Interactions of Thermally Responsive Polyelectrolyte Latices with Low Molar Mass Organic Molecules Studied by Light Scattering

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Received September 30, 1997; Revised Manuscript Received January 5, 1998

ABSTRACT: A latex of a thermally responsive polyelectrolytic copolymer of *N*-isopropylacrylamide, methyl methacrylate, and methacrylic acid was synthesized by emulsion polymerization. An aqueous latex of the copolymer and its mixtures with low molar mass organic compounds, ibuprofen, protocatechuic acid, and ephedrine, were studied by dynamic light scattering at temperatures from 20 to 80 °C. In this temperature range, pure copolymer latex particles gradually collapse with increasing temperature. The interactions of low molar mass compounds with the copolymer strongly depend on the ratio of hydrophilic and hydrophobic structural units in the polymer and in the low molar mass compounds. Ibuprofen and protocatechuic acid were strongly bound to the copolymer latex, causing a decrease in the particle size and a sharpening of the volume transition of the latex particles. Ephedrine is a water-soluble substance, and it was bound to the polymer particles below the lower critical solution temperature LCST. Binding of ephedrine to the polymer led to aggregate formation; the aggregates break down when temperature is increased above the LCST.

Introduction

In the last 2 decades, polymeric materials used in drug delivery in both their soluble and particulate forms have been widely studied.[1−3] Several hydrophilic polymers are close to the natural components of the body and, whether of natural or synthetic origin, can be tailored to the desired physicochemical properties. Thus, polymeric materials may be used to provide a slow release of the drug, either bound covalently or mixed into the polymer. To design and develop new and more specific delivery systems, biocompatible copolymers of hydrophilic and hydrophobic monomers with the appropriate composition are sythesized to control the rate of drug release.

Poly(*N*-isopropylacrylamide), PNIPAAM, has attracted wide interest in biomedical applications because it exhibits a well-defined lower critical solution temperature (LCST) in water around 32 °C. PNIPAAM expands and swells when cooled below the LCST, and it shrinks and collapses when heated above the LCST.[4,5] This behavior owes to the hydrophobicity of PNIPAAM chains due to the presence of isopropyl side groups. When NIPAAM is copolymerized so as to obtain the right balance of hydrophobic and hydrophilic monomers and to adjust the number of electric charges in the chain,[6−9] its LCST as well as other physical properties can be manipulated. Previous studies in our laboratory[10−12] showed that if the amount of acidic monomer copolymerized with NIPAAM is kept low enough, the aqueous solutions of the copolymers show both the characteristics of polyelectrolytes and the LCST behavior of the homopolymer PNIPAAM. Therefore, if NIPAAM copolymers are used as drug delivery matrixes, they can be expected to act as intelligent materials responsive to the enviromental changes of human bodies, such as temperature, solvent composition, pH, and ionic strength.[2]

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To obtain a deeper insight into the interaction between the polymers and certain low molar mass organic compounds at a molecular level, it would be beneficial to use a monodisperse polymer. Often, by solution polymerization the molar masses of the resulting polymers are low and the molar mass distributions are wide. However, by aqueous radical emulsion polymerization, monodisperse colloidal PNIPAAM particles have been obtained, which show a discontinuous and reversible volume transition with temperature.[13,14] Emulsion polymerization has been used also in the present work to prepare monodisperse polymer latices.

Dynamic light scattering (DLS), used to study the size of particles dispersed in a liquid,[15−17] allows one to investigate hydrodynamic interactions, kinetics of unstable states (aggregation), the structure and dynamics of metastable states, and the interplay between phase transition and gelation phenomena.[18,19]

This report describes the synthesis and characterization of monodisperse aqueous latices composed of *N*-isopropylacrylamide (NIPAAM), methyl methacrylate (MMA) and methacrylic acid (MAA). The purpose of the paper is to evaluate the difference in the interactions between the polymer latices and the low molar mass organic compounds ibuprofen, protocatechuic acid, and ephedrine, chosen as model substances because of their significant difference in hydrophobicity. The binding of these substances to the polymer latices has been studied by dynamic light scattering as a function of temperature and the scattering angle.

