Preparation and Characterization of pH-Responsive Poly(methacrylic acid-*g*-ethylene glycol) Nanospheres

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ABSTRACT: Poly(methacrylic acid-*g*-ethylene glycol) (P(MAA-*g*-EG)) has been studied extensively in our laboratory due to its extremely promising applications in the biomedical and pharmaceutical fields. It exhibits pH-responsive interpolymer complexes that make it a promising candidate as an oral carrier for peptide and protein drug. We have developed a photoinitiated free-radical precipitation polymerization method to produce P(MAA-*g*-EG) nanospheres with relatively narrow size distributions. The influence of various reaction parameters, such as total monomer concentration in water, comonomer molar feed ratios, cross-linking agent concentration, and polymerization time, on the particle size and size distribution was investigated. P(MAA-*g*-EG) nanospheres with a relatively narrow size distribution could be produced in the size range 150−650 nm depending on the monomer concentration and comonomer molar feed ratio. The P(MAA-*g*-EG) nanospheres exhibited a pH-responsive swelling behavior. Increasing the concentration of the cross-linking agent during polymerization produced P(MAA-*g*-EG) nanospheres that swelled to a lesser degree. The morphology of the P(MAA-*g*-EG) nanospheres was investigated using cryogenic scanning electron microscopy.

Introduction

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of therapeutic peptide and protein drugs. Currently, most protein drugs are administered parentally, by subcutaneous, intravenous, or intramuscular injections. Because of the short half-life of proteins in blood, multiple injections are necessary for therapeutic effectiveness. Therefore, significant efforts have been directed toward the development of protein formulations and delivery methods compatible with noninvasive routes of administration, such as pulmonary, nasal, oral, vaginal, rectal, ocular, buccal, and transdermal formulations.[1−5]

Of the above noninvasive routes, oral delivery remains the most desirable method for chronic drug treatment. However, despite years of continuous research, oral delivery of protein and peptide drugs continues to challenge scientists. There are two main barriers that inhibit the effective delivery of these protein drugs: (i) the instability of the proteins in the gastrointestinal (GI) tract due to degradation by digestive enzymes; (ii) the poor absorption of the drug across the intestinal lining. Therefore, most oral drug delivery methods are focused on protecting the drug in the GI tract until it reaches its site of greatest absorption and prolonging its residence time at that site while the drug is being delivered.

Polymeric particulate systems have been extensively investigated to overcome the above barriers.[6,7] In these systems, the protein drug is either encapsulated or entrapped within the polymeric carrier. In designing these particulate systems, factors such as particle size, type and composition of polymer, and adjuvants affect the performance of the system. Particle size and particle size distribution affect parameters such as drug release kinetics, degradation rate of the particles, biodistribution, delivery options, and interaction of the particulate carrier with biological membranes.[8] In particular, the size of the particles used as carriers is important, as it has been shown[1] that nanospheres in th erange of 200−600 nm are more likely to pass across intestinal epithelial cells by paracellular transport.

Most methods of nanoparticle production are based on polymerization of a monomer in various media. These polymerization-based methods often require the use of large amounts of organic solvents or unsafe stabilizers (surfactants) that could result in toxic side affects if not properly removed. An important requirement for pharmaceutical usage is that the nanoparticles are free of any potentially toxic impurities. Therefore, alternative or modifications of existing polymerization methods that are free of toxic organic solvents and use safe stabilizers are being investigated.

In this work, the production of poly(methacrylic acid-*g*-ethylene glycol) nanospheres for oral delivery of proteins was investigated. In past research, P(MAA-*g*-EG) hydrogels were synthesized in the form of films by a free radical bulk polymerization.[9] A suspension polymerization technique using silicon oil as the suspending phase was used to produce P(MAA-*g*-EG) microspheres.[10] For the first time, nanospheres of P(MAA-*g*-EG) have been produced using an UV-initiated free radical precipitation polymerization method in water.

