of this is the fact that, at higher contents of modifier, the uptake and release properties of the modified polymer is affected: the copolymer loses its ability to accommodate solubilised guests above the LCST if a critical content of hydrophilic modifier is used [45] but retains solubility of probes below the conformational transition upon use of relatively low concentrations of hydrophobic monomer [28].

In an effort to overcome this potential drawback as far as the controlled release capabilities are concerned, we have been considering alternative strategies for manipulation of the conformational switch whilst maintaining the magnitude of the transition. Our initial findings are discussed in the next section.

4.3. Effect of polymer architecture

Recently, by consideration of the thermodynamics involved in the process, we have suggested that polymer architecture should have a bearing on the LCST. Since ΔG_m is influenced by both ΔH_m and ΔS_m we have argued [46] that altering the polymer topography will affect the overall entropy of the system while the enthalpic contribution to the free energy remains constant. Manipulation of the entropy term is possible by varying the number of chain ends within the system, which has lead us to investigate the thermal response of graft copolymers based on NIPAM through TRAMs.

4.3.1. Styrene-NIPAM graft copolymers

Graft copolymers with styrene backbones and NIPAM "arms" have been synthesised by a macromonomer route [47]. These have been labelled with fluorescent reporter groups located either in the styryl segments or within the amide units. The molecular weight data (listed in Table 1) was derived from GPC analysis and will be published in due course [42]. Photophysical techniques, such as TRAMs, have been used to investigate the conformational behaviour of these novel polymer systems in aqueous media. The cloud point data as a result of optical transmittance measurements are also listed in Table 1 for two graft samples containing ca. 16 mol% styrene: one is fluorescently tagged in the backbone [i.e. ACE-STY(16)-g-NIPAM] while the second contains an ACE label in the NIPAM grafts [i.e. STY(14)-g-NIPAM-ACE]. Also shown for comparison, is the equivalent data for a linear sample of a similar styrl composition. Intriguingly, by consideration of the data in Table 1, it is clear that incorporation of ca. 16 mol% styrene into the graft copolymer serves to shift the LCST (as sensed by cloud point measurements) to a much higher temperature than that of the linear sample. This provides an early indication of the influence of the entropic term on the LCST behaviour.

Time-resolved anisotropy experiments were performed on the two ACE labelled samples as a function of temperature. The data are plotted in Arrhenius form in Fig. 5. Examination of the dynamic behaviour, as sensed by TRAMs, of each fluorescently tagged site within the graft sample provides revealing information regarding the thermal response of the system. As far as STY(14)-g-NIPAM-ACE is concerned, at temperatures below 35 °C, the relatively short τ_c observed (ca. 5 ns) implies that the grafts adopt a flexible, open structure under these conditions. It can be concluded that attachment of these units to the styrene backbone has little influence on their mobility. The NIPAM "arms" undergo a conformational collapse of a similar magnitude to that of the linear homopolymer except that the onset temperature is ca. 5 °C higher at 37 °C. This onset of the conformational collapse of the grafts coincides with the LCST of the system as sensed by cloud point measurements (cf. Table 1). Consideration of the styrene labelled backbone data at temperatures below 35 °C (cf. Fig. 5), reveals that τ_c is always superior to that of the NIPAM grafts over the same temperature range (presumably a degree of coiling exists within the backbone as a consequence of intramolecular styrene aggregation in order to minimize contact with the aqueous phase). In addition, since τ_c is invariant with temperature, it can be concluded that the conformation of the core is unaffected and remains partially collapsed between 5 and 65 °C. Further examination of Fig. 5 reveals that the value of τ_c obtained for backbone motion at temperatures in excess of 35 °C is always inferior to that of the NIPAM grafts: this implies that the label detects limited motion within the core as opposed to motion of the entire collapsed graft copolymer globule itself (if the labelled backbone was capable of detecting motion of the collapsed globule, τ_c would be comparable to that obtained for the labelled NIPAM grafts over a similar temperature range). Presumably, ingress of water into the styrl core results in a degree of fluidity which allows restricted movement of the segments as sensed by the ACE label.

