Volumetric Studies of Aqueous Polymer Solutions Using Pressure Perturbation Calorimetry: A New Look at the Temperature-Induced Phase Transition of Poly(*N*-isopropylacrylamide) in Water and D_2O

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ABSTRACT: We report the first application of pressure perturbation calorimetry (PPC) to determine the hydration properties of poly(*N*-isopropylacrylamide) (PNIPAM) in H_2O and in D_2O as the solutions undergo a temperature-induced phase transition. The technique, which measures the heat change resulting from a pressure change above a solution of PNIPAM placed in a microcalorimeter cell, yields the temperature dependence of the coefficient of thermal expansion, α_p, of the polymer in solution and the change in volume of the solvation layer around the polymer chain. In the temperature ranges below and above the phase transition, α_p of PNIPAM in H_2O increased linearly with temperature. It underwent a sharp increase at the transition temperature, T_{max}, then rapidly decreased. The phase transition was accompanied by an increase in the partial specific volume of the hydrated polymer. This increase was significantly higher for solutions of PNIPAM in D_2O, compared to H_2O. A study by PPC of the phase transition of hydrophobically modified PNIPAM samples that undergo micellization in water demonstrated that the hydration of the polymeric micelles varies significantly as a function of the degree of hydrophobic substitution and length of the alkyl group linked to the polymer.

Introduction

The temperature-induced phase transition of aqueous solutions of poly(*N*-isopropylacrylamide) has fascinated many scientists ever since it was reported by Heskins and Guillet in 1968.[1] The interest in this polymer stems from its potential applications in biotechnological devices and drug delivery vehicles[2] as well as its use as model polymer to understand the fundamental physics of the polymer coil-to-globule collapse.[3] Experimentally, a plethora of techniques has been employed to study this phenomenon. They range from measurements of macroscopic properties, such as turbidimetry,[4] high-sensitivity scanning microcalorimetry,[4−6] and pycnometry in the case of PNIPAM gels,[7] to experiments probing molecular interactions, such as light scattering,[8−12] small-angle neutron scattering,[13] fluorescence spectroscopy,[14] and atomic force microscopy.[15] It is now well accepted that the solution properties of PNIPAM in water are the results of the rather complex polarity of the polymer functionalities. Below the phase transition temperature (cloud point, CP) the amides groups "imbibe"[16] water molecules via hydrogen bonding. As the solution temperature exceeds the cloud point, these hydrogen bonds break; water is expelled by the polymer as it undergoes a coil-to-globule collapse and eventually phase-separates to form visible particles.

Changes in hydration are undoubtedly one of the major driving forces in the phase transition of PNIPAM aqueous solutions. Surprisingly, there have been few direct, macroscopic, studies of the hydration properties of this polymer. This deficiency reflects, in part, the inherent difficulty to resolve an overall observable into the hydration contribution of a particular solute domain. Moreover, relatively few macroscopic techniques are sensitive to solute hydration in dilute aqueous solutions. The phenomenon has been under intense scrutiny, however, in the case of globular proteins in their native and unfolded states using various experimental and computational techniques.[17−20] The volumetric characteristics of proteins and amino acids, such as their partial molar volume and adiabatic compressibility, have proven to reflect sensitively solute−solvent interactions.[21−23] In particular, advances in acoustic techniques have made it possible to conduct high precision compressibility measurements on small volumes of dilute protein and amino acid solutions over a range of temperatures and pressures.[23,24] Differential scanning density measurements provide another means to determine the partial specific volumes and the thermal expansion coefficient of protein solutions, albeit of relatively high concentrations (between 5 and 30 g L^{-1}).[25]

It turns out that volumetric properties of dilute polymer solutions can be measured easily by a calorimetric technique, known as pressure perturbation calorimetry (PPC),[26] which measures the heat change resulting from a pressure change above a polymer solution. This heat change can be used to calculate the coefficient of thermal expansion of the solute, and its temperature dependence. The technique can be exploited to obtain the changes in the *volume of the solvation layer* around a polymer chain before and after a phase transition. As such it differs in its outlook from techniques commonly used by polymer chemists to observe phase transition phenomena, such as scattering measurements or fluorescence techniques that always focus on the polymer itself.

