Microcalorimetric Study of the Temperature-Induced Phase Separation in Aqueous Solutions of Poly(2-isopropyl-2-oxazolines)

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ABSTRACT: The effect of temperature on aqueous solutions of poly(2-isopropyl-2-oxazoline) (PIPOZ) samples of molecular weights ranging from 1900 to 5700 g mol<sup>-1</sup> was monitored by turbidimetry, high-sensitivity microcalorimetry (HS DSC), and pressure perturbation calorimetry (PPC) from 10 to 80 °C. The polymers were soluble in cold water and underwent phase separation at  $T_{\rm CP} \sim 45-63$  °C, depending on their molecular weight. The phase transition was endothermic, with an enthalpy change ranging from 0.36  $\pm$  0.01 to 1.16  $\pm$  0.01 kcal mol<sup>-1</sup>. The coefficient of thermal expansion of PIPOZ in water ( $\alpha_{\rm pol}$ ), determined by PPC, underwent a sharp increase at the temperature corresponding to the onset of phase transition, reaching a maximum value at  $T \sim T_{\rm CP}$ . Microcalorimetric measurements were carried out with solutions of PIPOZ samples in D<sub>2</sub>O and in aqueous NaCl solutions. The thermodynamic and volumetric changes associated with the phase transition of aqueous PIPOZ solutions are compared to those of aqueous solutions of two related polymers, poly(vinylcaprolactam) and poly(*N*-isopropylacrylamide) (PNIPAM), a polymer structurally related to PIPOZ that undergoes a phase transition in water at ~31 °C.

## Introduction

A large body of work has been devoted over the past decades toward the development of drug delivery systems based on polymeric micelles featuring longevity in blood circulation.<sup>1-6</sup> In most studies, these drug vehicles consist of a hydrophilic poly(ethylene glycol) (PEG) outer corona, since this polymer prevents nonspecific adsorption of the polymeric micelles to proteins and cells, thus allowing the micelles to evade recognition by the reticuloendothelial system.<sup>3,7,8</sup> There has been little investigation into the use of hydrophilic coronaforming polymers other than PEG to create micellar drug delivery systems. Charged hydrophilic polymers have been used for delivery to mucosal surfaces, such as the respiratory, gastrointestinal, and urogenitary tracts.<sup>9</sup> Polymeric micelles with a corona made up of natural or semisynthetic polysaccharides are investigated as well as vehicles in the oral administration of poorly water soluble drugs.<sup>10,11</sup> Recently, polymeric micelles based on block copolymers of 2-ethyl-2-oxazoline (PEOZ) and  $\epsilon$ -caprolactone<sup>12</sup> were evaluated as drug carriers and shown to have low cytotoxicity and hemolytic activity.13

In contrast to PEG or polysaccharide systems, poly-(2-alkyl-2-oxazolines), synthesized by living cationic polymerization, can be tailored through the choice of not only the end groups but also the side chains.<sup>14–16</sup> The length of the alkyl substituents controls to a large extent the relative hydrophilicity of the poly(2-alkyl-2-oxazolines), and only few oxazolines, such as the 2-methyl-, 2-ethyl-, and 2-isopropyl-2-oxazolines, lead to polymers soluble in water at room temperature.<sup>17</sup> The biocom-

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patibility and interfacial characteristics of poly(2-methyl-2-oxazolines) (PMOZ) and PEOZ have been assessed in various biotechnological applications.<sup>18–20</sup> Lipid vesicles doped with poly(2-ethyl-2-oxazoline) lipopolymers exhibited enhanced blood circulation times compared to ordinary phospholipid vesicles.<sup>21</sup> The ability of the PMOZ and PEOZ chains to elicit beneficial properties when grafted onto the surface of liposomes has been explained by the conformational mobility of the polymers and the tendency of their repeat units to form hydrogen bonds with water.<sup>21</sup> However, quantitative information on the interactions of poly(2-alkyl-2-oxazolines) with water is scarce compared to the wealth of data dealing with the behavior of PEG in water.<sup>22</sup>

Among the various poly(2-alkyl-2-oxazolines), PEOZ has attracted the most attention by far. Aqueous solutions of PEOZ exhibit a cloud point around 62-65 °C depending on molecular weight and concentration.<sup>23</sup> The cloud point temperature is affected by the presence of cosolvents, such as dioxane, and of salts, decreasing in the presence of sodium chloride but increasing upon addition of tetrabutylammonium bromide.24 Studies of PMOZ and PEOZ in water by light scattering revealed that their second virial coefficients decrease with increasing solution temperature, an indication of the involvement of water/polymer hydrogen bonds in the mechanism of the temperature-induced phase separation.<sup>25,26</sup> The phase transition of aqueous PEOZ solutions was investigated over the entire water/polymer composition domain. Concentrated PEOZ solutions obey the "classical" Flory-Huggins miscibility behavior: the cloud point shifts to lower polymer concentration as the molecular weight of the polymer increases ( $M_{\rm vis}$  22 000-170 000 g mol<sup>-1</sup>).<sup>25,27</sup> As pointed out by Lin et al.,<sup>24</sup> the repeat unit of PEOZ is isomeric to that of poly(N,N)dimethylacrylamide) (Figure 1), and as such, there are similarities in the solubility and miscibility character-