Experimental Section

Materials. Monomer *N*-isopropylacrylamide (NIPAAM) purchased from Polysciences, Inc., was purified by recrystallization with hexane. The comonomers methyl methacrylate (MMA, Fluka) and methacrylic acid (MAA, Polysciences were used without further purification, as were the initiator, potassium persulfate (KPS, Merck), and surfactant, sodium dodecyl sulfate (SDS, Fluka). The water used for polymerization and light scattering measurements was deionized in an Elgastat UHQ-PS purification system. Low molar mass

S0024-9297(97)01437-X CCC: $15.00
Published on Web 02/18/1998

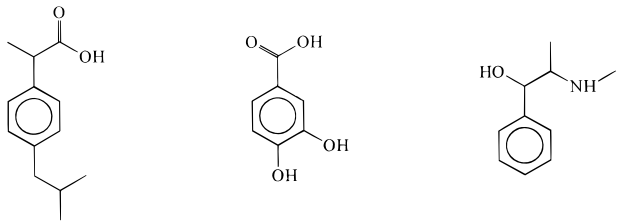

Figure 1. Structural formulas of the low molar mass compounds.

substances ibuprofen, protocatechuic acid, and ephedrine were all from Sigma and their structures are shown in Figure 1.

Copolymer Synthesis. The emulsion polymerization was carried out in a 1-L thermostated double-wall reactor fitted with a reflux condenser, a mechanical Teflon stirrer, and a nitrogen inlet/outlet. A total of 3 g of monomers, either only NIPAAM or a mixture of NIPAAM, MMA, and MAA (the molar ratio of the monomers was NIPAAM/MMA/MAA = 80:15:5) and SDS (0.2 g/L) dissolved into 190 mL of deionized water were added into the reactor. The solution was heated to 70 °C and stirred at 200 rpm for 30 min with a nitrogen purge to remove oxygen. Then, KPS (0.6 g/L) dissolved in 10 mL of water was added to start the polymerization and the reaction was carried out at 70 °C for 4 h. After the copolymerization, the resulting products were purified to remove small molar mass impurities by dialysis against purified water for a period of a week. The polymers were dried at room tempeature. In the text, the PNIPAAM homopolymer is designated EP1and the copolymer of NIPAAM, MMA, and MAA is designated EP2.

Copolymer Characterization. (a) IR Spectroscopy. Infrared spectra of homopolymer EP1 and copolymer EP2 samples dispersed in KBr were recorded on a Nicolet 205 FT-IR spectrometer.

(b) ^{13}C NMR Spectroscopy. ^{13}C NMR spectra of the polymers were measured with a 200 MHz Varian Gemini 2000 spectrometer using 3 wt % aqueous (D_2O) polymer solutions.

(c) Dynamic Light Scattering. Dynamic light scattering was studied using a Brookhaven Instruments BI-200SM Goniometer and BI-9000AT multi-τ digital correlator with 522 time channels. The light source was Spectra Physics 127 helium/neon laser (633 nm, 35mW). Time correlation functions were analyzed with a Laplace inversion program (CONTIN). The samples for dynamic light scattering were the pure aqueous EP2, as well as the aqueous mixtures of EP2 and ibuprofen (I-EP2), protocatechuic acid (P-EP2), or ephedrine (E-EP2), respectively. The samples were prepared by mixing 0.4 mg of dry EP2 into 5 mL of water or by mixing 0.4 mg dry EP2 *and* 0.2 mg of the respective low molar mass substance into 5 mL of water. The aqueous mixtures were allowed to equilibrate at room temperature for 2 weeks before being used. The intensity−intensity time correlation functions $g_2(t,q)$ in the self-beating mode were measured at the scattering angle 60° at temperatures ranging from 20 to 80 °C. At each temperature, the samples were allowed to equilibrate 1 h before the measurement. Measurements were also conducted at various scattering angles from 30° to 135° for the sample I-EP2 at different temperatures. As in the vast majority of cases, the CONTIN program produces no artifact peaks and yields reasonably accurate moments,[5] and thus CONTIN was chosen to study the hydrodynamic character of the polyelectrolyte latices.