We present here the PPC experimental setup and the thermodynamic equations relating the observables to the coefficient of thermal expansion of polymer solutions

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Table 1. Physical Properties and Thermodynamic Characteristics of the Polymers Investigated

polymer	composition NIPAM/C_n	M_v	solvent	cloud point (°C)[a]	T_{max} (°C)	Δc_p (cal mol^{-1} K^{-1})	ΔH (kcal mol^{-1})
PNIPAM	1/0	300 000	H_2O	31.8	33.7	−15	1.4
PNIPAM	1/0	300 000	D_2O	33.7	34.3	−30	1.5
PNIPAM-C_{10}/200	240/1	370 000	H_2O	30.2	31.0	−20	1.5
PNIPAM-C_{14}/100	108/1	370 000	H_2O	28.8	30.5	−15	1.2
PNIPAM-C_{14}/200	220/1	380 000	H_2O	23.2	28.8	−15	1.2
PNIPAM-C_{18}Py	206/1	380 000	H_2O	31.7	31.2	−20	1.1

[a] Determined by turbidimetry[27] (polymer concentration, 1.0 g L^{-1}; heating rate, 0.5 °C min^{-1}).

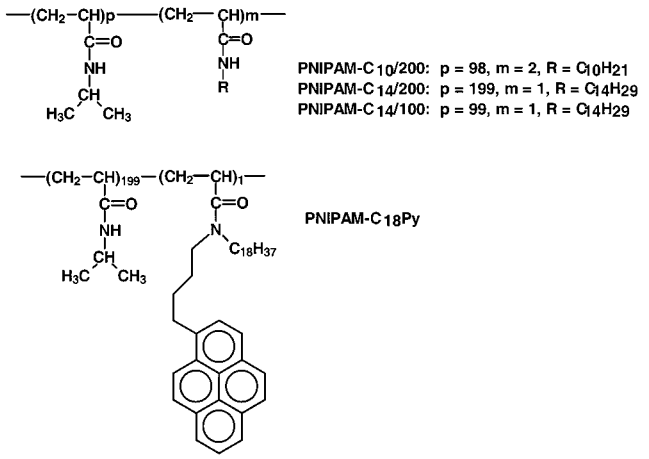

Figure 1. Chemical structure of the polymers investigated.

and the partial volume changes that accompany a phase transition. We describe a study by PPC of the phase transition of PNIPAM in water. To assess the role of solvent/polymer hydrogen bond in the formation and properties of the hydration layer of PNIPAM aqueous solutions, PPC measurements were performed on PNIPAM solutions in light (H_2O) and heavy (D_2O) water. These two solvents are chemically identical, yet their physical properties differ significantly.[27] The differences between H_2O and D_2O are believed to stem from differential energetics of intermolecular hydrogen bonds. The lengths of hydrogen bonds in the two liquids are essentially the same, but a "hydrogen" bond in D_2O is about 5% stronger than a hydrogen bond in H_2O.[27] Overall, D_2O is a "more structured" liquid than light water, although this order breaks down faster with temperature.

PPC measurements were carried out also on solution of hydrophobically modified PNIPAM's (HM-PNIPAM) bearing 0.5−1 mol % of an alkyl chain ranging in size from *n*-decyl to octadecyl. Four polymers, PNIPAM-C_{10}/200, PNIPAM-C_{14}/200, PNIPAM-C_{14}/100, and PNIPAM-C_{18}Py (Figure 1), were selected for this study. Previous studies by dynamic light-scattering and fluorescence spectroscopy have indicated that all four polymers form micellar assemblies in water via association of the hydrocarbon chains, although the degree of association varies significantly with the nature of the hydrophobic group.[28] The results of PPC measurements on solutions of the four polymers in water are evaluated in terms of the importance of hydrophobic association on the hydration layer surrounding the polymer chain.