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**Figure 1.** Chemical structure of the repeat units of poly(2ethyl-2-oxazoline) (PEOZ), poly(*N*,*N*-dimethylacrylamide), poly-(2-isopropyl-2-oxazoline) (PIPOZ), and poly(*N*-isopropylacrylamide) (PNIPAM).

istics of the two polymers; yet, there are also important differences. Although both polymers are soluble in water, only PEOZ aqueous solutions exhibit a cloud point below 100 °C, when the polymer molecular weight exceeds a threshold value of  $\sim 10\ 000\ g\ mol^{-1}.^{27}$  In this respect, one may note that the repeat unit of poly(2isopropyl-2-oxazoline) (PIPOZ) is isomeric to that of poly(N-isopropylacrylamide) (PNIPAM) (Figure 1), a water-soluble polymer that exhibits a cloud point in water at 31 °C.<sup>28</sup> Indeed, PIPOZ in water presents a cloud point, as revealed by turbidity studies.<sup>29</sup> The cloud point of a solution of PIPOZ ( $M_n$  16 700) ranges from 39 to 36 °C, depending on polymer concentration. Addition of NaCl depresses the solution cloud point, while surfactants such as sodium dodecyl sulfate and n-dodecyltrimethylammonium chloride have the opposite effect. From these data, one detects similarities in the solution properties of PIPOZ and PNIPAM. Nonetheless, cloud point values give little indication on the differences in the state of hydration of the two polymers.

As part of a project aimed at the development of novel PIPOZ-based thermoresponsive polymeric micelles, we prepared recently a series of PIPOZ oligomers terminated with a hydroxyl group. To understand the solution properties of the ultimate polymeric micelles, we set about to study the properties of aqueous solutions of PIPOZ as they undergo phase transition. Two techniques, high-sensitivity differential scanning calorimetry (DSC) and pressure perturbation calorimetry (PPC), were employed. From the DSC measurements, we retrieve the temperature and the enthalpy of the phase transition of solutions of oligomers of various molecular weights and polydispersities. From the PPC results, we derive the volumetric properties of PIPOZ solutions as they undergo a phase transition. Pressure perturbation calorimetry measures the heat absorbed or released by a solution due to a sudden pressure change at constant temperature.<sup>30</sup> The heat can be used to calculate the coefficient of thermal expansion of the solute and its temperature dependence. The technique has been exploited to obtain the changes in the volume of the solvation layer around proteins before and after unfolding,<sup>31</sup> to monitor the volume changes accompanying the pretransition and transition of phospholipid bilayers,<sup>32</sup> and to evaluate the volume changes of the solvation layer during the coil/globule collapse in water of PNIPAM,<sup>33,34</sup> hydrophobically modified PNIPAM samples,<sup>33,34</sup> and poly(vinylcaprolactam).<sup>35</sup>

Solutions of PIPOZ in  $H_2O$ ,  $D_2O$ , and aqueous NaCl solutions were examined by the two calorimetry tech-

niques in order to assess the relative importance of water/polymer hydrogen bond formation and polymer/polymer hydrophobic interactions. Light and heavy water are chemically identical, yet their physical properties differ significantly.<sup>36</sup> The dissimilarities between H<sub>2</sub>O and D<sub>2</sub>O are believed to stem from differences in the intermolecular hydrogen bonds energies. The lengths of hydrogen bonds in the two liquids are about the same, but a "hydrogen" bond in D<sub>2</sub>O is about 5% stronger than a hydrogen bond in H<sub>2</sub>O. Overall, D<sub>2</sub>O is a more "structured" solvent than light water.<sup>37</sup>

In a first section, we present the preparation of the oligomers and describe their chemical characteristics obtained from <sup>1</sup>H NMR spectroscopy, MALDI–TOF mass spectrometry, and gel permeation chromatography (GPC). Then, we report the results of the microcalorimetry study of PIPOZ samples, placing particular emphasis on the effect of the polymer chain length on its behavior during the solution phase transition. Taken together, the results suggest a description of the PIPOZ/ water interactions, which will be compared to the currently accepted view of the phenomena taking place in aqueous solutions of PNIPAM, the chemical isomer of PIPOZ.