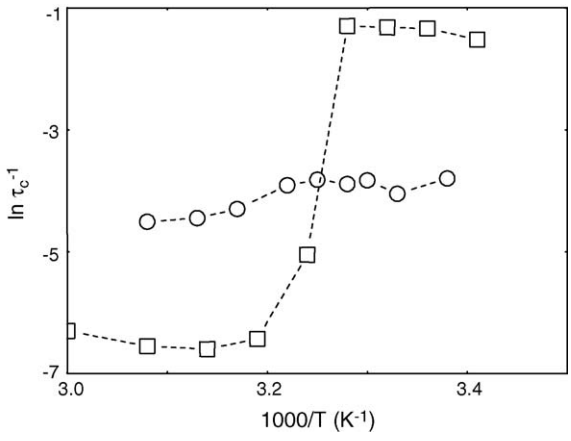


Fig. 5. Arrhenius representation of the rate of macromolecular motion, τ_c^{-1} for ACE-STY(16)-g-NIPAM (◯) and STY(14)-g-NIPAM-ACE (□), respectively.

The implications of these data are clear as far as the conformational behaviour of the styrene graft copolymers is concerned:

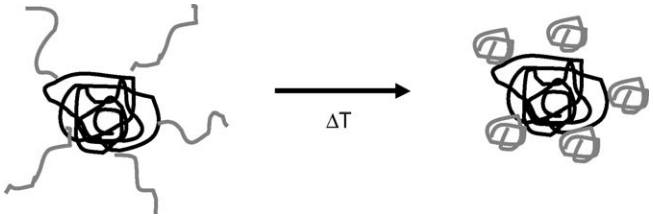
(i) The NIPAM "arms" are flexible and extended below the LCST of the system. At 37 °C, these units (the LCST) collapse onto the styrene core to form a compact globule.
(ii) The styrene backbone forms a relatively compact core at all temperatures studied in the current work.

A model, which describes the thermoresponsive behaviour of styrene-NIPAM, is shown in Scheme 1.

These observations have consequences as far as uptake and release of low molar mass material is concerned in that the styrene-NIPAM graft copolymers solubilise organic guests but do not show [42] the desired thermoresponsive release properties: solubility of probes is retained across the entire temperature range. This is a direct consequence of the existence of intramolecular styrene aggregates (as sensed by TRAMs) which serve to create solubilising domains below the LCST. To fashion thermally responsive systems which both retain the magnitude of the transition and have the desired controlled release properties, we present in the final section, anisotropy data from a series of DMAC-NIPAM graft copolymers.

4.3.2. DMAC-NIPAM graft copolymers

In an effort to create responsive graft copolymers which will release material at the LCST, we have used a more hydrophilic monomer (DMAC) to which NIPAM units have been grafted [46] (it would be envisaged that solubilising pockets below the



Scheme 1.

LCST will be prevented with this combination of constituent monomers).

We present preliminary TRAMs data here which show the level of control which can be exercised over the thermal response of the branched copolymers through variation of the number of NIPAM units grafted onto the DMAC backbone and the length of the grafts. In addition, we have fluorescently tagged either the DMAC backbone or NIPAM grafts to monitor the thermal response of each of the sites within the macromolecule via TRAMs. A fuller account of the synthesis (again by a macromonomer technique [47]) and characterization of these polymers will be published in due course [46]. Table 1 lists the molecular weight data derived from GPC analysis [46].