Results and Discussion

The composition of the copolymer used was chosen to produce a hydrophobically modified polyelectrolyte latex showing the thermal behavior typical to PNIPAAM. Thus, the comonomer content was kept low. Consequently, the analysis of the polymer structure is not straightforward, and the composition given in the experimental part refers to the feed in the polymerization mixture.

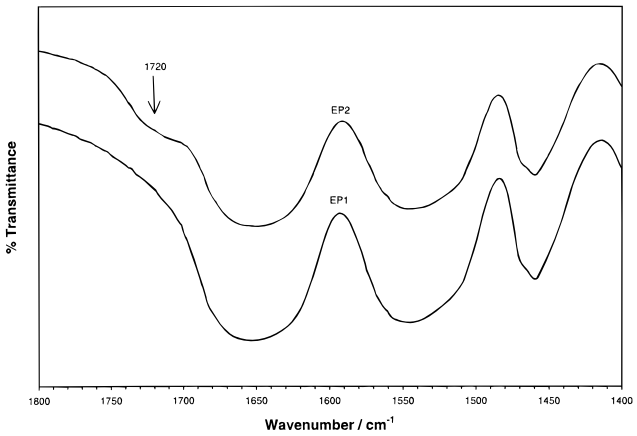

Figure 2. FTIR spectra (1800−1400 cm^{-1}) of homopolymer PNIPAAM (EP1), *bottom*, and copolymer of NIPAAM, MMA, and MAA (EP2), top.

Figure 2 shows the 1400−1800 cm^{-1} region of the FTIR spectra of EP1 and EP2 synthesized by emulsion polymerization. In the spectrum of EP2, a band at 1720 cm^{-1} corresponds to the C=O stretching in MMA and MAA.

The ^{13}C NMR spectra of EP1 and EP2 dispersed in D_2O are almost identical. However, in the spectrum of EP2, a small broad shoulder around 21.5 ppm is observable in the band of the methyl substituents of the polymer (22 ppm) which may be assigned to the methyl group in both MMA and MAA. It may be concluded that copolymerization has taken place, although the ratio of the repeating units was not exactly obtainable.

Interaction of the Copolymer with Low Molar Mass Substances. The interaction of low molar mass organic substances with an aqueous polymer is based on the balance between hydrophobic and hydrophilic forces. By adjustment of the ratio of hydrophobic and hydrophilic structural units in polymers, the binding and the release of organic substances into and from polymers are supposed to be possible to control. The application of responsive polymers such as PNIPAAM and its derivatives gives a possibility to affect the binding of low molar mass substances by environmental stimuli.

A hydrophobic drug, ibuprofen (2-(4-isobutylphenyl)-propionic acid) is widely used for acute relief of pain and for treatment of chronic diseases such as rheumatoid arthritis and other rheumatoid conditions. A hydrophilic (water soluble) drug ephedrine (1-phenyl-1-hydroxy-2-methylaminopropane) is known as both an α- and β-adrenergic agonist. Controlled and targeted release systems for these substances have a noticeable fundamental and practical significance. In the past, the effect of various polymers on the controlled release of ibuprofen has been studied.[20−24] Protocatechuic acid (3,4-dihydroxybenzoic acid) is not used as a drug but was chosen as a model compound because it is only sparingly soluble in water.

Polyelectrolytic copolymers of *N*-isopropylacrylamide (NIPAAM) are environmentally sensitive polymers whose conformation changes from an extended coil to a collapsed globule at the LCST. In the following, we will discuss the interactions of the copolymer EP2 with ibuprofen, protocatechuic acid, and ephedrine below and above LCST investigated by dynamic light scattering.