Experimental Section

Materials. Deionized water was obtained from a Millipore Milli-Q water purification system. Deuterium oxide (99.8%) was purchased from Aldrich-Sigma Chemicals. The polymers, PNIPAM, PNIPAM-C_{14}/200, PNIPAM-C_{14}/100, PNIPAM-C_{10}/200, and PNIPAM-C_{18}Py (Figure 1) were prepared as described previously.[28] Their chemical compositions and physical characteristics are listed in Table 1. The partial specific volume of PNIPAM was calculated from the linear relationship $\overline{V_p}(t) = 0.842 + 0.0014t$, where $\overline{V_p}(t)$ is the partial specific volume of PNIPAM and t the temperature in °C.[6] The same value of $\overline{V_p}(t)$ was used for the hydrophobically modified PNIPAM samples as well.

Methods. Differential Scanning Calorimetry (DSC). DSC measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc.) at an external pressure of ca. 180 kPa. The cell volume was 0.517 mL. The heating rate was 1.5 °C min^{-1}, and the instrument response time was set at 5.6 s. Data were corrected for instrument response time and analyzed using the software supplied by the manufacturer. The polymer concentration was 1.0 g L^{-1}.

Pressure Perturbation Calorimetry (PPC). PPC measurements were performed on a VP-DSC microcalorimeter equipped with a pressure perturbation accessory (MicroCal Inc). The pressure applied during the compression cycle was 500 kPa. The reference cell and sample cell volumes were identical (0.517 mL). The polymer concentration was 5.0 g L^{-1}.

Principle. The polymer solution is placed in the sample cell and the solvent (H_2O or D_2O) is placed in the reference cell of the microcalorimeter fitted with a PPC accessory (Figure 2). The filling tubes for each cell open into a common pressure chamber containing a sensor, from which data are transmitted to a computer for storage. The calorimetric baseline is allowed to equilibrate at constant temperature and at pressure P_1. The excess pressure is then changed to P_2, causing heat to be absorbed in both cells. Since the solutions in the sample cell and in the reference cell are identical except for the small amount of dissolved polymer in the sample cell counterbalanced by the corresponding volume of solvent in the reference cell, differential heats are quite small. The compression and decompression peaks are of identical size and opposite sign. The differential heat values, ΔQ_{rev}, are obtained by integrating each peak. The temperature is then changed, the baseline is allowed to equilibrate, and the compression/decompression cycle is repeated.

Derivation of the Equations. Single-Component System. From the second law of thermodynamics, the heat change dQ_{rev} for a reversible process carried out at constant temperature T is related to the entropy change dS by

$$dS = \frac{dQ_{rev}}{T} \tag{1}$$

Differentiation of eq 1 with respect to P at constant T gives

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = T\left(\frac{\partial S}{\partial P}\right)_T \tag{2}$$

Using the Maxwell relation, $(\partial S/\partial P)_T = -(\partial V/\partial T)_P$, where V is the volume of the system, and substituting into eq 2 gives

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = -T\left(\frac{\partial V}{\partial T}\right)_P = -TV\alpha \tag{3}$$

where α is the coefficient of thermal expansion of the system, $\alpha = 1/V(\partial V/\partial T)_P$. Integration of eq 3 at constant temperature and over a small pressure range gives eq 4, where it is assumed

