Monodisperse Nanoparticles of Poly(ethylene glycol) Macromers and *N*-Isopropyl Acrylamide for Biomedical Applications

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ABSTRACT: Poly(ethylene glycol)-based nanoparticles have received significant attention in the field of biomedicine. When they are copolymerized with pH- or temperature-sensitive comonomers, their small size allows them to respond very quickly to changes in the environment, including changes in the pH, ionic strength, and temperature. In addition, the high surface-to-volume ratio makes them highly functionalized. In this work, nanoparticles composed of temperature-sensitive poly(*N*-isopropylacrylamide), poly(ethylene glycol) 400 dimethacrylate, and poly(ethylene glycol) 1000 methacrylate were prepared by a thermally initiated, free-radical dispersion polymerization method. The temperature-responsive behavior of the hydrogel nanoparticles was characterized by the study of their particle size with photon correlation spectroscopy. The size of the nanoparticles varied from 200 to 1100 nm and was a strong function of the temperature of the system, from 5 to 40°C. The thermal, structural, and morphological characteristics were also investigated. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 87: 1678–1684, 2003

Key words: hydrogels; dispersions; gels; ionomers; drug delivery

INTRODUCTION

Dispersion polymerization has received a great deal of attention as a method of preparing micrometer-sized monodisperse polymer particles.[1–10] Latex particles are formed from an initially homogeneous reaction mixture in the presence of a suitable steric stabilizer. The advantages of preparing particles with the dispersion polymerization method include (1) a fast reaction rate by a simple free-radical dispersion polymerization, (2) the elimination of organic solvents from the system, and (3) the ability to produce spherical monodisperse particulate systems. The absence of organic solvents in this process eliminates toxicity concerns; this is especially important for biomedical applications.

Solvent selection is the most important factor in dispersion polymerization, and it especially affects the nucleation period. The solvent must be a thermodynamically good solvent for the monomer, the initiator, and the steric stabilizer. Accordingly, the reaction mixture is homogeneous at the onset, and the polymerization is initiated in this homogeneous solution. However, for the dispersion to be achieved, the same liquid must serve as a thermodynamically poor solvent for the oligomer and polymer formed. During the course of the reaction, the liquid changes from a solvent to a dispersing medium, and this results in the precipitation of the polymer chains to form reaction nuclei.

Therefore, monomers and oligomers diffuse into the reaction nuclei, and the reaction continues inside them. Depending on the compatibility of the medium for the resulting oligomers and polymers, phase separation can occur at an early stage, leading to control over the particle size. Another factor that affects the particle size in dispersion polymerization is the temperature of the reaction, which is directly related to the compatibility of the continuous phase.[11–15]

Several other factors play an important role in controlling the kinetics, colloidal stability, particle size, and molecular weight during dispersion polymerization. These factors include (1) the stabilizer or surfactant, (2) the monomer, and (3) the type of initiator used. Key aspects of the polymerization mechanism are the stabilization process, the role of the solvent or

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dispersing agent, the nucleation process, and the particle formation process. Tseng et al.[5] studied the effects of solvent selection and the concentrations of monomers, stabilizers, and initiators on the size of polymer particles formed in dispersion polymerization.

The system used to form hydrogel nanoparticles in this work is based on the free-radical dispersion polymerization of unsaturated monomers forming two phases in which the polymerization process can take place: monomer-swollen polymer particles and a continuous phase. At the reaction onset, the monomers are completely miscible with the continuous phase. However, as the reaction proceeds, no constant concentration of the monomer in polymer particles can be expected.[12–15] The partitioning of monomers, oligomers, and initiators between the reaction nuclei and the continuous phase plays an important role in the polymerization process. It can result in a desirable layered composition across the polymer particles, with poly(ethylene glycol) (PEG) chains predominating on the surface.

The objectives of this work were to synthesize PEG-containing nanoparticulate systems;[16–19] to investigate the effects of the monomer concentration, monomer composition, reaction time, reaction temperature, and initiator concentration on nanoparticle formation; and to study the swelling behavior of the ensuing nanoparticles.