## **Experimental Section**

**Materials.** Water was deionized with a Millipore Milli-Q water purification system. Isobutyric acid, 2-aminoethanol, and methyl *p*-tosylate were purchased from Wako Pure Chemical Industries. Deuterium oxide (99.8%) was purchased from Aldrich-Sigma Chemicals. PNIPAM was prepared by RAFT polymerization, using a trithiocarbonate RAFT agent.<sup>38</sup>

**Instrumentation.** NMR spectra were recorded on a Bruker ARX-400 400 MHz spectrometer. UV/vis spectra were measured with a Hewlett-Packard 8452A photodiode array spectrometer equipped with a Hewlett-Packard 89090 temperature controller. Gel permeation chromatography (GPC) measurements were performed with a TOSOH HLC-8220 system equipped with a TOSOH differential refractometer, two TOSOH Super HHZ columns (4000 and 3000) eluted with DMF containing triethylamine (30 mM), and LiCl (10 mM) (flow rate  $0.8 \text{ mL min}^{-1}$ ,  $40^{\circ}$ C) and calibrated with poly(ethylene glycol) standards used to determine the molecular weights. MALDI-TOF mass spectra were recorded with a Reflex III (Bruker). All spectra were obtained in positive ion mode, and ionization was performed with a 337 pulsed (3 ns pulse width,  $10^{6}-10^{7}$ W cm<sup>-2</sup>) nitrogen laser. An external calibration was performed using poly(ethylene glycol) (MeO-PEG-OH) of M<sub>w</sub> 5000 (NOF Corp.). To prepare the matrix, a solution of the polymer in THF (20  $\mu$ L, 6 mg mL<sup>-1</sup>) was mixed with a solution of 8,9trihydroxyanthracene in THF (20  $\mu$ L, 10 mg mL<sup>-1</sup>) by sonication for a few minutes. A solution of lithium trifluoroacetate in THF (2  $\mu$ L, 2 mg mL<sup>-1</sup>) was added to the matrix. The resulting mixture was shaken briefly and applied to the probe  $(2 \ \mu L)$ 

**Preparation of 2-Isopropyl-2-oxazoline**.<sup>39</sup> Isobutyric acid (132 g, 1.5 mol) was added to 2-aminoethanol (92 g, 1.5 mol) under stirring. The resulting salt was heated slowly to 230 °C, a temperature higher than its melting point. The liquid was refluxed for 48 h, followed by azeotropic distillation at 110 °C. The distillate was diluted in dichloromethane (350 mL) to separate water from 2-isopropyl-2-oxazoline. The aqueous layer was extracted repeatedly with dichloromethane. The combined organic layers were concentrated in vacuo. The residue was purified by repeated distillations in the presence of CaH<sub>2</sub>, yielding dry 2-isopropyl-2-oxazoline (72 g, b.p. 138 °C). <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$ ): 1.11 (d, 6 H, (CH<sub>3</sub>)<sub>2</sub>CH–), 2.49 (m, 1 H, (CH<sub>3</sub>)<sub>2</sub>CH–), 3.79 (t, 2 H, NCH<sub>2</sub>CH<sub>2</sub>O), 4.18 ppm (t, 2H, NCH<sub>2</sub>CH<sub>2</sub>O).

**Polymerizations. Hydroxy-Terminated Poly(2-isopropyl-2-oxazolines).** A solution of 2-isopropyl-2-oxazoline (9.74 g, 86 mmol) and methyl *p*-tosylate (0.30 g, 1.0 mmol) in nitromethane (30 mL) was stirred at 40 °C under argon. Aliquots of the polymerization solution (1.0 mL) were taken at various time intervals (48, 72, 120, and 168 h). They were cooled to room temperature and poured into a mixture of aqueous NaOH (1.5 mL, 1.0 M) and methanol (1.5 mL). The resulting solution was dialyzed against water for 2 days. The polymer was isolated by freeze-drying. <sup>1</sup>H NMR (CDCl<sub>3</sub>,  $\delta$ ): 1.1 (br s, [(CH<sub>3</sub>)<sub>2</sub>CH–], 2.66 and 2.89 (br m, [(CH<sub>3</sub>)<sub>2</sub>CH–], 3.08 (s, terminal CH<sub>3</sub>), 3.54 (br s, –(CH<sub>2</sub>–CH<sub>2</sub>–N)–).

**Cloud Point Determinations.** Cloud points were determined by spectrophotometric detection of the changes in turbidity ( $\lambda = 600$  nm) of aqueous polymer solutions (1.0 g L<sup>-1</sup>) heated at a constant rate (1 °C min<sup>-1</sup>) in a magnetically stirred UV cell. The value reported is the temperature corresponding to a decrease of 20% of the solution transmittance.

**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 0.52 mL. The heating rate was 1.0 °C min<sup>-1</sup>, 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 ranged from 0.5 to 2.0 g L<sup>-1</sup>.