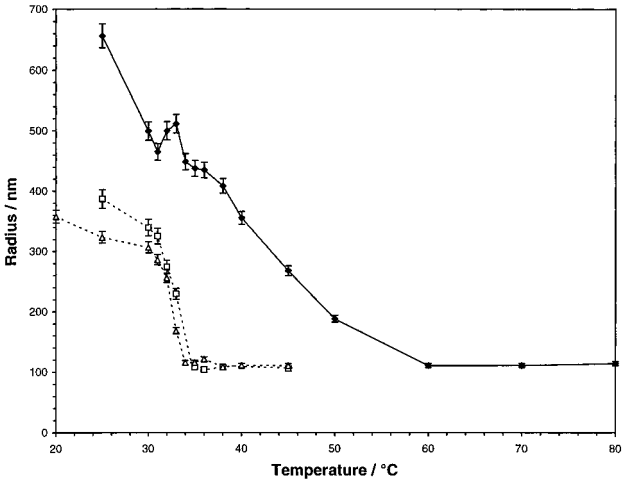

Figure 3. Hydrodynamic radius of the latex particles as a function of temperature in pure water for (◆) EP2, (□) I-EP2, (△) P-EP2. The width of the size distibution is indicated with bars.

The binding of the substances into EP2 has been studied by measuring the changes in the size and aggregation of the latex particles induced by the additives.

Interaction of the Copolymer with Hydrophobic Molecules: Ibuprofen and Protocatechuic Acid. Figure 3 shows the average hydrodynamic radii of the latex particles against temperature for the pure aqueous copolymer latex EP2 and for those containing added ibuprofen and protocatechuic acid, respectively, measured by dynamic light scattering at an angle of 60°. In the samples, the concentration of the copolymer EP2 is kept constant at 0.08 mg/mL and the concentration of ibuprofen and protocatechuic acid is 0.04 mg/mL, i.e., 50 wt % of the mass of the copolymer. All of the samples, EP2, I-EP2 and P-EP2, show a volume transition starting in the vicinity of the lower critical solution temperature of PNIPAAM, 31 °C. The transition range of EP2 is wide, from 31 to 60 °C and the radius decreases about 5-fold during the transition. The behavior is typical for the copolymers of NIPAAM; the transition range is known to broaden with increasing amount of hydrophilic comonomers.[18] The temperature range of the volume transition of the samples containing ibuprofen (I-EP2) or protocatechuic acid (P-EP2) is narrow, from 31 to 35 °C, and the radius decreases about 3-fold. It may be worth noting that the small maximum at 31−33 °C in the curve representing the R_h of EP2 is repeatable and probably owes to the different thermal behavior of the hydrophobic and hydrophilic structural units of the polymer.

As noted above, the thermal behavior of the EP2 latex particles is closely similar to the behavior of weakly charged NIPAAM copolymers in solution. The polymers switch from a hydrophilic and soluble form to a hydrophobic and less soluble form with increasing temperature due to the balance between the hydrophobic and electrostatic forces.

In Figure 3, it can be seen that below the LCST the particle size in both I-EP2 and P-EP2 is smaller than that in EP2. Above the transition range, the radii of all three latices reach a stable value of about 110 nm. Thermal behavior of the EP2 sample, as well as I-EP2 and P-EP2 samples, is schematically described in Figure 4. Negative charge on the surface of the particle owes to the carboxyl groups of MAA as well as to the anionic surfactant SDS. These charges repel each other and

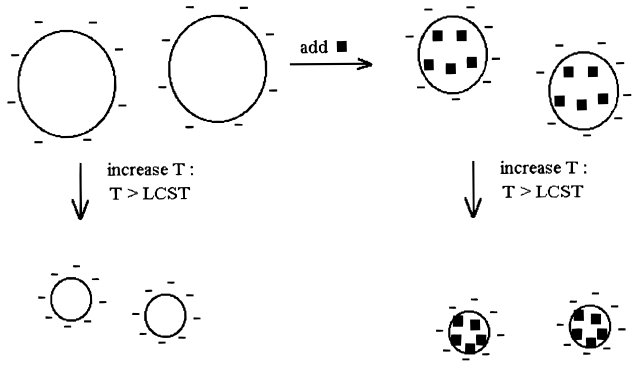

Figure 4. Schematic illustration of the thermal behavior of the latex EP2, as well as the latex with added ibuprofene or protocatechuic acid. The hydrophobic additive is marked with ■.