EXPERIMENTAL

Synthesis

A free-radical dispersion polymerization method was used to prepare nanoparticles of poly[*N*-isopropyl acrylamide-*co*-poly(ethylene glycol) 1000 methacrylate] [P(NIPAAm-*co*-PEGMA)]. The crosslinker was poly(ethylene glycol) 400 dimethacrylate (PEG-DMA).[20,21]

Before the reaction, *N*-isopropyl acrylamide (NIPAAm; Fischer Scientific, Pittsburgh, PA) was recrystallized in benzene/hexane. PEGDMA and poly(ethylene glycol) 400 monomethacrylate (PEGMA; Polysciences, Warrington, PA) were used as received. In a typical experiment, NIPAAm, PEGDMA, and PEGMA (70/20/10 w/w/w) were dissolved in an appropriate amount of deionized and distilled water to form a 2% aqueous solution. The mixture was bubbled with argon for 30 min for the removal of any dissolved oxygen.

The monomer mixture was then heated to 85°C in a silicone-oil temperature bath. Ammonium persulfate (Aldrich Chemicals, Milwaukee, WI) was added to the system to act as a thermal initiator, and the polymerization process was allowed to continue for 45 min. The resulting dispersion was purified for 5 days with a regenerated cellulose dialysis membrane with a 14-kDa cutoff molecular weight (Spectrum Laboratories, Rancho Dominquez, CA).

Nanoparticle characterization

Differential scanning calorimetry (DSC; DSC 2910, TA Instruments, New Castle, DE) was used to study the glass-transition behavior of the nanoparticulate system. In a typical experiment, the nanoparticle dispersion was dried for 12 h at 80°C and then for 24 h in a vacuum oven at 40°C. The dried sample was then pulverized, and 10 mg of the sample was sealed in an aluminum DSC pan and heated from 0 to 150°C at a rate of 5°C/min.

The effect of the temperature on the particle size of the nanoparticulate system was studied with photon correlation spectroscopy (PCS; N4 Plus, Coulter, Miami, FL). Each dispersion sample was diluted with deionized and distilled water to fit the required frequency count between 5×10^4 and 10^6. The measurements were taken at a 90° incident angle. The PCS studies were used to calculate the average particle sizes of the nanoparticles at different temperatures.

The morphology of the nanoparticles was examined with transmission electron microscopy (TEM; FEI/Philips CM-10, Portland, OR). Uranyl acetate (Aldrich Chemicals) was used as the staining agent. In a typical experiment, a droplet of the P(NIPAAm-*co*-PEGMA) nanoparticulate dispersion [2% (w/w)] was spread onto the surface of a 300-mesh copper carbon grid (Electron Microscopy Sciences, Fort Washington, PA). The dispersion was allowed to air-dry for 30 min. Two droplets of the staining solution [2% (v/v) aqueous solution of uranyl acetate] were then added to the copper carbon grid. The sample was then air-dried and vacuum-oven-dried at 30°C for 24 h. The dried specimen was clamped onto a TEM specimen rod, inserted into the sample chamber, and observed at 80 kV.

RESULTS AND DISCUSSION

Nanoparticle synthesis

Free-radical dispersion polymerization was employed to synthesize nanoparticles of the copolymers of IPAAm and various PEG-containing monomers. The types of PEG-containing macromonomers and NIPAAm employed in this work are hydrophilic. As both PEG and PNIPAAm have relatively low lower critical solution temperatures (LCSTs),[22–27] by increasing the reaction temperature above the LCST, we could balance the thermodynamic properties to achieve a thermodynamically poor solvent for the polymer chains while maintaining a thermodynamically good solvent for the monomers. The reaction temperature used here was 85°C, well above the LCSTs of both PEG[22,23] and PNIPAAm[24–27]

Typically, the monomer composition was kept constant at a 70:20:10 NIPAAm/PEGDMA/PEGMA weight ratio. The reaction temperature and time were

