Preparation and Characterization of Thermal- and pH-Sensitive Nanospheres

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INTRODUCTION

Thermal and pH-sensitive polymers are being used in many applications ranging from drug delivery to separations (1–6). Polymers with temperature-sensitive or pH-sensitive properties are responsive to external stimuli involving changes in temperature, pH, or ionic composition. In most cases, the response to such external stimuli is manifested in the changes of polymer swelling and the occurrence of a volume phase transition. As such, when used as drug delivery systems, these polymers can provide time-varying modulations of drug release not achievable by other release mechanisms. Most of the reported studies have been based on polymers containing segments of either thermal-sensitive poly(*N*-isopropylacrylamide) [poly(NIPAm)] or pH-sensitive poly(acrylic acid) [poly(AA)] or poly(methacrylic acid) [poly(MAA)]. More recently, the combination of both thermal and pH-sensitive properties into a drug delivery polymer matrix has been attempted (7). While these approaches provide additional mechanisms for release rate modulation, the response time for these polymer matrices has been generally very long (hours), primarily because of the finite sizes of the samples studied (1–3). In addition to the employment of a heterogeneous polymer structure (7), another useful way to reduce the response time of these stimuli-sensitive polymers would be to reduce their sizes. Tanaka and co-workers (8) have studied the thermal phase transition behavior of submicron gel beads of ionized poly(NIPAm), however, the beads were polydispersed and the effect of pH and ionic strength on the phase volume transition was not investigated. In this study, we report the synthesis of polymeric nanospheres which are not only nearly monodispersed but also both thermal and pH sensitive, and the characterization of their equilibrium properties, such as the volume phase transition and drug loading, as a function of polymer ionic content, temperature, pH, and ionic strength. These nanoparticles may be potentially useful for drug delivery or diagnostic applications.

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MATERIALS AND METHODS

Preparation of Nanospheres

Poly(*N*-isopropylacrylamide-co-methacrylic acid) [poly(NIPAm/MAA)] nanospheres were prepared by an aqueous dispersion polymerization process (9,10) using *N,N'*-methylenebisacrylamide (BIS) as the cross-linking agent, potassium persulfate (KPS) as the initiator, and sodium dodecyl sulfate (SDS) as the stabilizer. Monomer mixtures containing recrystallized NIPAm and inhibitor free MAA were dissolved in distilled water to a total concentration of 135 m*M*. The NIPAm-to-MAA mole ratio was varied from 1:0 to 1:1. After the incorporation of 6.4 mol% of BIS, a small amount of SDS was then added to the reaction mixture to a concentration level of 0.4 m*M*, well below the reported CMC for SDS (~8 m*M*). After purging the reaction mixture with nitrogen, the polymerization was initiated by the addition of a small amount of concentrated KPS solution to give a total concentration of KPS in the reactor of 2.1 m*M*. The polymerization was carried out under nitrogen blanket at 70°C for 4 hr at 200 rpm. Typically, nanospheres in the form of latices with a polymer concentration of about 1.5 wt% were prepared. More concentrated latices were obtained by ultracentrifuge (Beckman L5-65B).

Characterization of Nanospheres

The volume phase transition of the resulting latices was studied by measuring the changes in transmittance (turbidity) as a function of either temperature or pH. The transmittance at 400 nm was measured on a HP8452 diode array UV/Vis spectrophotometer. Water-jacketed cells and a constant-temperature bath (Haake D8-G, accurate to ±0.02°C) were used for all transmittance measurements. For the particle diameter and volume determination, a Brookhaven BI-90 dynamic light scattering particle sizer was used. All measurements were carried out either in distilled water or in buffers. In the latter case, Sørensen phosphate buffers were employed for pH 5–8 and a potassium biphthalate buffer was used for pH 4. The desired ionic strength of a buffer was adjusted by the addition of a calculated amount of NaCl.

Drug Loading

Using theophylline as a model drug, a small finite volume of an aqueous drug solution of known drug concentration was added to a fixed amount of latex. The mixture was allowed to stand at 25°C for 24 hr before being separated by ultracentrifuge. The amount of theophylline loading in the latex was determined from the UV assay of the supernatant at 272 nm. Similar experiments were also carried out at 40°C. In this case, the collapse of the nanospheres at this elevated temperature alleviated the need for ultracentrifuge.