make the latex stable in water. On the other hand, the hydrophobic parts of the copolymer fill the interior of the particle. Ibuprofen and protocatechuic acid are weak acids and only sparingly soluble in water. When these substances are added into aqueous EP2, they prefer to dissolve in the polymer particles. Because of the attraction between the low molar mass substances and EP2 polymer, the added substances enhance the collapse of the EP2 copolymer particles, as is shown in Figure 4. Therefore, the added molecules not only decrease the particle size of EP2 at room temperature but also make the collapse of EP2 particles more distinct. At elevated temperatures, after the volume transition, the particles reach a new stable state and the particle size of the latices has a constant value.

Below the volume transition temperature, the particle size of I-EP2 is higher than that of P-EP2 at the same temperature. However, the difference is small and may be due to a difference in the molar concentrations of the low molar mass additives. The concentration of protocatechuic acid (2.6×10^{-4} mol·L^{-1}) used in the experiment is slightly higher than that of ibuprofen (1.94×10^{-4} mol·L^{-1}). The dependence of the particle size on the concentration of added substances will be studied more closely in the future.

The size of the polymer particles of the sample I-EP2 was measured at various scattering angles as a function of temperature. The result is shown in Figure 5. It may be seen how the particle size gradually decreases with increasing temperature, up to 33 °C. Above this temperature, the particles suddenly collapse and reach a stable size. The particle size is independent of the scattering angle, except at temperatures ≤31 °C. The very high values of the particle size observed at low temperatures with increasing scattering vector are clearly erroneous and due to the large size of the studied particles ($qR_h \gg 1$). At $T \leq 31$ °C with a high scattering vector q the size distribution calculated by CONTIN was bimodal. However, narrow monomodal distributions were always observed at low q. At $T \geq 32$ °C the distribution was monomodal and showed no angle dependence. The result allows one to conclude that the sample consists of monodisperse polymer particles. The width of the size distribution is indicated with bars in Figures 3 and 5.

Interaction of the Copolymer with Ephedrine. Above it was concluded that both ibuprofen and protocatechuic acid bind into the EP2 latex particles causing a decrease of the particle size. Owing to the increased hydrophobicity of the particles loaded with these sub-

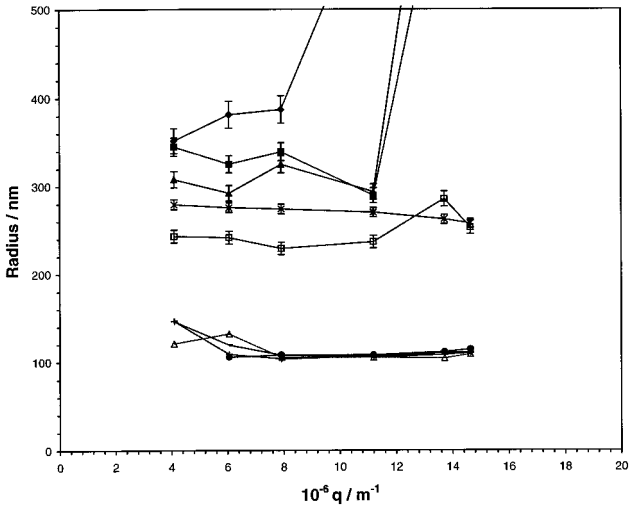

Figure 5. Hydrodynamic radius as a function of q for the copolymer latex with added ibuprofen, I-EP2, at various temperatures: (◆) 25 °C; (■) 30 °C; (▲) 31 °C; (×) 32 °C; (□) 33 °C; (●) 35 °C, (+) 36 °C; (−) 38 °C; (△) 45 °C. The width of the size distribution is indicated with bars.