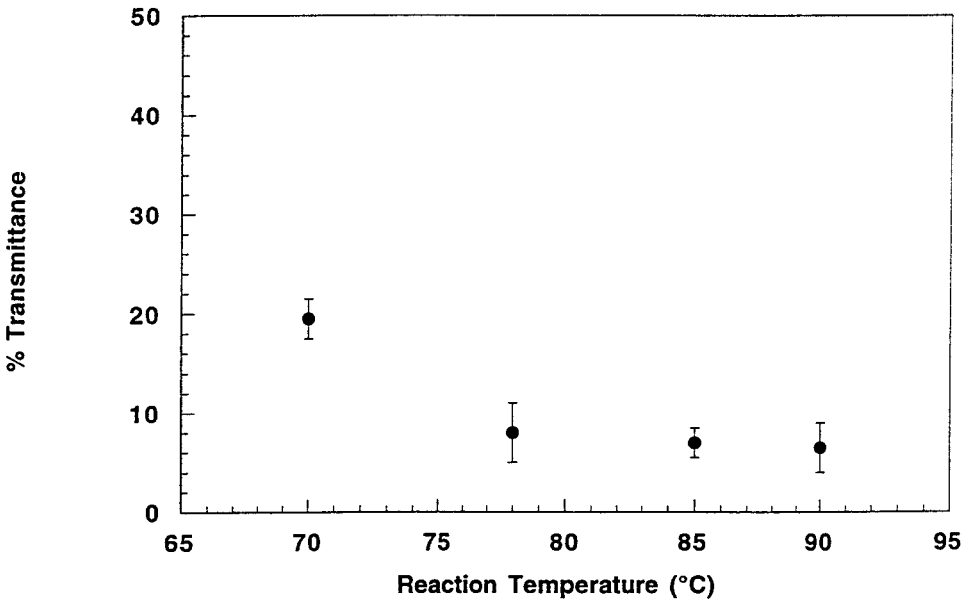

Figure 1 Light transmittance (600 nm) through a nanoparticle dispersion produced by the reaction of NIPAAm/PEGDMA/PEGMA in a 70:20:10 ratio and at different reaction temperatures. The dispersion polymerization was carried out for 45 min by thermally initiated, free-radical polymerization.

also held constant at 85°C and 45 min, respectively. The amount of ammonium persulfate added to the system was fixed at 2% (w/w) of the total amount of monomers added. The effect of the monomer concentration (in the dispersed phase) on the nanoparticle formation was studied. When the amount of the monomer used was 1% (weight of monomers/weight of solution), only a slight turbidity was observed. At a 2% concentration, a blue-white dispersion was observed, whereas at a 3% concentration, a highly concentrated solid white dispersion was noted. At a 4% concentration and higher, a solid gel was observed instead of a microparticulate dispersion. The maximum amount of the monomer used was 2%, yet this still ensured the formation of a fine nanoparticulate dispersion.

In these reactions, PEG was not only an important biological component but also a steric stabilizer. In this nanoparticulate system, the addition of PEG chains resulted in increased agglomeration. As PNIPAAm had a lower LCST than the PEG-containing structures, increasing the amounts of PEG in the system increased the LCST of the overall system. Therefore, some of the polymer chains interconnected before the system collapsed and were stable. This was even observed when the monomer concentration was 4% or higher.

Latex dispersions produced by dispersion polymerization in the absence of any stabilizers are not sufficiently stable and may coagulate during their formation. Particle stabilization in dispersion polymerization is, of course, a steric stabilization process. Good stabilizers for dispersion polymerization are polymer and oligomer compounds with relatively low solubility in the polymerization medium and moderate affinity for the polymer particles.[6]

When conventional stabilizers consisting of a polar or ionic head group and a nonpolar tail were employed in our dispersion polymerization, some difficulties were encountered. For example, the stabilizers were held on the particle surface only by physical forces. These conventional stabilizers interfered with adhesion to a substrate and were leached out upon contact with water. However, amphiphilic macromonomers such as the PEGMA used here exhibited the typical properties of conventional surfactants but also had an unsaturated reactive polymerizable group, which allowed PEGMA to be incorporated into the surface layer of the polymer particles by copolymerization with the comonomers. In this case, PEGMA was bound to the particle surface and, therefore, was prevented from subsequently migrating, yet it was able to stabilize the polymer particles.