Fig. 6 shows the effect of both the label location and the number of grafts on the thermoresponsive behaviour of a copolymer sample containing grafts of molecular weight of 5000 g mol^{-1} [DMAC-g-NIPAM(5000)]. When ACE is located in the backbone [ACE-DMAC-g-NIPAM(5000)] (cf. Fig. 6a) and only ca. 1 mol% of grafts is present, Arrhenius behaviour is observed (i.e. the rate of segmental motion increases as the temperature is raised). This implies that the backbone is expanded and flexible under these conditions and does not undergo a conformational transition (indeed, this behaviour is very similar to that of linear PDMAC [48]). When the label is located in the graft (DMAC-g-ACE-NIPAM) with ca. 1 mol% of branches, a dramatic transition occurs (cf. Fig. 6b). Presumably, the cloud point (35.5 °C) observed in Table 1 for this degree of branching is driven by collapse of the NIPAM grafts. In general, increasing the degree of branching has little effect on the relaxation characteristics when the label is located in the grafts (cf. Fig. 6b). However, more dramatic thermoresponsive behaviour is apparent upon increasing the branch content when the label is sited in the backbone (cf. Fig. 6a): clearly it can be concluded that increasing the number of NIPAM grafts within the polymer increases the hydrophobicity of the sample and serves to induce a conformational transition in the DMAC backbone. (Since PDMAC itself is relatively hydrophilic, its LCST would be expected to be greater than 100 °C. Copolymerisation with a more hydrophobic monomer will change the hydrophobic to hydrophilic balance within the polymer reducing the LCST to below the boiling point of water. Such behaviour has been predicted by theory [23] for linear samples and observed experimentally in our laboratories through simple copolymerization of DMAC with styrene [49].)

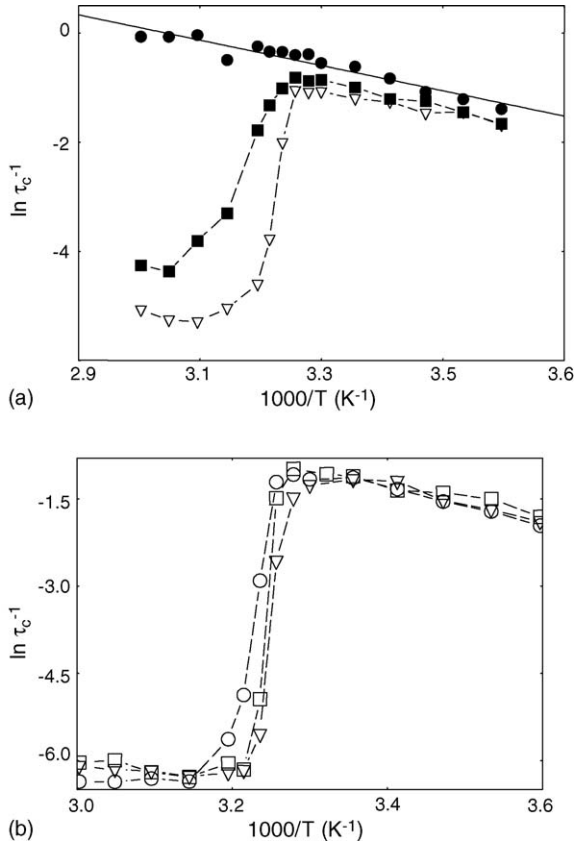
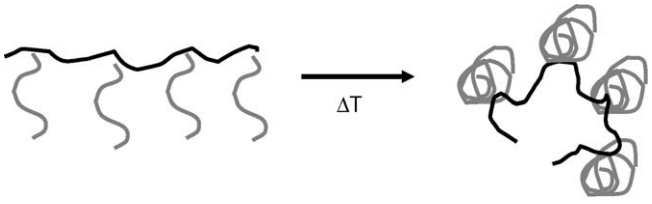


Fig. 6. Effect of label location and number of grafts on the rate of segmental relaxation (τ_c^{-1}) as function of T^{-1} for DMAC-g-NIPAM(5000): (a) label in the backbone, 1 mol% graft (●); 10 mol% graft (■); 20 mol% graft (▽) and (b) label in the graft, 1 mol% graft (◯); 10 mol% graft (□); 20 mol% graft (▽).