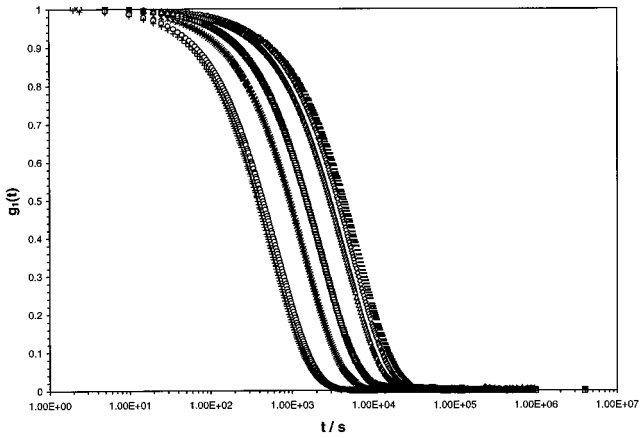

Figure 6. Normalized correlation functions $g_1(t)$ measured at various temperatures for EP2 in pure water: (−) 25 °C; (◇) 30 °C; (△) 35 °C; (□) 45 °C; (*) 50 °C; (○) 60 °C; (+) 70 °C.

stances, the volume transition becomes much sharper than that in the pure polymer. The next question to be discussed is how a hydrophilic substance, ephedrine, interacts with the polymer.

Figure 6 shows the normalized correlation functions $g_1(t)$ of EP2 against logarithmic time, measured at temperatures from 25 to 70 °C. The correlation functions are monomodal over the entire temperature range. The relaxation time decreases with increasing temperature indicating a decrease in the particle size, shown already in Figure 3.

Figure 7 shows the temperature dependence of the correlation functions $g_1(t)$ of the aqueous mixture of EP2 and ephedrine, E-EP2. The measurements were done at the scattering angle 60°. At temperatures ≤35 °C, bimodal correlation functions are observed. The relaxation time of the fast decay is almost constant at low temperatures. The amplitude of the slow process increases with increasing temperature. However, at temperatures above 35 °C, only one relaxation mode is apparent and the relaxation time starts to decrease with increasing temperature. It seems evident that below the LCST, ephedrine induces aggregation of the EP2 particles but that the aggregates break down when the particles start to shrink.

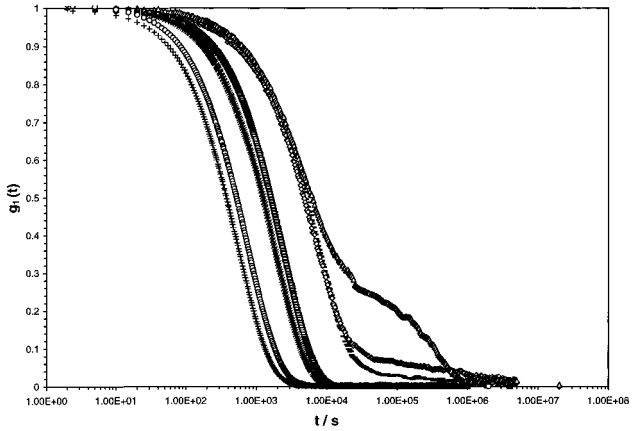

Figure 7. Normalized correlation functions $g_1(t)$ measured at various temperatures for the copolymer latex with added ephedrine, E-EP2, in pure water: (−) 25 °C; (◇) 30 °C; (△) 35 °C; (□) 45 °C; (*) 50 °C; (○) 60 °C; (+) 70 °C.