The effect of the reaction temperature on the extent of the reaction was also studied. The reaction mixture turned opaque when the nanoparticles were formed. This phenomenon was used to study the importance of the reaction temperature in these studies. Ultraviolet–visible spectrophotometry was used to analyze the transmittance of a diluted dispersion at a 600-nm wavelength.

In the modified free-radical dispersion polymerization employed in this work, the reaction temperatures varied from 50 to 90°C. At temperatures between 50 and 70°C, a clear gel was observed. The transmittance of the dispersion as a function of the reaction temperature is shown in Figure 1. There were no significant

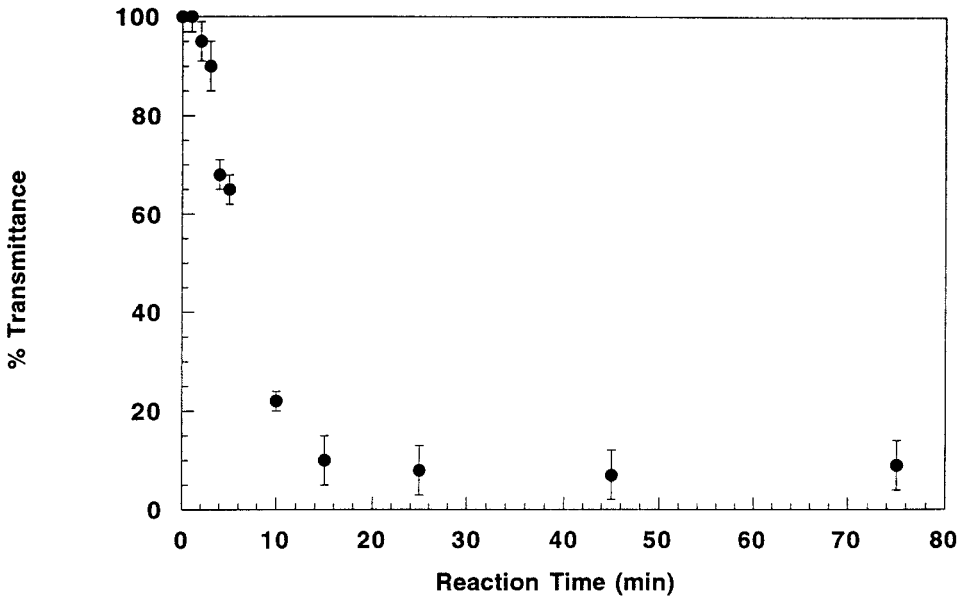

Figure 2 Light transmittance (600 nm) through a nanoparticle dispersion produced by the reaction of NIPAAm/PEGDMA/PEGMA in a 70:20:10 ratio and at different reaction times. The dispersion polymerization was carried out at 85°C by thermally initiated, free-radical polymerization.

variations among the transmittances of the dispersions obtained by reactions in the 78, 85, and 90°C system. However, below 50°C, the polymer particles did not collapse, and so no nucleation was observed. This effect of temperature on the process was agreed with the hypothesis that the LCST of the polymers triggered the nucleation process. The LCST phenomenon was observed by a very sharp transition, such as that for pure PNIPAAm.

The effect of the reaction time on the extent of polymerization is shown in Figure 2. In these experiments, the monomer composition (70:20:10), the concentration in the dispersing fluid [2% (w/w)], the initiator concentration [0.04% (w/w)], and the reaction temperature (85°C) were kept constant. The light transmittance of the dispersions at 600 nm was analyzed to evaluate the effect of the reaction time. A significant difference was observed in the light transmittance through a dispersion that was allowed to react up to 10 min. Beyond 10 min, there was no significant difference in the transmittance. This fast reaction was very typical for a free-radical polymerization. On the basis of these results, the reaction time was set at 45 min.