For DMAC-g-NIPAM(5000), when 10–20 mol% of grafts are present, two distinct conformational transitions occur within the polymer: one less intense associated with collapse of the backbone (cf. Fig. 6a), the other as a result of contraction of the grafts (cf. Fig. 6b) which is comparable in magnitude to that of linear PNIPAM. This is an intriguing observation and implies that above the LCST, a partially coiled water swollen backbone structure exists coupled to compact globular arms as shown in Scheme 2.

From the "Arrhenius" plots in Fig. 6, the onset temperature for each transition was estimated: when 10 mol% of grafts is present the DMAC backbone transition occurs at 40–45 °C (cf. Fig. 6a) while the grafts collapse at 34–36 °C (cf. Fig. 6b). The cloud point was determined to be ca. 35 °C whether the sample was labelled in the graft or backbone (cf. Table 1). This implies that the cloud point results from a merging of the two transitions but is dominated by collapse of the NIPAM grafts. Similarly, when the branch content reaches 20 mol% the backbone collapses at 38–40 °C (cf. Fig. 6a) and the grafts at 34–36 °C (cf. Fig. 6b). Again, the cloud point (34.5 °C, Table 1) is dominated by contraction of the grafts. It is interesting to note that with the current data (irrespective of whether the label is sited in the backbone or graft) we see no evidence for any partial collapse between ca. 17–30 °C prior to the conformational change at ca. 35 °C. Such behaviour has been observed previously through use of intensity measurements from a dansyl label [18] and fluorescence energy transfer experiments [35] on grafted NIPAM samples. Our data infer that between ca. 15–33 °C both the labelled grafts (cf. Fig. 6b) and the backbone (cf. Fig. 6a) undergo normal Arrhenius type behaviour (i.e. the rate of segmental motion increases



Scheme 2.

until the onset of the conformational change. In addition, the backbone and the grafts adopt an open chain conformation until the LCST).

When the length of the NIPAM graft is reduced to 2000 g mol^{-1}, similar behaviour to that of DMAC-g-NIPAM(5000) is observed: time-resolved anisotropy experiments provide evidence for two distinct conformational transitions [46]. However, it would appear that the DMAC backbone is more flexible when the shorter grafts are present since, in general, a faster rate of segmental motion is observed above the LCST than for the DMAC-g-NIPAM(5000) samples. Furthermore, evidence from fluorescence quenching experiments coupled with that of TRAMs indicate that the shorter NIPAM branch (which contains less hydrophobicity) results in a more open, expanded backbone structure above the LCST. Such would be expected since more low molecular weight grafts would be required to induce an appreciable conformational transition in the DMAC units. Only when 20 mol% of branches are present is a change in conformation apparent in the backbone [46].

Finally, it is worthy of note that the magnitude of the conformational transition of DMAC-g-ACE-NIPAM(5000) when 20 mol% of labelled graft is present is comparable to that of PNIPAM but larger than that of a similar content liner copolymer [ACE-DMAC(20)-NIPAM] (cf. Fig. 7) (the onset of the LCST occurs at a similar temperature for both the graft and linear copolymer sample). This provides strong evidence that one of our major objectives has been achieved: to raise the LCST yet maintain the magnitude of the transition. These findings have major implications as far as the controlled release properties are concerned. The graft sample is capable of solubilising pyrene above the LCST and releasing the probe to the aqueous phase when the temperature is lowered [46]. In addition, we have fluorescence energy transfer evidence which suggests [46] that pyrene is solubilised preferentially within the collapsed grafts and released to the aqueous phase when the temperature is lowered below the LCST of the system: these experiments were performed using an ACE label as the donor and a pyrene probe as the acceptor. Since this provides information regarding the proximity of D to A it is subsequently possible to determine the site within the graft copolymer which the probe is preferentially solubilised. These results will be published in due course [46] but, essentially, have revealed that for DMAC-g-NIPAM, when a labelled graft is used, pyrene is sequestered almost exclusively in the NIPAM unit at 50 °C. Little energy transfer is apparent when ACE is located in the backbone. These data confirm the model proposed from the TRAMs: above the LCST a relatively open DMAC structure exists coupled to compact, globular NIPAM grafts.