RESULTS AND DISCUSSION

Unlike the emulsion polymerization process [such as the one used by Tanaka and co-workers (8)], which involves an initial reaction mixture consisting of two separate phases, the dispersion polymerization process employed here starts

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with an initially homogeneous solution of the reaction mixture. As the polymerization progresses, polymer particles insoluble in the dispersion medium are formed and stabilized in the latex by the surface charge from the initiator and the dispersion stabilizer (9,10). The particle formation is believed to be governed by a homogeneous nucleation process, which can produce a very narrow particle size distribution provided that the particles have been formed very early in the polymerization process and subsequent growth has taken place without the formation of additional particles (11).

Particle Size and Particle Size Distribution

Table I summarizes the results obtained from dynamic light scattering at 20 and 50°C. Where the intensity mean particle diameter, the geometric standard deviation (GSD), and the volume swelling ratio at these two temperatures are tabulated as a function of polymer composition. The particle diameter ranges from 114 to 413 nm, depending on the polymer composition and experimental temperature. At a fixed temperature, the particle diameter appears to follow an increasing trend with the MAA content. On the other hand, at any given polymer composition, a shrinkage in particle diameter with up to ninefold volume change is noted when going from 20 to 50°C. This is the result of going above the lower critical solution temperature (LCST) of the poly(NIPAm) segments (ca. 31–33°C) (2). The magnitude of such shrinkage, as expressed by the tabulated volume ratio, actually decreases with increasing MAA content. In all cases, a fairly narrow particle size distribution with GSD in the range of 1.2–1.5 has been achieved, indicative of a fast rate of polymerization.

TRANSITION TEMPERATURE

Effect of Polymer Composition

Results of the transmittance measurements in distilled water as a function of temperature and MAA content are shown in Fig. 1, where the transmittance can be related to the refractive index and diameter of the nanoparticles. The transmittance readings (Fig. 1), normalized with respect to that at 10°C, indicate that the volume phase transition of the suspended nanoparticles is continuous, and the magnitude of the volume change at transition decreases with increasing MAA content. The latter observation is further supported by the volume swelling ratios obtained from dynamic light scattering (Table I). These observations are consistent with Tanaka's volume phase transition results in pure water on other ionized poly(NIPAm) gels (12). Figure 2 shows the corresponding volume phase transition observed in pH 7.4 buffer (0.25 *M* ionic strength). In this case, being in an ionized state and at a high ionic strength, the sharpness of the volume phase transition is enhanced for nanospheres with the lowest MAA content which resulted in the coagulation of suspended nanoparticles. However, the transition temperature moves higher and the magnitude and sharpness of the volume change at transition decrease with increasing MAA content. The slightly increasing trend with temperature in the relative transmittance (Fig. 2) is thought to result from additional particle swelling because of the increased osmotic pressure of those polymers with the highest ionic content.

Table I. Particle Size and Particle Size Distribution of Poly(NIPAm/MAA) Nanospheres in Distilled Water

	20°C		50°C		
NIPAm: MAA	Diam. ± σ[a] (nm)	GSD	Diam. ± σ (nm)	GSD	Volume ratio, $V_{20°C}/V_{50°C}$
1:0	301 ± 4	1.3	155 ± 3	1.3	7.32
I:0.05	234 ± 5	1.5	114 ± 3	1.3	8.65
1:0.1	287 ± 9	1.3	148 ± 3	1.3	7.29
1:0.2	340 ± 4	1.2	168 ± 2	1.2	8.29
1:0.4	357 ± 1	1.5	188 ± 2	1.2	6.85
1:0.7	413 ± 5	1.5	321 ± 7	1.3	2.13
1:1	326 ± 8	1.4	244 ± 1	1.3	2.39

[a] σ is the standard deviation of intensity mean diameter based on five repetitions of measurements.