Precipitation polymerization for production of submicrometer particles is often associated with limited solubilty characteristics as polymerization takes place, leading to conditions similar to those of dispersion. The polymerization begins in the homogeneous mixture of the monomer, initiator, and solvent, but as the polymerization progresses, the resulting polymer precipitates

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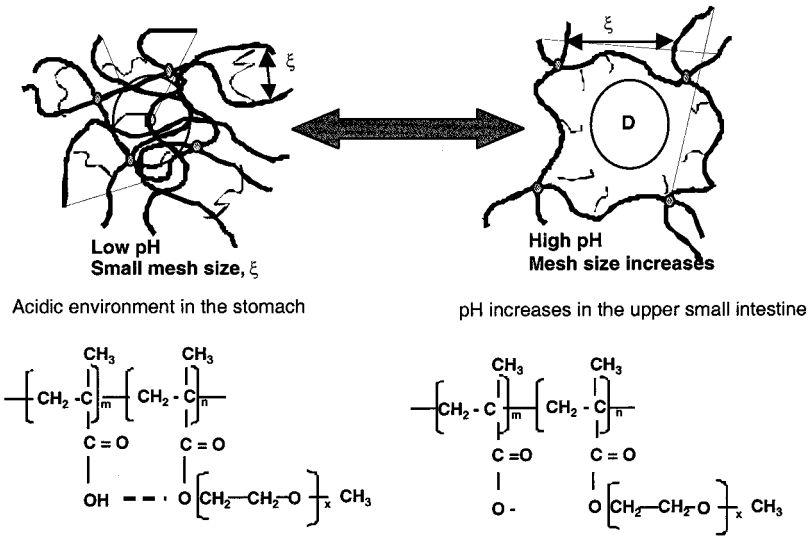

Figure 1. Effect of interpolymer complexation on the swelling behavior of P(MAA-*g*-EG) hydrogels. Schematic of the complexation and decomplexation behavior of P(MAA-*g*-EG).

from the solution forming nanospheres. This technique was first used in the biomedical/pharmaceutical field by Kreuter and Speiser[11] to produce poly(methyl methacrylate) nanospheres for pharmaceutical applications. Since their work, dispersion polymerization has been used in a number of other systems to produce nanoparticles for drug delivery.[12−14]

Poly(methacrylic acid-*g*-ethylene glycol), henceforth designated P(MAA-*g*-EG), is a copolymer of poly(methacrylic acid) (PMAA) and poly(ethylene glycol) monomethacrylate (PEGMA) that forms interpolymer complexes in response to changes in pH of environmental fluids.[15] Particulate systems that respond to pH changes are ideal carriers for protein drug delivery because they are able to take advantage of the large change in pH from the stomach to the intestine. At low pH values, the P(MAA-*g*-EG) hydrogels assume a hydrophobic structure with a low degree of swelling and permeability. Therefore, it is able to protect the protein drug from degradation as it travels through the stomach. When the pH increases in the intestine, the hydrogel swells and its permeability increases, allowing the drug to diffuse out (Figure 1). Recent research has demonstrated the effectiveness of P(MAA-*g*-EG) hydrogels in the delivery of insulin to rats.[16] Nanospheres of these hydrogels can improve its effectiveness as a carrier for protein drugs. Smaller particles allow more intimate interaction with the intestinal mucosa, resulting in a longer residence time, and a uniform shape and narrow size distribution will result in reproducible drug release kinetics.

The aim of this research was to produce P(MAA-*g*-EG) hydrogels as nanospheres using a precipitation/dispersion polymerization method in water and to characterize their swelling behavior. During the polymerization, variations in reaction parameters, such as monomer concentration, polymerization time, and cross-linking agent concentration, were investigated to understand their effect on particle formation, particle size, particle size distribution, and particle stability. Characterization of the polymer system included equilibrium swelling studies as a function of pH and cross-linking agent concentration during the polymerization process. The particle size and size distribution of the P(MAA-*g*-EG) nanospheres was assessed using photon correlation spectroscopy, and the morphology of the P(MAA-*g*-EG) nanospheres was investigated using cryogenic scanning electron microscopy.

Experimental Section

Materials. The monomers used were methacrylic acid (MAA, Polysciences Inc., Warrington, PA), poly(ethylene glycol) monomethyl ether monomethacrylate (PEGMA, Polysciences Inc., Warrington, PA) with a PEG molecular weight 1000, tetraethylene glycol dimethacrylate as a cross-linking agent (TEGDMA, Polysciences Inc., Warrington, PA), and 1-hydroxycyclohexyl phenyl ketone (Irgacure 184, CIBA-GEIGY Corp., Hawthorne, NY) as the photoinitiator. Prior to use, MAA was vacuum distilled at 54 °C/25 mmHg to remove the inhibitor, hydroquinone. PEGMA and TEGDMA were used as received without any further purification.