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 (0.52 mL). The polymer concentration was 5 0.0 g  $L^{-1}\!.$  Control experiments were performed, namely water (sample cell) vs water (reference cell), H<sub>2</sub>O vs D<sub>2</sub>O, D<sub>2</sub>O vs D<sub>2</sub>O, NaCl in water vs NaCl in water, and water vs NaCl in water. The PPC accessory has been described in detail elsewhere.<sup>30</sup> Briefly, it applies a pressure of 500 kPa, then a pressure release to ambient pressure, to the sample, which is kept at constant temperature. The temperature of the sample cell is kept constant by compensation of the heat change caused by the pressure jump. After equilibration, an upward pressure jump (500 kPa) is performed. The heat peaks caused by the compression and decompression are opposite in sign but agree in absolute value. A large number of compression/decompression cycles are performed at each temperature.

The coefficient of thermal expansion of a polymer solute in a solvent is obtained from eq  $1^{\rm 33}$ 

$$\alpha_{\rm pol} = \alpha_{\rm solv} - \frac{\Delta Q_{\rm rev}}{T \Delta P m_{\rm pol} \bar{V}_{\rm pol}} \tag{1}$$

where  $\alpha_{pol}$  and  $\alpha_{solv}$  are the thermal expansion coefficients of the polymer and the solvent, respectively,  $\Delta Q_{rev}$  corresponds to the heat consumed or released upon the small pressure changes,  $\Delta P$  is the change in pressure, *T* is the temperature,  $\bar{V}_{pol}$  is the partial specific volume of the polymer, and  $m_{pol}$  is the mass of polymer in solution. The partial specific volumes of the polymers were determined by an increment method based on the group contribution theory developed to estimate  $\bar{V}$  of aqueous systems and estimated to be accurate within 2%.<sup>40</sup> Thus, for PIPOZ  $\bar{V} = 0.888$  cm<sup>3</sup> g<sup>-1</sup> and for PNIPAM  $\bar{V} = 0.894$  cm<sup>3</sup> g<sup>-1</sup>.

The change in volume of the solvation layer of the polymer  $(\Delta V)$  during a phase transition is obtained by integration of the curve of the changes in the coefficient of thermal expansion with temperature (eq 2), assuming that  $\Delta V$  is small compared to  $\overline{V}$ , the intrinsic volume of the polymer. A complete derivation of the equations has been reported previously.<sup>30,33</sup> Data were analyzed using the software supplied by the manufacturer.

$$\frac{\Delta V}{V} = \int \alpha \, \mathrm{d} \, T \tag{2}$$

Results

**Preparation and Characterization of the Polymers.** The oligomers were synthesized by living cationic



**Figure 2.** Reaction scheme for the preparation of poly(2-isopropyl-2-oxazolines) and structure of the polymer.

 
 Table 1. Polymerization Conditions and Characteristics of the Poly(2-isopropyl-2-oxazolines)

polymer	[M <sub>0</sub> ]/ [I <sub>0</sub> ] <sup>a</sup>	time (h)	$M_{ m n}{}^b$ (g mol <sup>-1</sup> )	$M_{ m w}/M_{ m n}^{b}$	$M_{ m n}{}^c$ (g mol <sup>-1</sup> )	DP <sup>c</sup>
PIPOZ <sub>17</sub> -OH PIPOZ <sub>21</sub> -OH PIPOZ <sub>41</sub> -OH PIPOZ <sub>50</sub> -OH PNIPAM	86 86 86 86	48 72 120 168	1700 2400 4300 5700 13000	1.05 1.04 1.03 1.03 1.16	1900 2400 4600 5650	17 21 41 50

<sup>a</sup> Initial monomer/initiator feed ratio. <sup>b</sup> From GPC measurements. <sup>c</sup> From <sup>1</sup>H NMR spectra in CDCl<sub>3</sub>.



**Figure 3.** MALDI–TOF mass spectrum of  $PIPOZ_{50}$ -OH obtained after 168 h polymerization. An expanded view of the spectrum ranging from 6100 to 6400 emu is given in the inset.

polymerization, initiated with methyl *p*-tosylate, of 2-isopropyl-2-oxazoline, followed by termination with a NaOH/methanol solution yielding hydroxyl-terminated chains (Figure 2). Polymerization conditions were selected to achieve the narrow molecular weight polydispersity and low polymer molecular weight required by the ultimate application of the PIPOZ chains as drug delivery vehicles. One set of samples (PIPOZ<sub>n</sub>-OH, n = 17, 21, 41, and 50; Table 1) was obtained by sampling a single polymerization mixture at various time intervals. This method provided oligo(2-isopropyl-2-oxazolines) of narrow polydispersity index, as determined by GPC and MALDI–TOF mass spectrometry (Table 1).