The stability of a latex is governed by the interparticle potential energy which determines how strongly the latex particles repel each other in the dispersed state and also how much thermal or mechanical energy it would take to force them together to form a coagulum.[25] The polymer EP2 is a weak polyacid and its latices are colloidally stabilized by the anionic charges of the carboxyl groups of MAA as well as by SDS remaining on the surface of the particles. Ephedrine is a water-soluble weak base, and thus, when it is added into an EP2 latex at room temperature it will be bound to the surface of the polymer. Because of the binding of ephedrine, the repulsion between polymer particles is reduced and the attractive interparticle potential increases. As a result the polymer latex loses its stability and starts to aggregate. It should be noted that the fast decay in the correlation functions shown in Figure 7 is characterized by a relaxation time that is of the same order of magnitude as the relaxation time in pure EP2. This indicates that ephedrine as such does not change noticeably the size of the individual polymer particles. Above the LCST the aggregates break down while the particles start to shrink. Although in an indirect way, this finding suggests that the ephedrine molecules are detached from the surface of the particles. The process is reversible: when temperature is lowered back to room temperature, the aggregates start to form up. The particle size distribution of E-EP2 below and above the critical temperature is shown in Figure 8.

Conclusions

Linear polyelectrolyte copolymer of *N*-isopropylacrylamide (NIPAAM), methyl methacrylate (MMA), and methacrylic acid (MAA) has been synthesized by emulsion polymerization.

The aqueous latex EP2, and the mixtures of EP2 with ibuprofen, protocatechuic acid, and ephedrine, respectively, have been studied by dynamic light scattering. Aqueous EP2 shows the LCST behavior but its phase transition temperature region is wide, ranging from 31 to 60 °C.

The interactions of the low molar mass substances with the polymer strongly depend on their hydrophobicity. Hydrophobic substances ibuprofen and protocatechuic acid dissolve into the EP2 latex particles. Because of the hydrophobic attraction between the copolymer and the added molecules, ibuprofen and pro-

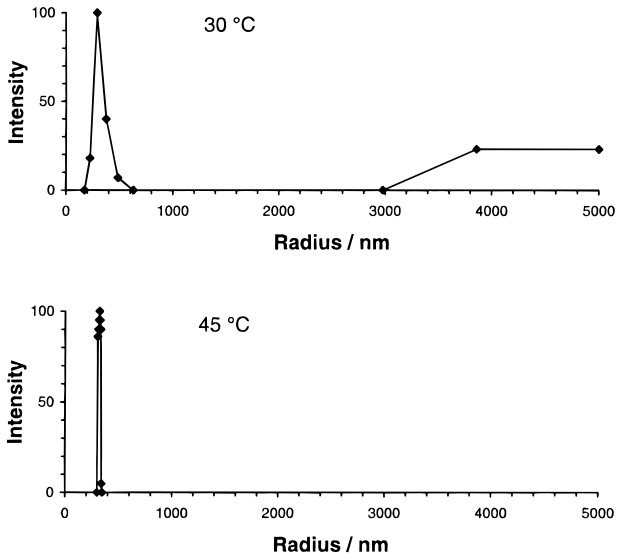

Figure 8. Distibution of the hydrodynamic radii of the latex particles with added ephedrin (E-EP2) below and above the critical temperature.

tocatechuic acid decrease the dimensions of the copolymer particles and make the volume transition of the copolymer sharp, occurring at temperatures ranging from 31 to 35 °C.

On the other hand, hydrophilic ephedrine dissolves into water. The interaction between ephedrine and EP2 takes place close to the charged surface of the polymer particles. Below LCST ($T \leq 35$ °C), ephedrine is bound to the polymer latex and causes the aggregation of the particles. The size of the individual polymer particles does not change due to the binding of ephedrine. Above LCST ($T \geq 45$ °C), ephedrine is cleaved from the polymer to water and the aggregates disappear.

It has been shown how the binding of low molar mass substances to polymer particles may be regulated by adjusting the solubilities of the polymer and the added substances. The distribution of the low molar mass compounds between polymer and water will be studied more closely in the future using macroscopic thermosensitive gels to determine quantitatively the bound and released substances.

The present work reveals new insights into the design of drug delivery systems by using hydrophobic interactions in a fairly hydrophilic polymer latex. These systems may find applications where new ways are needed to administer drugs into a body in a highly targeted manner.

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MA9714373