Polymerization. MAA and PEGMA were combined in an amber glass bottle in molar feed ratios of 1:1, 2:1, 4:1 and 1:2 mol of MAA per mol of EG. In a typical reaction, TEGDMA was added to the mixture in the amount of 0.75 mol % of total monomer, and the photoinitiator, 1-hydroxycyclohexyl phenyl ketone (Irgacure 184, CIBA-GEIGY Corp., Hawthorne, NY), was dissolved in the monomer mixture in the amount of 0.5 wt % of total monomer.

Following the complete dissolution of the monomers and initiator, 0.4 g of the mixture was added dropwise to 50 mL of deionized water in a 125 mL glass Erlenmeyer flask. The reaction flask was sealed with a rubber stopper, and nitrogen was bubbled through the reaction mixture for 20 min to remove any dissolved oxygen, which acts as a free radical scavenger.

After the nitrogen purge, the sealed reaction flask was exposed to ultraviolet light (Efos Acticure Ultraviolet/Visible spot cure system, Mississauga, Ontario) at an intensity of approximately 100 mW/cm^2 for 15 min. After polymerization, the resulting nanosphere dispersion was washed by dialysis in deionized water using a regenerated-cellulose membrane with a molecular weight cutoff of 25 000 (Spectra Por 7, Spectrum Laboratories Inc., Rancho Dominguez, CA). The purification process lasted 5 days, changing the water twice daily.

The washed nanosphere suspension was transferred to 50 mL centrifuge tubes, and 5 w/v% of Pluronic F-68 (10%) solution (Sigma Chemical Co., St. Louis, MO) was added to the suspension then vortexed for 15 s. Pluronic F-68 served as a steric stabilizer for our system. Pluronic is a triblock copolymer of propylene oxide and ethylene oxide. It has been shown to play a role in stabilization of nanoparticles.[17] Pluronic stabilized our nanosphere suspension by shielding the surface

Table 1. Standard Method for the Precipitation Polymerization of MAA and PEGMA and the Reaction Parameters Studied

parameter	standard method	variations
MAA/EG molar feed ratio	1:1	2:1, 4:1, 1:2
total monomer concentration in water (g/mL)	0.008	0.001, 0.004, 0.016, 0.032, 0.060, 0.10
reaction time (min)	15	5, 15, 30, 60, 120, 240
TEGDMA concentration (mol %)	0.75	1.0, 2.0, 5.0

charges on our nanospheres and providing steric stabilization between the nanospheres.

Samples were then prepared for freeze-drying. The samples were frozen by immersing the centrifuge tube containing the suspension in liquid nitrogen for approximately 1 min or until the solution was frozen. The centrifuged tube was sealed with Parafilm and placed in the Labconco freeze-dry system (model 77500, Kansas City, MO) for 36 h. The dried particles were stored in a desiccator until further use.

Characterization. Characterization of the particle size and size distribution as a function of the various reaction parameters was evaluated using photon correlation spectroscopy. The various reaction parameters studied are listed in Table 1. The suspensions were tested after polymerization using the Coulter N4 Plus submicrometer particle sizer (Coulter Corporation, Miami, FL) at an angle of 90° at 20 °C ± 0.1. The nanosphere suspensions in amounts between 0.5 and 10 μL, depending on the concentration of the suspension, were added to the sample cuvette, which was prefilled with filtered deionized water. The deionized water was filtered with a 0.45 μm filter to remove dust from the diluent. The cuvette was then capped and inverted a few times to disperse the sample uniformly in the diluent. The filled cuvette was placed in the sample compartment and the intensity of the sample was checked. To avoid multiple scattering by the sample, the intensity reading must fall in the range of 5×10^4 to 1×10^6 counts per second. If the intensity reading was too high, the sample was diluted until it fell within the appropriate range. Size measurements were performed in triplicate.

Cryogenic SEM was used to investigate the morphology of the nanospheres in their hydrated state. The nanospheres were viewed using a JEOL JSM-840 SEM equipped with a Hexland T1000 cold stage. The cold stage keeps the samples frozen during observation. The samples were prepared for imaging by placing a droplet of the nanosphere suspension onto a copper sample stud. The suspension was frozen by mechanically plunging the sample stud into liquid nitrogen. The frozen sample was fractured followed by partial sublimation of the water at −70 °C for 30 s in order to reveal the nanospheres that were embedded in the frozen water. The fractured samples were sputter-coated with Gold for 4 s and then transferred to the SEM chamber. The samples were imaged at −140 °C using a 4 kV accelerating voltage. Image analysis was performed using the IPLab Scientific Image Processing 3.2.4 software (Scanalytics, Inc., Fairfax, VA).