The mass spectrum of PIPOZ-OH ( $M_n$  6190) presented in Figure 3 is dominated by one main ion population. The spacings of the  $\Delta m/z$  signals, 113.192, are in good agreement with the expected mass of one monomer unit. The major population of signals was attributed to the Li<sup>+</sup> adduct and the minor population of signals to the Na<sup>+</sup> adduct. On the basis of the mass spectra, it can be assumed that all oligomers bear the desired endfunctionalities, namely a methyl group at one chain end and either a hydroxyl or a primary amine group at the other chain end.

The degree of polymerization of the oligomers was calculated from the <sup>1</sup>H NMR spectra of the samples in



Figure 4. <sup>1</sup>H NMR spectrum of PIPOZ<sub>21</sub>-OH in CDCl<sub>3</sub>.

Table 2. Thermodynamic Parameters of the Poly(2-isopropyl-oxazolines)

solvent	$T_{\rm CP}(^{\circ}{ m C})^a$	$T_{\text{onset}} (^{\circ}\text{C})^{b}$ (DSC)	$T_{\max} (^{\circ}C)^{b}$ (DSC)	$\Delta H (\text{kcal mol}^{-1})^b$ (DSC)	$T_{\max} (^{\circ}C)^{c}$ (PPC)	$\Delta V V (\%)^c$
H <sub>2</sub> O	$72.5\pm0.2$	$73.1\pm0.2$	$76.5\pm0.3$	$0.36\pm0.01$	$65.0\pm0.3$	0.43
$D_2O$		$68.9\pm0.2$	$75.2\pm0.3$	$0.39\pm0.01$	$63.0\pm0.3$	0.70
$H_2O$	$62.8\pm0.2$	$61.4 \pm 0.2$	$64.0\pm0.3$	$0.72\pm0.01$	$52.5\pm0.3$	0.64
$D_2O$	$60.3\pm0.2$	$58.9\pm0.2$	$62.1\pm0.3$	$0.85\pm0.01$	$53.0\pm0.3$	1.00
$H_2O$	$51.3\pm0.2$	$51.3\pm0.2$	$54.0\pm0.3$	$0.90\pm0.01$	$45.2\pm0.3$	0.84
$D_2O$		$45.2\pm0.2$	$51.3\pm0.3$	$1.16\pm0.01$	$47.5\pm0.3$	1.30
$H_2O$	$48.1\pm0.2$	$46.5\pm0.2$	$48.0\pm0.3$	$1.35\pm0.01$	$45.0\pm0.3$	1.10
$D_2O$		$46.9\pm0.2$	$47.0\pm0.3$	$1.43\pm0.01$	$43.0\pm0.3$	1.80
NaCl (0.5 M)		$37.1\pm0.2$	$40.6\pm0.3$	$1.61\pm0.01$	$37.0\pm0.3$	1.30
NaCl (1.0 M)		$33.5\pm0.2$	$35.0\pm0.3$	$1.76\pm0.01$	$32.0\pm0.3$	1.40
H <sub>2</sub> O		$24.5\pm0.2$	$32.6\pm0.3$	$0.80\pm0.01$	$32.0\pm0.3$	0.90
$D_2O$						
NaCl (0.5 M)		$18.4 \pm 0.2$	$25.6\pm0.3$	$1.20\pm0.01$	$26.0 \pm 0.3$	1.00
NaCl (1.0 M)		$13.6\pm0.2$	$20.1\pm0.3$	$1.37\pm0.01$	$21.0\pm0.3$	1.20
H <sub>2</sub> O	$32.7^{d}$		$31.7^{d}$	$0.45^{d}$		
	$\begin{array}{c} \text{solvent} \\ H_2O \\ D_2O \\ H_2O \\ D_2O \\ H_2O \\ D_2O \\ H_2O \\ D_2O \\ NaCl (0.5 \text{ M}) \\ NaCl (1.0 \text{ M}) \\ H_2O \\ D_2O \\ NaCl (0.5 \text{ M}) \\ NaCl (1.0 \text{ M}) \\ H_2O \\ NaCl (1.0 \text{ M}) \\ H_2O \\ \end{array}$	$\begin{array}{c c} \mbox{solvent} & $T_{\rm CP}  (^\circ {\rm C})^a$ \\ \hline H_2 {\rm O} & 72.5 \pm 0.2 \\ D_2 {\rm O} & \\ H_2 {\rm O} & 62.8 \pm 0.2 \\ D_2 {\rm O} & 60.3 \pm 0.2 \\ H_2 {\rm O} & 51.3 \pm 0.2 \\ D_2 {\rm O} & \\ H_2 {\rm O} & 48.1 \pm 0.2 \\ D_2 {\rm O} & \\ Na {\rm Cl}  (0.5  {\rm M}) \\ Na {\rm Cl}  (1.0  {\rm M}) \\ H_2 {\rm O} & \\ D_2 {\rm O} \\ Na {\rm Cl}  (0.5  {\rm M}) \\ Na {\rm Cl}  (1.0  {\rm M}) \\ H_2 {\rm O} & \\ 32.7^d \end{array}$	$\begin{array}{c c} & T_{\rm cp}(^{\rm e}{\rm C})^a & T_{\rm onset}(^{\rm e}{\rm C})^b \\ ({\rm DSC}) \\ \hline \\ H_2{\rm O} & 72.5 \pm 0.2 & 73.1 \pm 0.2 \\ D_2{\rm O} & 68.9 \pm 0.2 \\ H_2{\rm O} & 62.8 \pm 0.2 & 61.4 \pm 0.2 \\ D_2{\rm O} & 60.3 \pm 0.2 & 58.9 \pm 0.2 \\ H_2{\rm O} & 51.3 \pm 0.2 & 51.3 \pm 0.2 \\ D_2{\rm O} & 45.2 \pm 0.2 \\ H_2{\rm O} & 48.1 \pm 0.2 & 46.5 \pm 0.2 \\ D_2{\rm O} & 46.9 \pm 0.2 \\ Na{\rm Cl}(0.5{\rm M}) & 37.1 \pm 0.2 \\ M_2{\rm O} & 24.5 \pm 0.2 \\ H_2{\rm O} & 32.7^d \\ \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