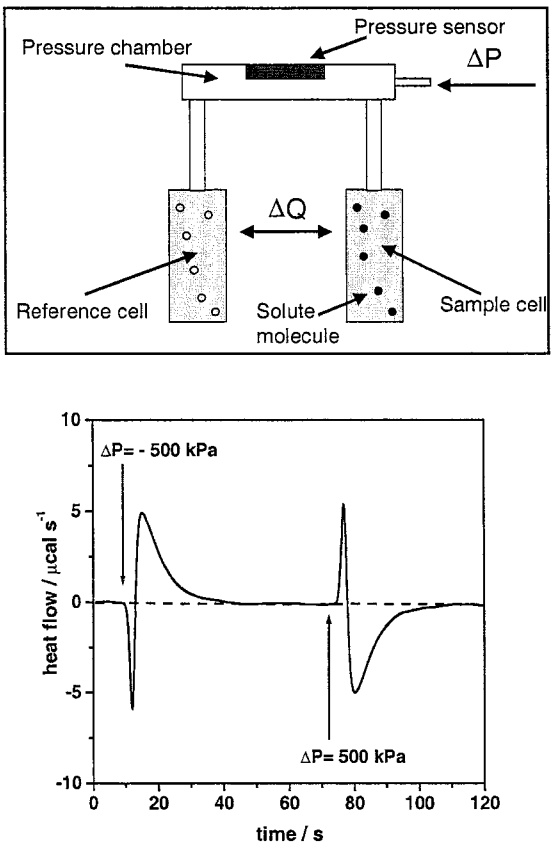

Figure 2. Schematic diagram of a pressure perturbation calorimetry (PPC) experimental setup. The open circles in the reference cell represent the volume occupied by solvent in this cell, which counterbalances the volume occupied by the solute molecules in the sample cell (top); experimental PPC trace registered for a solution of poly(*N*-isopropylacrylamide) (5.0 g L^{-1}) in H_2O, temperature = 20 °C (bottom).

that V and α are nearly invariant with a small pressure change, a good approximation for all liquids:

$$Q_{rev} = -TV\alpha\Delta P \tag{4}$$

The Case of a Two-Component System. When a solution is formed by dissolving a polymer of mass m_p in a solvent of mass m_s, the total volume, V_{total}, of the resulting solution is given by eq 5, where $\overline{V_p}$ is the partial specific volume of the polymer in solution and V_S is the specific volume of pure solvent in solution:

$$V_{total} = m_S V_S + m_P \overline{V_P} \tag{5}$$

The partial specific volume of the polymer includes not only the intrinsic volume of the polymer, but also any volume changes induced in the solvent as a result of interactions with the solute. Differentiating eq 5 with respect to temperature at constant pressure yields

$$\left(\frac{\partial V_{total}}{\partial T}\right)_P = m_S\left(\frac{\partial V_S}{\partial T}\right)_p + m_P\left(\frac{\partial \overline{V_P}}{\partial T}\right)_P \tag{6}$$

and after substituting the right-hand side of eq 6 into eq 3 we obtain

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = -T\left[m_S\left(\frac{\partial V_s}{\partial T}\right)_P + m_P\left(\frac{\partial \overline{V_P}}{\partial T}\right)_P\right] \tag{7}$$

Multiplying and dividing the first term in brackets by V_S and multiplying the second term by $\overline{V_P}$ converts eq 7 into eq 8,

where α_s and α_p are the coefficients of thermal expansion of the solvent and the polymer, respectively:[29]

$$\left(\frac{\partial Q_{rev}}{\partial P}\right)_T = -T[m_s V_s \alpha_s + m_p \overline{V_P}\alpha_p] \tag{8}$$

According to eq 8, the heat arising from pressure perturbation of a solution can be viewed as the sum of heats arising from the perturbation of the solvent and from the perturbation of the solvated polymer. Integrating eq 8 over a small pressure range leads to eq 9:

$$Q_{rev} = -T[m_s V_s \alpha_s + m_p \overline{V_p}\alpha_p]\Delta P \tag{9}$$

In a differential calorimetric experiment using polymer solution in the sample cell and pure solvent in the reference cell, when both cells are subjected to the same ΔP, the net heat ΔQ_{rev} will be equal to the difference between eq 9 for the sample cell and for the reference cell, giving eq 10, which can be rewritten as eq 11:

$$\Delta Q_{rev} = -T\Delta P[m_p \overline{V_p}\alpha_p - m_p \overline{V_p}\alpha_s] \tag{10}$$

$$\alpha_p = \alpha_s - \frac{\Delta Q_{rev}}{T\Delta P m_p \overline{V_p}} \tag{11}$$

The volume change ΔV, which accompanies a transition in a system, is obtained by integration of the curve of the changes in the coefficient of thermal expansion with temperature, as indicated in eq 12, where we assume that ΔV is small compared to V. The value ΔV is expressed as a percent of V.

$$\frac{\Delta V}{V} = \int \alpha_p \, dT = \int \frac{1}{V}\left(\frac{dV}{dT}\right) dT \tag{12}$$

Note that there are three temperature-dependent parameters which are treated as temperature independent variables in the data analysis. They are as follows: (1) V_{total}, the total solution volume; this value related to the expansion of the cell itself is only marginally dependent on temperature in the range studied; (2) the polymer concentration, m_p, which will decrease with increasing temperature as the solvent expands; (3) the polymer partial volume, V_p, which will increase with temperature according to its thermal coefficient of expansion. The last two factors work in opposite directions and tend to cancel. The error introduced in the determination of α is of ~1% over the temperature range 0−100 °C, which is well within the experimental errors.