The effect of the initiator concentration on the yield of the dispersion polymerization was also investigated. The monomer composition was fixed at 70:20:10 NIPAAm/PEGDMA/PEGMA. The monomer concentration [2% (w/w)], reaction temperature (85°C), and reaction time (45 min) were also constant. No significant difference in the yield was observed for the polymerizations with initiator concentrations of 0.5, 1, 1.5, and 2% (w/w) of the total amount of monomer charged, as shown in Figure 3. It was concluded that, within the range of initiator concentrations studied, there was no significant effect of the initiator concentration on the yield of the dispersion polymerization.

There was a significant effect of the initiator concentration on the size of the nanoparticles obtained, as shown in Figure 4. The largest nanoparticles (550 nm) were obtained from a dispersion produced with a 0.5% (w/w) initiator concentration, whereas the smallest nanoparticles (450 nm) were obtained from a dispersion produced with a 2% (w/w) initiator concentration, as measured with PCS at 25°C. As the amount of the initiator increased, there were more propagating polymer chains, and this resulted in more reaction nuclei. Because the amount of the monomer was constant for all the samples, the system with more reaction nuclei produced smaller particles.

Therefore, we selected a set of reaction parameters that would allow the synthesis of a nanoparticulate system with a high yield and yet no significant agglomeration. These reaction parameters were a 2% (w/w) monomer concentration in the aqueous solution, a 70:20:10 NIPAAm/PEGDMA/PEGMA monomer composition, a 0.04% (w/w) initiator concentration [2% (w/w) of the total monomers] in an aqueous solution, a reaction temperature of 85°C, and a reaction time of 45 min.

Nanoparticle characterization

The glass-transition temperature of the dried nanoparticles was studied with DSC. As the temperature increased at 5°C/min from 0 to 145°C, the heat flow

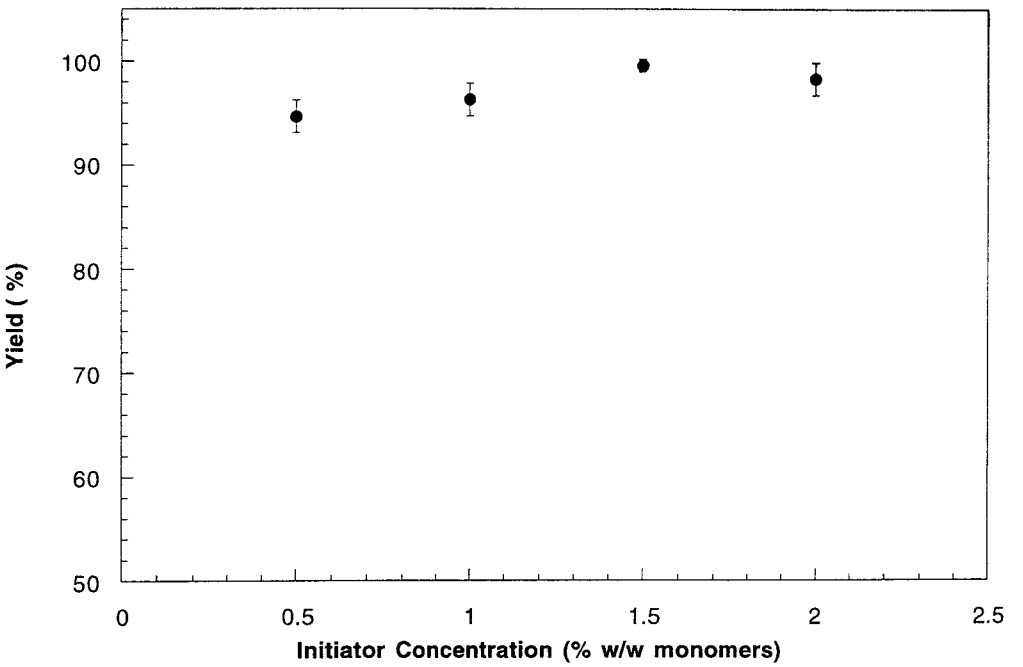

Figure 3 Yield of a nanoparticle dispersion produced by the reaction of NIPAAm/PEGDMA/PEGMA in a 70:20:10 ratio as a function of the initiator (ammonium persulfate) concentration. The monomer concentration was fixed at 2% (w/w). The reaction was conducted at 85°C for 45 min.