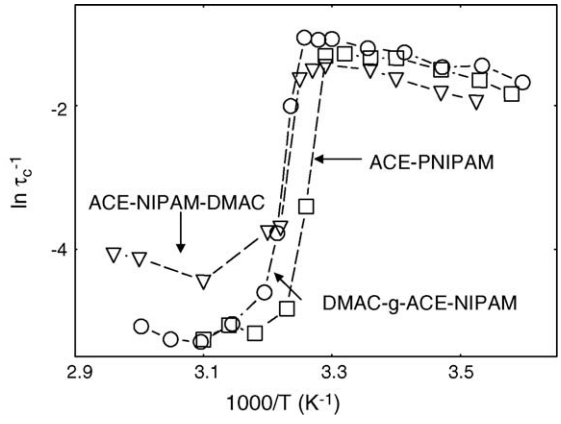


Fig. 7. Comparison of the thermal response of ACE-PNIPAM (□); DMAC-g-NIPAM(5000)-ACE (○) and ACE-DMAC(20)-NIPAM (▽), respectively.

5. Conclusions

(i) Time-resolved (fluorescence) anisotropy measurements have proven to be a useful tool in monitoring the thermal response of NIPAM-based systems.
(ii) Simple free radical copolymerization changes the hydrophobic to hydrophilic balance of PNIPAM which allows manipulation of the LCST across a wide temperature range. However, the magnitude of the transition is reduced upon increasing levels of modification which also impacts upon the controlled release capabilities of the system.
(iii) Changing the polymer architecture permits fine tuning of the conformational switch whilst maintaining the magnitude of the transition. Such entropic control should provide scope for increased diversity of application of NIPAM-based polymers in the future.

Acknowledgements

The authors gratefully acknowledge support from EPSRC in the form of fellowships to C.K.C. and R.R. and in the provision of beamtime at the SRS, Daresbury (UK).

References

[1] I. Soutar, L. Swanson, Eur. Polym. J. 29 (1993) 371.
[2] J.R. Ebdon, D.M. Lucas, I. Soutar, L. Swanson, Macromol. Symp. 79 (1994) 167.
[3] I. Soutar, L. Swanson, Polymer 35 (1994) 1942.
[4] I. Soutar, L. Swanson, Macromolecules 27 (1994) 4304.
[5] J.R. Ebdon, B.J. Hunt, D.M. Lucas, I. Soutar, L. Swanson, A.R. Lane, Can. J. Chem. 73 (1995) 1982.
[6] C.K. Chee, S. Rimmer, I. Soutar, L. Swanson, Polymer 38 (1997) 483.
[7] N.J. Flint, S. Gardebrecht, L. Swanson, J. Fluorescence 8 (1998) 343.
[8] I. Soutar, L. Swanson, N.J. Flint, R. Haywood, J. Fluorescence 8 (1998) 327.
[9] K.P. Ghiggino, K.L. Tan, in: D. Phillips (Ed.), Polymer Photophysics, Chapman and Hall, 1985 (Chapter 7).
[10] F.M. Winnik, Chem. Rev. 93 (1993) 587.
[11] I. Soutar, L. Swanson, Applications of luminescence spectroscopy to the study of polymers, in: N.S. Allen (Ed.), Current Trends in Polymer Photochemistry, Ellis Horwood Publishers, 1995.
[12] I. Soutar, L. Swanson, to be published.
[13] J.S. Scarpa, D.A. Mueller, I.M. Klotz, J. Am. Chem. Soc. 89 (1967) 6024.
[14] M. Heskins, J.E. Guillet, J. Macromol. Sci. Chem. 2 (1969) 1441.
[15] H.G. Schild, Prog. Polym. Sci. 17 (1992) 163.
[16] C.K. Chee, S. Rimmer, I. Soutar, L. Swanson, Polymer 42 (2001) 5079.
[17] R. Yoshida, K. Uchida, Y. Kaneko, K. Sakai, A. Kikuchi, Y. Sakurai, T. Okano, Nature 374 (2002) 240.
[18] E. Yoshinari, H. Furukawa, K. Horie, Polymer 46 (2005) 7741.