Results and Discussion

The interpretation of PPC data requires one to know the temperature of the phase transition investigated. This value is determined readily by performing a temperature scan of the polymer solution using the microcalorimeter under normal conditions. As the thermal transition of PNIPAM in water has been studied previously by microcalorimetry, we limit our description to a brief summary of the results obtained with solutions of PNIPAM in H_2O and in D_2O. Figure 3 shows the temperature dependence of the partial heat capacity c_p of a solution of PNIPAM in the two solvents. The plots yield three thermodynamic parameters related to the phase transition: the temperature of the maximum heat capacity, T_{max}, the heat of the transition, ΔH, and the difference of the heat capacity of PNIPAM solutions before and after the phase transition (Δc_p). For the solution in H_2O, T_{max} (33.7 ± 0.2 °C) is slightly higher than the cloud point determined spectrophotometrically by changes in solution transmittance (31.8 ± 0.3 °C). The heat of transition (ΔH) per NIPAM unit is 1.4 ±

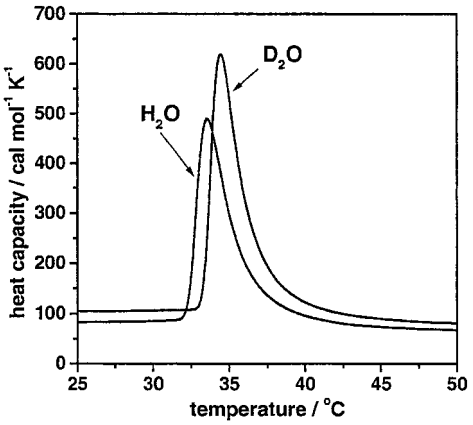

Figure 3. Microcalorimetric endotherms for aqueous solutions of PNIPAM (1.0 g L^{-1}) in H_2O and D_2O. Heating rate: 1 °C min^{-1}.

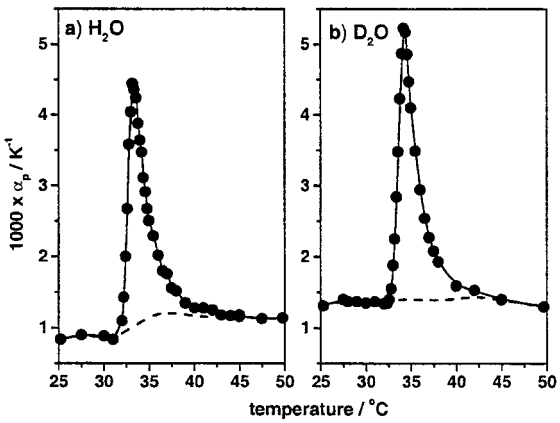

Figure 4. Temperature dependence of the coefficient of thermal expansion (α_p) of PNIPAM in H_2O (a) and D_2O (b). Polymer concentration: 5.0 g L^{-1}. The dotted line represents the progress baseline.

0.2 kcal mol^{-1}, a value in agreement with previous reports.[30,31] The heat capacity of the solution at high temperature is smaller than the heat capacity of the solution at temperatures lower than the phase transition ($\Delta c_p = -15$ cal mol^{-1} °C^{1-}), an observation interpreted as evidence that the number of water−polymer contacts decreases during the phase transition, in analogy with the refolding of proteins upon heating after cold denaturation.[32]