experienced a secondary transition at 95°C. The heat-flow transition at 95°C was caused by the endothermic relaxation of polymer chains from the glassy state to the rubbery state. The endothermic response to the relaxation of the hydrogel was caused by the increase in the system entropy as the degree of freedom of the polymer chain increased.

The effect of the swelling-medium temperature on the hydrodynamic particle size of the nanoparticulate system was studied with PCS. The results are shown

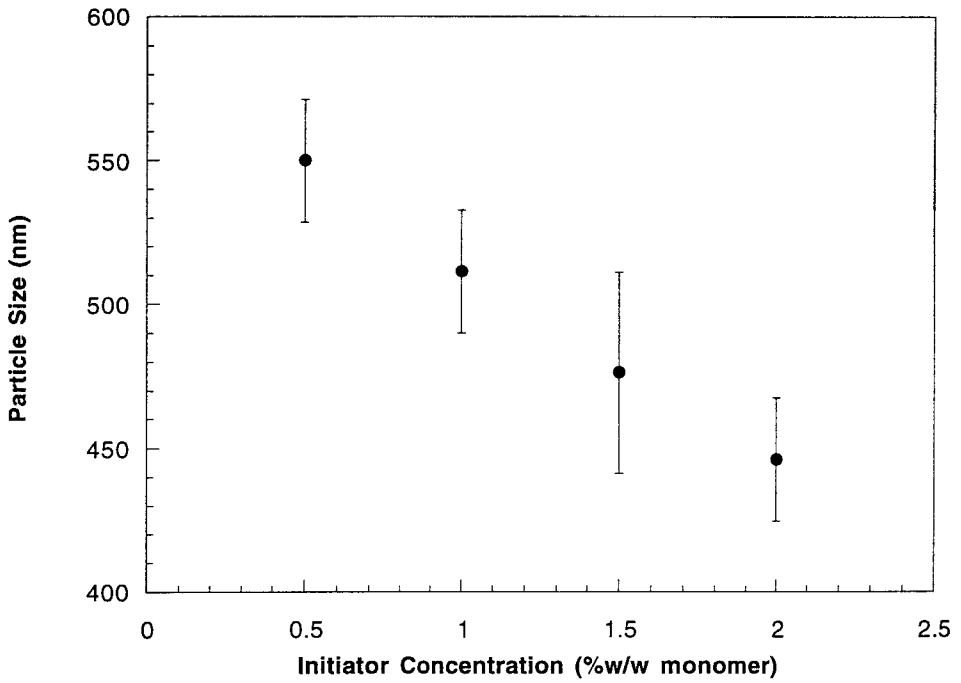

Figure 4 Particle size of a nanoparticle dispersion produced by the reaction of NIPAAm/PEGDMA/PEGMA in a 70:20:10 ratio as a function of the initiator (ammonium persulfate) concentration, which was measured with PCS at 25°C. The dispersion polymerization was carried out at 85°C for 45 min by thermally initiated, free-radical polymerization.