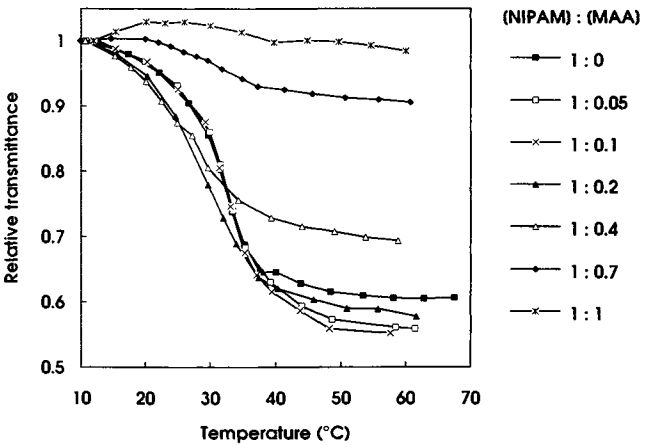

Fig. 1. Effect of MAA content on the thermal phase transition of poly(NIPAm/MAA) nanospheres in distilled water.

Effect of pH

The results in Fig. 3 show the effect of pH (at a constant ionic strength of 0.05 *M*) on the thermally induced volume

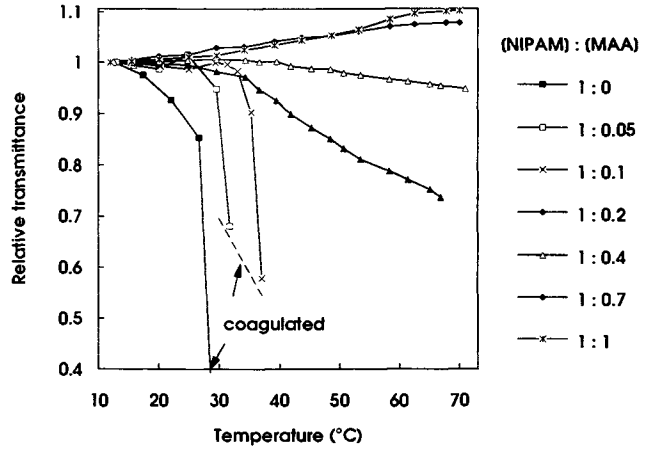

Fig. 2. Effect of MAA content on the thermal phase transition of poly(NIPAm/MAA) nanospheres in pH 7.4 buffer (ionic strength, 0.25 *M*).

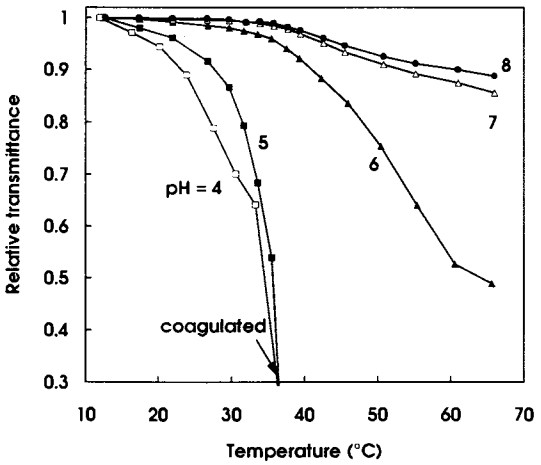

Fig. 3. Effect of buffer pH on the thermal phase transition of poly(NIPAm/MAA) nanospheres (NIPAm:MAA = 1:0.1) at ionic strength 0.05 *M*.

phase change in poly(NIPAm/MAA) at a NIPAm:MAA ratio of 1:0.1. It is seen that the transition temperature increases, but the sharpness of the transition diminishes, with pH. At lower pH's (pH 4 and 5), the sharp volume phase transition generally leads to a significant collapse of the suspended nanoparticles above the transition temperature.

Effect of Ionic Strength

Figure 4 displays the influence of ionic strength (at a constant pH of 7.4) on the volume phase transition of the same polymer studied in Fig. 3. It is clear that a sharper transition and a relative lower transition temperature have resulted from the increasing ionic strength. In this case, the decrease in ion osmotic pressure difference between the nanoparticle and the solution due to the increase in solution osmotic pressure tends to lower the degree of polymer swelling, which may have facilitated the collapse of the suspended nanoparticles.