The T_{max} value obtained for the PNIPAM solution in D_2O is higher by ∼ 2 °C, compared to that registered for the PNIPAM solution in H_2O. This observation is in agreement with light scattering studies of the PNIPAM coil-to-globule collapse in D_2O and H_2O,[12] which have shown that the heat-induced polymer collapse takes place between 31.7 and 33.9 °C in D_2O, a temperature range higher by ∼ 2 °C than the temperature of coil-to-globule collapse of PNIPAM in H_2O.[10] We note also that the difference of heat capacity measured before and after the phase transition (Δc_p) is nearly double in D_2O, relative to H_2O. The exact origin of this enhancement is not clear, but, as in the case of protein denaturation, it may be related to the difference in the solvent accessible surface area (ASA) before and after the phase transition.[21,22] The fact that Δc_p is larger in deuterated water would imply that the chain dimensions of PNIPAM for $T < T_{max}$ are larger in D_2O. Indeed, light scattering studies of fractionated PNIPAM in D_2O and H_2O have demonstrated that, well below the phase transition temperature, the unperturbed PNIPAM chains are more extended in D_2O than in H_2O.[12] The thermodynamic data obtained for solutions in water of the HM-PNIPAM samples are listed in Table 1, together with the values recorded for solutions of PNIPAM. As reported earlier the cloud points and transition temperatures of HM-PNIPAM's are slightly lower than those of PNIPAM.[28]

PPC scans were carried out next with solutions of PNIPAM in H_2O and D_2O yielding the changes with temperature of the thermal expansion coefficient, α_p, shown in Figure 4. The plots can be divided into three temperature ranges. Below the transition temperature, $15 < T < 30$ °C, α_p increases nearly linearly with temperature. Around the phase transition temperature, $30 < T < 40$ °C, α_p undergoes a sharp increase, reaches a maximum at T_{max}, and then rapidly decreases with increasing temperature, to reach a value nearly equal to that registered below the transition temperature. The thermal expansion coefficient continues to increase slightly in the highest temperature domain probed, $45 < T < 80$ °C, an effect which may indicate aggregation of the collapsed chains upon further heating. The traces recorded for PNIPAM in H_2O and D_2O are similar in their general features, but differ significantly in terms of the relative values of α_p before and after the phase transition. In the case of D_2O solutions, α_p remains nearly constant, whereas in the H_2O solutions, α_p after the transition is significantly higher than for $T < 30$ °C. Values of α_p for PNIPAM in H_2O and D_2O at 15, 25, and 50 °C, as well as $\Delta\alpha_p = \alpha_{p50} - \alpha_{p15}$, are listed in Table 2. The temperature dependences of the thermal expansion of pure H_2O and D_2O were determined under the same conditions. The values agree well with the literature values[33] also listed in Table 2 for comparison. Note that the coefficient of thermal expansion of water

Table 2. Thermodynamic Characteristics of the Polymers and Solvents Determined by Pressure Perturbation Calorimetry

polymer	solvent	T_{max} (°C)	$\Delta V/V$ (%)	α_{p15} (K^{-1})	α_{p25} (K^{-1})	α_{p50} (K^{-1})	$\Delta\alpha_p = \alpha_{p50} - \alpha_{p15}$ (K^{-1})
PNIPAM	H_2O	33.7	1.01	0.76×10^{-3}	0.88×10^{-3}	1.28×10^{-3}	0.52×10^{-3}
PNIPAM	D_2O	34.3	1.32	1.42×10^{-3}	1.32×10^{-3}	1.30×10^{-3}	-0.12×10^{-3}
PNIPAM-C_{10}/200	H_2O	31.0	1.44	0.92×10^{-3}	1.02×10^{-3}	1.06×10^{-3}	0.14×10^{-3}
PNIPAM-C_{14}/200	H_2O	30.5	1.43	0.97×10^{-3}	1.03×10^{-3}	1.08×10^{-3}	0.11×10^{-3}
PNIPAM-C_{14}/100	H_2O	28.8	1.12	0.75×10^{-3}	0.96×10^{-3}	0.96×10^{-3}	0.21×10^{-3}
PNIPAM-C_{18}Py	H_2O	29.5	1.04	0.86×10^{-3}	0.96×10^{-3}	0.93×10^{-3}	0.07×10^{-3}
	H_2O[a]			1.503×10^{-4}	2.578×10^{-4}	4.571×10^{-4}	3.068×10^{-4}
	H_2O[b]	---	---	1.507×10^{-4}	2.571×10^{-4}	4.578×10^{-4}	*3.071* $\times 10^{-4}$
	D_2O[a]	---	---	0.561×10^{-4}	1.838×10^{-4}	4.104×10^{-4}	3.511×10^{-4}
	D_2O[b]	---	---	0.475×10^{-4}	1.846×10^{-4}	4.290×10^{-4}	3.815×10^{-4}

[a] Values determined by PPC. [b] Values given in italics are taken from or calculated based on: Kell, G. S. *J. Chem. Eng. Data* **1967**, *12*, 66.

is much smaller than that of the polymer throughout the temperature range probed in our experiments.