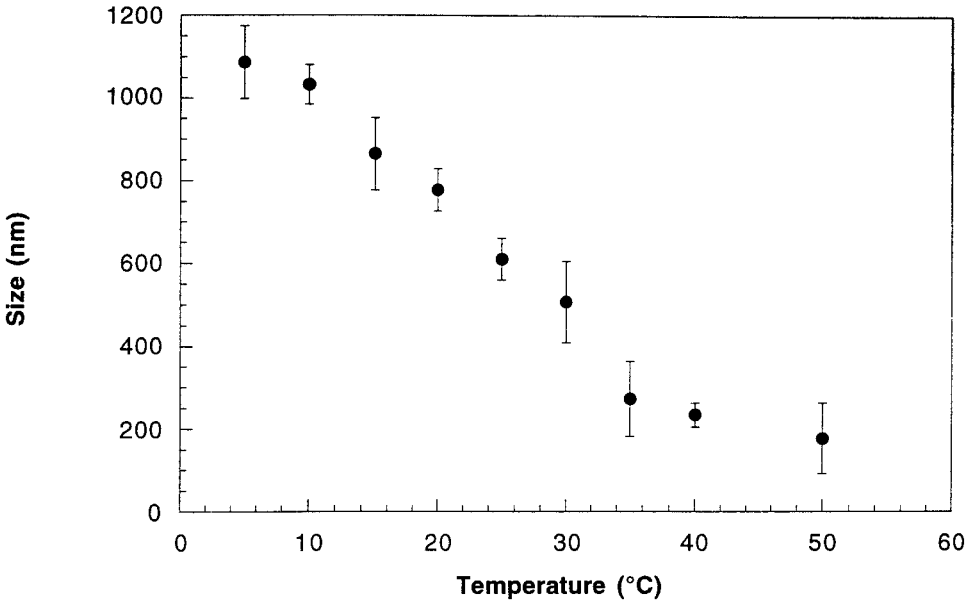

Figure 5 Particle size of a nanoparticle dispersion produced by the reaction of NIPAAm/PEGDMA/PEGMA in a 70:20:10 ratio as a function of the temperature of the swelling medium. The dispersion polymerization was carried out at 85°C for 45 min by thermally initiated, free-radical polymerization.

in Figure 5. The polymers used in this system exhibited LCST behavior with a change in the experimental temperature.[27–32] Indeed, the particle size of the nanoparticles varied from 200 to 1100 nm at different temperatures, as shown in Figure 5.

When the particles were swollen at 5°C, their size was 1100 nm. The size remained constant until 10°C before it changed drastically to 200 nm at 35°C. There was an order of magnitude difference in the size of the nanoparticles as the temperature increased from 5 to 50°C.

Hydrophobic interactions and hydrogen bonding were the major contributors to this temperature sensitivity. Hydrophobic interactions arise between nonpolar molecules in water. Up to a certain temperature, the hydrophobic groups of the polymer chain are shielded by water molecules, which are arranged in a certain pattern to form a cage around the group. When the temperature was increased, this cage of immobile water molecules was partially lost, and the protection of the hydrophobic groups was weakened. This may be the reason that the hydrophobic interaction increased as the temperature was increased. These explanations can be extended to explain the temperature sensitivity in our system. However, a broad transition, from about 10 to 35°C, was observed in this system instead of the typical sharp transition of a pure PNIPAAm gel.[17] This was due to the fact that the PEG-containing macromer was copolymerized with NIPAAm, leading to a shifted and broadened LCST of the system.

The morphology of nanoparticles stained with uranyl acetate was observed with TEM, as shown in Figure 6. A monodisperse, spherical nanoparticulate system was observed without any trace of agglomeration. This was in agreement with the hypothesis that the PEG added to the system also acted as the