<sup>*a*</sup> From turbidity measurements (polymer concentration: 1.0 g L<sup>-1</sup>). <sup>*b*</sup> From DSC measurements (polymer concentration: 1.0 g L<sup>-1</sup>). <sup>*c*</sup> From PPC measurements (polymer concentration: 5.0 g L<sup>-1</sup>). <sup>*d*</sup> From ref 44.

chloroform-D, using the broad singlet at  $\delta$  1.11 ppm, ascribed to the resonance of the methyl protons of the isopropyl group of the repeat unit, together with the singlet at  $\delta$  3.08 ppm, attributed to the resonance of the terminal methyl protons (Figure 4). The number-average molecular weights derived from integrations of these <sup>1</sup>H NMR signals are in excellent agreement with the molecular weights determined by GPC (Table 1) and MALDI–TOF mass spectra (Figure 3).

**Phase Transition of the Oligomers in Water. Turbidity Measurements.** The fastest method to determine the cloud point of a polymer solution consists of measuring the changes in turbidity as the solution is heated at a constant rate. The temperature of turbidity onset, defined here as the cloud point temperature,  $T_{CP}$ , decreases with increasing PIPOZ molecular

weight (Table 2). The turbidity increased sharply as the temperature exceeded this onset value. To ascertain the reversibility of the phase transition, polymer solutions heated to 70 °C were kept at this temperature for 30 min and then cooled to room temperature at constant cooling rate. The solutions became clear in all cases, but the temperature for which 80% transmission was recovered was lower by  $\sim 2$  °C than  $T_{CP}$  recorded during a heating scan. The cloud point temperature of a solution of PIPOZ<sub>21</sub>-OH (1.0 g  $L^{-1}$ ) was determined in light and heavy water: it was lower by  $\sim 1.5$  °C in D<sub>2</sub>O compared to  $H_2O$  (Table 2). Note that this trend is in agreement with an observation made by Chen et al. in their study of a polydisperse sample of PEOZ ( $M_w$ 116 000). They reported that use of  $D_2O$  in place of  $H_2O$ yielded a cloud point some 3 °C lower.<sup>25</sup>



Figure 5. Microcalorimetric endotherms for aqueous solutions of PIPOZ-OH samples in water. Polymer concentration: 1.0 g  $L^{-1}$ . Heating rate: 60 °C  $h^{-1}$ .

High-Sensitivity Differential Calorimetry. The changes with temperature of the partial excess heat capacity  $C_p$  of aqueous solutions of several PIPOZ-OH samples (1 g L<sup>-1</sup>) are presented in Figure 5. The thermograms are endothermic, broad, and markedly asymmetric, with a sharp increase in heat capacity on the low-temperature side (onset of the transition,  $T_{onset}$ ) and a gradual decrease of the heat capacity for temperatures higher than a maximum temperature  $T_{\text{max}}$ . For all polymers the values of  $C_p$  were the same, within experimental error, before and after the transition. Neither changing the concentration of the solution, from 0.5 to 5 g  $L^{-1}$ , nor varying the scanning rate, from 10 to 90 °C/h, affected the shape of the thermograms. Therefore, the characteristic times of the transitions are shorter than those of the thermal equilibration of the cells. The reproducibility of the thermograms was demonstrated during the second and third heating at the same heating rate. The transition observed is completely reversible.