The change in volume of the solvation layer of the polymer (ΔV) corresponding to the collapse of the chain can be extracted from PPC scans, by integration of the changes in α_p as a function of temperature (see eq 12, Experimental Section), assuming that the intrinsic volume occupied by a polymer chain remains constant in the temperature range studied. The volume change is expressed as $\Delta V/V$ in percent of the total polymer volume, using the area defined by the peak in a PPC scan and by the progress baseline (dashed line, Figure 4). Values of $\Delta V/V$ for PNIPAM in H_2O and D_2O and for the HM-PNIPAM samples in H_2O are listed in Table 2. We note some important differences among the samples. First, for solutions of PNIPAM, the amplitude of the volume change is larger by ~20% in D_2O, compared to H_2O. Second, in the case of the HM-PNIPAM's in H_2O, it appears that the perturbation to solvent brought about by the hydrophobic substituent becomes less important with increasing length of the hydrocarbon chain, e.g., $\Delta V/V = 1.44\%$ in the case of a PNIPAM-C_{10}/200 and $\Delta V/V = 1.04\%$, in the case of a solution of PNIPAM-C_{18}Py. The latter value is identical, within experimental error, to the volume change measured for a solution of PNIPAM in water ($\Delta V/V = 1.01$).

Considering first the differences noted for PNIPAM solutions in light and heavy water, it can be argued that the enhanced change in hydration volume in D_2O results from a combination of two effects. On one hand, our measurements of Δc_p (Table 2), together with the light-scattering results of Wu and co-workers,[12] indicate that PNIPAM coils ($T < 30$ °C) are more extended in D_2O; hence, there may be a higher level of ordering of D_2O associated with a polymer chain, hence a larger volume of directly interacting water. On the other hand, the larger volume change may simply indicate that as the polymer contracts at the phase transition temperature, the water molecules expelled from the hydration layer occupy a larger volume of "bulk solvent" in the case of D_2O, a more structured solvent than H_2O.

The origin of the differences observed among the solutions of hydrophobically modified polymers may be traced to differences of the micellar assemblies. Previous studies based on fluorescence probe experiments revealed that in the case of PNIPAM-C_{10}/200, the polymer chains form loose assemblies that can be readily disrupted, whereas the polymeric micelles formed by PNIPAM-C_{18}Py, in contrast, are best viewed as consisting of a hydrophobic core consisting of associated octadecyl chains, surrounded by a corona of PNIPAM chains.[28] Thus, the hydration of PNIPAM-C_{18}Py occurs via interactions between the NIPAM units and water molecules and not between water molecules and the hydrophobic substituents which form a micellar core from which water is all but excluded. It is not surprising therefore that the volume increment at the phase transition of a solution of PNIPAM-C_{18}Py is the same as that observed for unmodified PNIPAM. In contrast, PNIPAM-C_{10}/200 forms loosely packed micellar aggregates in water and the decyl chains remain in contact with water molecules below the phase transition. Hence the volume of water released at the phase transition is comparatively higher.

Conclusions

Our results suggest that pressure perturbation calorimetry provides new insights into the solvation properties of amphiphilic polymers in water. The real strength of the technique becomes apparent when the data are used in conjunction with results generated by techniques probing the size, conformation, and association of the polymer chains, such as static and dynamic light-scattering or fluorescence spectroscopy. This study focused on the temperature-dependent hydration of PNIPAM in H_2O and D_2O, a phenomenon that has been observed by various methods previously. It raised fundamental questions about the molecular nature of the different hydration of polar and nonpolar groups in D_2O and H_2O. A systematic investigation of low-molecular compounds, polymers of narrow molecular weight distribution, as well as copolymers of controlled composition may clarify these issues.

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