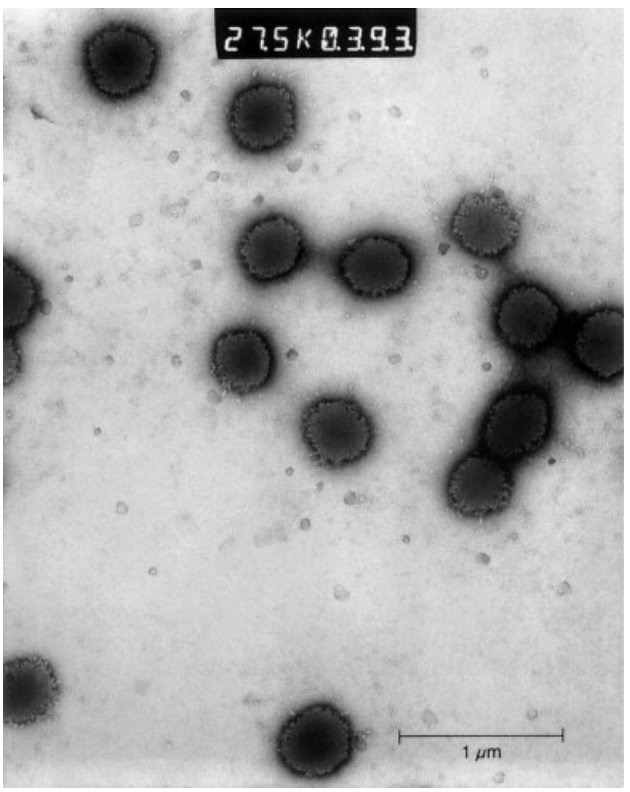

Figure 6 TEM micrograph of dry nanoparticles produced by the reaction of NIPAAm/PEGDMA/PEGMA in a 70:20:10 ratio and stained with uranyl acetate at a magnification of ×27,500. The average size of the nanospheres was 330 nm.

surfactant, and so monodisperse, spherical nanoparticles without any agglomeration were produced. The size of the nanoparticles measured directly from the TEM image was about 330 nm, which was in the range of particle sizes measured with PCS. However, the two sizes could not be compared because the size obtained from TEM was the size of the dried nanoparticle.

Nanoparticulate formation using the dispersion polymerization technique, involving several types of monomers, produced a nonuniform composition as a function of the radius of the particles. A core–shell structure was observed, with a solid core and a loose shell. This nonuniformity was caused by the unequal reactivity ratio of each comonomer being different. Therefore, one of the comonomers was depleted first before the polymerization was completed. More specifically, the core of the nanoparticles consisted of PNIPAAm-rich networks, whereas the shell was mostly PEG-rich networks. PNIPAAm has a lower LCST than PEG. Therefore, networks rich in PNIPAAm would collapse first to form the core before PEG-rich networks were incorporated. Moreover, PNIPAAm-rich networks would form first because NIPAAm is more reactive than bulky PEG macromonomers.

The aforementioned experiments led us to a possible mechanism of particle formation. Initially, NIPAAm, PEGDMA, and PEGMA are dissolved in deionized water to form a homogeneous mixture. Once the temperature of the mixture has reached 85°C, the polymerization reaction is initiated by the addition of ammonium persulfate. The ensuing radicals rapidly attack the double bonds of the monomers, and the polymer chains propagate. The polymer chains keep growing until they reach a critical length, beyond which the solvent cannot solvate them anymore. Therefore, they precipitate and form nuclei.

CONCLUSIONS

Nanoparticulate systems exhibiting temperature sensitivity were synthesized with a thermally initiated free-radical dispersion polymerization. The effects of (1) the composition and concentration in the dispersed phase, (2) the initiator concentration, (3) the reaction time, and (4) the reaction temperature were studied and optimized. The optimal preparation conditions were (1) the 70:20:10 NIPAAm/PEGDMA/PEGMA monomer composition being dissolved in deionized water to form a 2% (w/w) solution, (2) the mixture being bubbled with argon and then heated to 85°C before ammonium persulfate was added in the amount of 2% (w/w) of the total amount of the monomer charged to initiate the reaction, and (3) the reaction being allowed to proceed for 45 min.

The particle size was investigated under various temperature conditions with PCS. Equilibrium swelling results showed that the nanoparticle experienced a deswelling transition when the temperature exceeded 10°C until 35°C. This temperature sensitivity, combined with the size of the particles in the nanometer range, allowed the particle size to be changed very rapidly.

In principle, this behavior is desirable because it allows the efficient incorporation of drugs into the nanoparticles for the development of drug delivery systems for possible pharmaceutical applications. Drug diffusion into the nanoparticles is enhanced by the increased size of the nanoparticles.

The morphology of the nanoparticles was also investigated with TEM. A monodisperse, spherical particulate system with an average size of 330 nm was observed without any agglomeration.

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