[19] I. Soutar, L. Swanson, R.E. Imhof, G. Rumbles, Macromolecules 25 (1992) 4399.
[20] A.J. Marsh, G. Rumbles, I. Soutar, L. Swanson, Chem. Phys. Lett. 25 (1992) 31.
[21] P. Wahl, Chem. Phys. 7 (1975) 210.
[22] M.D. Barkley, A.A. Kowalczyk, L. Brand, J. Chem. Phys. 75 (1981) 3581.
[23] L.D. Taylor, L.D. Cerankowski, J. Polym. Sci.: Polym. Chem. 13 (1975) 2551.
[24] F.M. Winnik, H. Ringsdorf, J. Venzmer, Macromolecules 24 (1991) 1678.
[25] F.M. Winnik, H. Ringsdorf, J. Venzmer, Langmuir 7 (1991) 905.
[26] F.M. Winnik, H. Ringsdorf, J. Venzmer, Langmuir 7 (1991) 912.
[27] H.G. Schild, D.A. Tirrell, Langmuir 7 (1991) 1319.
[28] C.K. Chee, S. Rimmer, D.A. Shaw, I. Soutar, L. Swanson, Macromolecules 34 (2001) 544.
[29] I.C. Barker, J.M.G. Cowie, I. Soutar, L. Swanson, Macromolecules 36 (2003) 7765.
[30] A. Holtzer, M.F. Emerson, J. Phys. Chem. 73 (1969) 26.
[31] P.L. Privalov, S.J. Gill, Pure Appl. Chem. 61 (1989) 1097.
[32] Y. Maeda, T. Higuchi, I. Ikeda, Langmuir 16 (2000) 7503.
[33] Y. Maeda, H. Yamamoto, I. Ikeda, Macromolecules 36 (2003) 5055.
[34] I. Yamamoto, K. Iwasaki, S. Hirotsu, J. Phys. Soc. Jpn. 58 (1989) 210.
[35] F.M. Winnik, Polymer 31 (1990) 2125.
[36] T. Binkert, J. Oberreich, M. Meewes, R. Nyffenegger, J. Ricka, Macromolecules 24 (1991) 5806.
[37] F.M. Winnik, H. Ringsdorf, J. Venzmer, Macromolecules 23 (1990) 2415.
[38] H.G. Schild, M. Muthukumar, D.A. Tirrell, Macromolecules 24 (1991) 948.
[39] J. Cardwell, C.K. Chee, S. Rimmer, I. Soutar, L. Swanson, to be published.
[40] J. Elaissaf, J. Appl. Polym. Sci. 22 (1978) 873.
[41] Hg. Schild, D.A. Tirrel, J. Phys. Chem. 94 (1990) 4352.
[42] C.K. Chee, S. Rimmer, I. Soutar, L. Swanson, to be published.
[43] T.G. Park, A.S. Hoffman, Macromolecules 26 (1993) 5045.
[44] M. Shibayama, S. Mizutanians, S. Nomura, Macromolecules 29 (1996) 2019.
[45] I.C. Barker, J.M.G. Cowie, I. Soutar, L. Swanson, unpublished data.
[46] C.K. Chee, B.J. Hunt, S. Rimmer, R. Rutkaite, I. Soutar, L. Swanson, to be published.
[47] S. Rimmer, A.N. Mohd. Ramli, S. Lefevre, Polymer 37 (1996) 4135.
[48] I. Soutar, L. Swanson, F.G. Thorpe, C. Zhu, Macromolecules 29 (1996) 918.
[49] B. Collins, I. Soutar, L. Swanson, S.J. Wallace, to be published.