From plots of the partial heat capacity of PIPOZ solutions vs temperature, one can extract three thermodynamic parameters:  $T_{\text{onset}}$ ,  $T_{\text{max}}$ , and  $\Delta H$ , the enthalpy of the transition (Table 2). The values of  $T_{\text{onset}}$ and  $T_{\text{max}}$  follow the same trends as the values of  $T_{\text{CP}}$ : for a given polymer, they decrease with increasing concentration, and for solutions of identical concentration, they decrease with increasing polymer molecular weight (Figure 6). The enthalpy of transition, as well, exhibits a significant dependence on molecular weight: it increases from 0.36  $\pm$  0.01 kcal (mol of monomer unit)<sup>-1</sup> for the shortest oligomer to  $1.40 \pm 0.01$  kcal  $mol^{-1}$  for the longest (Figure 6). The latter value corresponds approximately to the energy required to break one hydrogen bond per repeat unit.41 It would appear, therefore, that the availability of the carbonyl groups in the PIPOZ chain to form hydrogen bonds with water increases with molecular weight. A similar observation was made by Chen et al.<sup>26</sup> in their light scattering studies of PEOZ in water: they report that, for solutions of this polymer in water, the excess enthalpy of dilution becomes more negative with increasing polymer molecular weight.

The phase transition of PIPOZ oligomers was affected by the presence of NaCl: increased electrolyte concentration decreased the phase transition temperature and increased the transition enthalpy (Table 2). Both phenomena are consistent with the well-known "saltingout" effect of NaCl and corroborate observations of



**Figure 6.** (top) Plots of the changes as a function of polymer concentration of the transition temperature,  $T_m$  (full circle), and of the cloud point, CP (full triangle), for aqueous solutions of PIPOZ<sub>41</sub>-OH. (bottom) Plots of the changes as a function of polymer molecular weight of the enthalpy of the transition,  $\Delta H$  (open circle), the temperature corresponding to the maximum in DSC enthalpograms,  $T_m$  (full circle), and the cloud point, CP (full triangle), for aqueous solutions of poly(2-isopropyl-2-oxazolines). Polymer concentration: 1.0 g L<sup>-1</sup>. Heating rate: 60 °C min<sup>-1</sup>.

Uyama and Kobayashi in their study of a PIPOZ sample of higher molecular weight<sup>29</sup> and of Lin et al. in their investigation of PEOZ.<sup>24</sup>

Thermograms recorded for polymer solutions in  $D_2O$  exhibit the same skewed shape as the thermograms of polymer solutions in  $H_2O$ , as exemplified in Figure 6 (top), where we show DSC scans for solutions of PI-POZ<sub>50</sub>-OH in heavy and light water. In all cases, the  $T_{onset}$  and  $T_{max}$  values recorded for solutions in  $D_2O$  are lower than those obtained for solutions in  $H_2O$ , and the differences between solutions in the two solvents increase as the molecular weight of the polymer decreases.

Pressure Perturbation Calorimetry. PPC scans were carried out next with solutions of PIPOZ<sub>n</sub>-OH (n = 17, 21, 41, and 50) in H<sub>2</sub>O and in D<sub>2</sub>O, yielding the changes with temperature of the thermal expansion coefficient,  $\alpha_{pol}$ , shown in Figure 7 (bottom), in the case of PIPOZ<sub>50</sub>-OH. The plot can be divided in three temperature ranges. Below  $T_{\text{onset}}$ , for 15 °C < T < 42 °C,  $\alpha_{\text{pol}}$  remains constant. It undergoes a sharp increase, reaches a maximum for  $T_{\text{peak}} = 45.0$  °C, and gradually decreases as the temperature further increases. PPC scans recorded with solutions of the same polymer in  $D_2O$  presented the same features (Figure 7, bottom), with slight differences in the temperatures of the maximum of  $\alpha_{pol}$ , that correspond to the slight shifts of  $T_{\text{onset}}$  and  $T_{\text{max}}$  already noted in the DSC scan (Figure 7, top). The changes in volume of the solvation layer of the polymer ( $\Delta V$ ), corresponding to the collapse of the chain and chain aggregation during the phase transition, can be extracted from PPC scans by integration of the changes in  $\alpha_{pol}$  as a function of temperature,



**Figure 7.** (top) Microcalorimetric endotherms for a solution of PIPOZ<sub>50</sub>-OH in H<sub>2</sub>O and in D<sub>2</sub>O. Polymer concentration 1 g L<sup>-1</sup>. (bottom) Temperature dependence of the coefficient of thermal expansion ( $\alpha_{pol}$ ) of PIPOZ<sub>50</sub>-OH in H<sub>2</sub>O (full circle) and in D<sub>2</sub>O (open circle). Polymer concentration: 5 g L<sup>-1</sup>. The dotted line corresponds to the progress baseline.

assuming that the intrinsic volume occupied by a polymer chain remains constant. The volume change, expressed as  $\Delta V V$  in percent of the partial volume of the polymer (eq 2), is taken as the area defined by the peak of the PPC scan and a progress baseline (dashed line, Figure 6, bottom) drawn from projections of the baselines in the pretransition and posttransition regions. Values of  $\Delta V/V$  for PIPOZ-OH samples in H<sub>2</sub>O,  $D_2O_2$ , and aqueous NaCl are listed in Table 2. Comparing samples of PIPOZ-OH of increasing molecular weight, we observe that the solvation volume change during the phase transition is extremely sensitive to the molecular weight of the oligomer, nearly doubling in value for solutions of PIPOZ<sub>50</sub>-OH, compared to the solutions of the shortest oligomer, PIPOZ<sub>17</sub>-OH. This significant effect corroborates the sensitivity to molecular weight of the enthalpy of transition (vide supra) and may be taken as added evidence that the number of hydrogen bond sites along the chain increases with molecular weight.