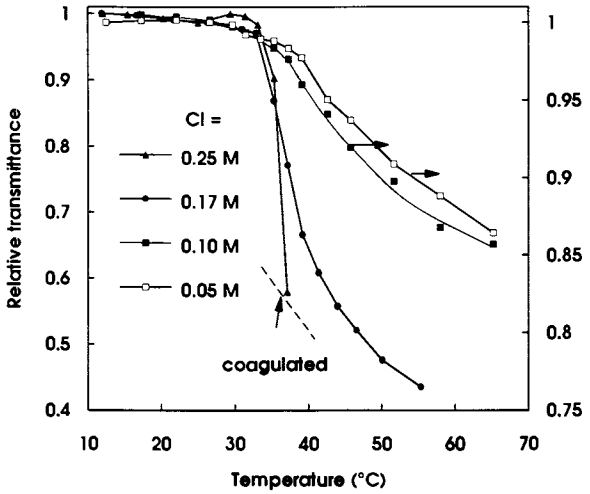

Fig. 4. Effect of ionic strength (CI) on the thermal phase transition of poly(NIPAm/MAA) nanospheres (NIPAm:MAA = 1:0.1) at pH 7.4.

Drug Loading

The drug loading based on dry polymer weight is plotted in Fig. 5 as a function of the theophylline concentration in the loading solution. Here, results from both 25 and 40°C are shown. As expected, the drug loading increases with the loading solution concentration at both temperatures. However, the loading is generally higher at 25°C due to the higher polymer swelling. In this case, the lower drug loading at 40°C is attributed to the loss of interstitial drug solution upon the collapse of suspended nanoparticles. Since the response time for these submicron particles is very small [microsecond range based on the square-length law proposed by Tanaka (8)], such temperature- or pH-induced collapses of the present nanospheres is expected to generate an instantaneous on–off type of drug release.

CONCLUSIONS

We have synthesized and characterized the equilibrium properties of a thermal- and pH-sensitive nanosphere system based on poly(NIPAm/MAA). The dispersion polymerization process employed here has resulted in particle sizes ranging from 114 to 413 nm, depending on the polymer composition and temperature. The larger particle sizes appear to occur with increasing MAA content. In all cases, a fairly narrow particle size distribution with a geometric standard deviation of 1.2–1.5 has been achieved. At temperatures above the LCST of the nanospheres, a shrinkage in particle diameter with up to ninefold volume changes is generally observed. The magnitude of such volume phase transition becomes smaller and the transition temperature moves higher with either increasing MAA content or increasing pH. At pH 7.4, the sharpness and magnitude of such transition tend to increase with either an increasing ionic strength or a lower MAA content. The isotherms for theophylline loading in these poly(NIPAm/MAA) nanospheres show a significant decrease in slope from 25 to 40°C. This is attributed to the loss of interstitial drug solution upon the collapse of suspended nanoparticles above the transition temperature.

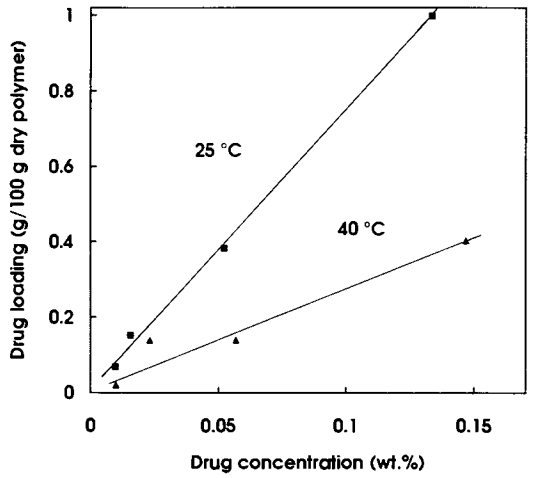

Fig. 5. Effect of temperature on the theophylline loading in poly(NIPAm/MAA) nanospheres (NIPAm:MAA = 1:0.1) as a function of loading solution concentration.

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