Equilibrium Swelling Studies. Equilibrium swelling studies were performed to characterize the pH-responsive behavior of the P(MAA-*g*-EG) nanospheres. The swelling behavior of the nanospheres was evaluated after the freeze-drying process. Swelling studies were also performed to study the swelling behavior of P(MAA-*g*-EG) nanospheres as a function of cross-linking agent concentration used during polymerization.

To determine the equilibrium swelling behavior, the freeze-dried nanospheres were swollen in dimethylglutaric acid/sodium hydroxide (DMGA) buffer solutions of pH 3.3, 4.0, 5.1, 6.1, and phosphate buffer solution (PBS) at pH 7.5. The ionic strength was kept constant for all solutions at $I = 0.1$ M by adding appropriate amounts of sodium chloride (Mallinckrodt Specialty Chemical Co., Paris, KY) to each solution. All buffer solutions were filtered with a 0.45 μm filter before adding them to the freeze-dried nanospheres.

A sample of 20 mg of freeze-dried nanospheres was resuspended in 2 mL of buffered solution. The suspensions were sonicated for 30 min to aid the resuspension process. The samples were allowed to equilibrate for 2 h before sizing.

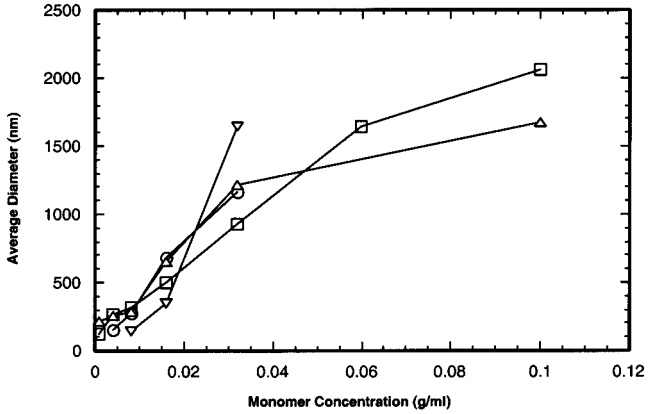

Figure 2. Effect of monomer concentration in water during polymerization on average diameter of P(MAA-*g*-EG) nanospheres prepared at MAA/EG molar feed ratios of (○) 1:1, (□) 2:1, (△) 4:1, and (▽) 1:2. Polymerization time = 15 min.

Particle size and size distribution was determined by PCS at angles of 62.6 and 90° at a temperature of 20 °C ± 0.1. At low pH values of 3.3 and 4.0, 10 μL of buffered suspensions was added to the sample cuvette, which was prefilled with the appropriate buffered solution. At the higher pH values, the entire buffered suspension was added to the sample cuvette. The intensity of the samples was checked, and if they registered within the range of 5×10^4 to 1×10^6 counts per second, the samples were ready for sizing. The samples were sized three to five times.

Equilibrium swelling studies were also performed on P(MAA-*g*-EG) nanospheres of cross-linking concentrations of 0.75, 1, 2, and 5 wt % of total monomer. The varied cross-linked samples were sized after freeze-drying in buffered solutions at pH values of 3.2 and 7.5. The nominal equilibrium volume swelling ratio, Q, was calculated as the ratio of the volume of the nanospheres in their swollen state at pH value of 7.5 divided by the volume of the nanospheres in their collapsed state at pH value of 3.3.

Results and Discussion

Polymerization. P(MAA-*g*-EG) nanospheres were prepared by a free radical precipitation/dispersion polymerization of MAA, PEGMA, TEGDMA, and water in the presence of Irgacure 184 as a free-radical photoinitiator. The reaction mixture began as a clear homogeneous mixture. After exposure to ultraviolet light for approximately 5 min, a faint opalescence was observed. As the polymerization proceeded, the opalescent liquid deepened in color resulting in a milky white solution, which contained a dispersion of P(MAA-*g*-EG) nanospheres.

Figure 2 shows the effect of monomer concentration, mass of monomer concentration per volume, on the average diameter of P(MAA-*g*-EG) nanospheres produced from the precipitation polymerization of MAA and PEGMA.