Considering next the values of  $\Delta V/V$  recorded in H<sub>2</sub>O and in  $D_2O$ , we note that for all samples the volume change in  $D_2O$  is larger by ~50% than the value recovered from measurements in H<sub>2</sub>O. The enhanced change in hydration volume in D<sub>2</sub>O, compared to H<sub>2</sub>O, results from a combination of two effects: (1) the PIPOZ coils may adopt a more extended conformation in  $D_2O$ , resulting in a higher level of ordering of the D<sub>2</sub>O molecules bound to the polymer chain and hence a larger volume of bound water, and (2) since  $D_2O$  is a more "structured" solvent than H<sub>2</sub>O, the water molecules expelled from the hydration layer occupy a larger volume of bulk solvent. The changes in the volume of hydration of the polymer during the phase transition are also affected by the presence of NaCl: the values of  $\Delta V/V$  increase with increasing salt concentration (Table

2), reflecting salt-induced changes in the hydration layer and in the polymer chain stiffness in solutions of temperature lower than the transition temperature.

## Conclusions

As noted in the Introduction, the repeat unit of poly-(2-isopropyl-2-oxazoline) is a structural isomer of the repeat unit of poly(*N*-isopropylacrylamide). These two polymers share a number of characteristics when dissolved in water: they both undergo a reversible heatinduced phase transition when brought to a temperature beyond a critical value. This temperature, detectable by changes in the transmission of the solution, or more accurately measured by DSC, depends on salt concentration and is different for solutions of the polymers in H<sub>2</sub>O and D<sub>2</sub>O. The phase transition of PNIPAM has been studied in great detail.<sup>42</sup> As the PIPOZ samples investigated here are monodisperse and of low molecular weight, we chose to compare their properties to those of PNIPAM oligomers, taking values from published reports. Microcalorimetric studies carried out independently with NIPAM oligomers bearing a carboxylic acid end group revealed that solutions of PNIPAM  $(M_{\rm n}$  1600, 2200, 2400, and 3500) have a transition temperature slightly higher than solutions of higher molecular weight PNIPAM (33.3 vs 31.5 °C) with a transition enthalpy of 0.45 kcal mol $^{-1}$   $L^{-1}\!\cdot\!\!{}^{43,44}$  These studies concur to indicate that, although the transition temperature,  $T_{\rm m}$ , is only slightly higher for NIPAM oligometric solutions, compared to  $T_{\rm m}$  of solutions of PNIPAM samples of higher molecular weight and wider polydispersity,<sup>33,45</sup> the transition peaks are wider and the transition enthalpies weaker in the case of NIPAM oligomers. The latter two features are observed in our study of PIPOZ, suggesting that they are related to the molecular weights of the samples rather than to their chemical constitution.

There are however several differences in the thermodynamic traits of aqueous solutions of PNIPAM and PIPOZ oligomers (Table 2). First, the molecular weight sensitivity of  $T_{\rm m}$  is pronounced in the case of PIPOZ and not PNIPAM. Second, the transition temperature of aqueous PNIPAM is higher in  $D_2O$ , compared to  $H_2O$ , whereas aqueous PIPOZ solutions exhibit the opposite effect. The latter situation seems to be a more common occurrence: it has been reported for solutions of hydroxypropylcellulose<sup>46</sup> and poly(N-vinylcaprolactam),<sup>35</sup> two polymers that possess a cloud point in water. The enthalpy of transition is higher in  $D_2O_1$ , compared to H<sub>2</sub>O, for solutions of PNIPAM or PIPOZ, but the enhancement in D<sub>2</sub>O is significantly larger in the case of PIPOZ. Third, thermograms recorded with PNIPAM solutions present a negative change in  $\Delta C_p$  ( $\Delta C_p = C_p(T > T_m) - C_p(T < T_m) = -63$  J mol<sup>-1</sup> K<sup>-1</sup>), while  $\Delta C_p \sim 0$  for PIPOZ. A negative change of  $C_p$  was reported also in studies of the phase transitions of various pluronic-type block copolymers.<sup>47</sup> Such a negative heat capacity change during the phase transition may be taken as an indication of diminished interaction between water molecules and polymer chains, before and after the transition.

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