As the monomer concentration increased, the average diameter of the resulting P(MAA-*g*-EG) nanospheres increased. However, increasing the monomer concentration adversely affected the stability of the forming P(MAA-*g*-EG) nanospheres. Aggregation of the P(MAA-

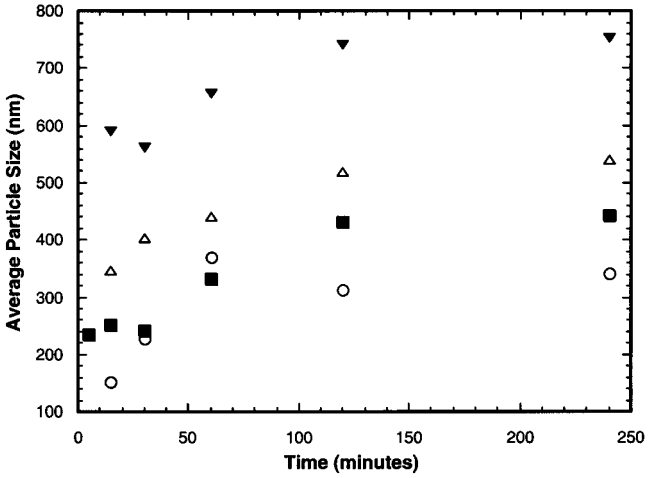

Figure 3. Effect of polymerization time on the average diameter of P(MAA-*g*-EG) nanospheres produced during the precipitation polymerization of at MAA/EG molar feed ratios (○) 1:2, (■) 1:1, (△) 2:1, and (▼) 4:1. Monomer concentration in water = 0.008 g/mL and 0.0125 g/m for the 4:1 sample.

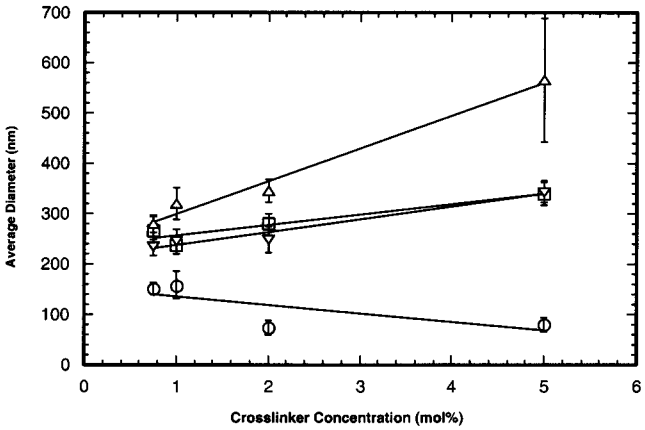

Figure 4. Effect of cross-linking concentration during precipitation polymerization of PEGMA and MAA on average diameter of the P(MAA-*g*-EG) nanospheres prepared from MAA/EG molar feed ratios of (○) 1:2, (□) 1:1, (△) 4:1, and (▽) 2:1 molar ratios. Polymerization time = 15 min. Monomer concentration in water = 0.008 g/mL.

g-EG) nanospheres prepared from MAA/EG molar feed ratios of 4:1 and 2:1 was observed at monomer concentrations above 0.06 g/mL, and aggregation of the P(MAA-*g*-EG) nanospheres increased with increasing monomer concentration in water.

Some aggregation of the P(MAA-*g*-EG) nanospheres prepared from the 1:1 MAA/EG molar feed ratio was observed at a monomer concentration of 0.032 g/mL. However, at a monomer concentration of 0.06 g/mL, a smooth polymer gel swollen with water containing a dispersion of P(MAA-*g*-EG) nanospheres was formed at the bottom of the reaction flask. It was concluded that solution polymerization had taken place at a monomer concentration higher than 0.06 g/mL during the polymerization of MAA and PEGMA prepared at the 1:1 MAA/EG molar ratio.

Precipitation polymerization of MAA and PEGMA prepared from the 1:2 MAA/EG molar ratio produced precipitates when the monomer concentration in the water was above 0.016 g/mL. At a monomer concentration of 0.06 g/mL most of the polymer formed by the polymerization of MAA and PEGMA at a MAA/EG molar feed ratio of 1:2 had precipitated out of the dispersion medium and had adhered to the bottom of the reaction flask.

Therefore, aggregation or precipitation of the resulting P(MAA-*g*-EG) nanospheres occurred due to the lack of adequate stabilization. At monomer concentrations below 0.016 g/mL, stable dispersions of P(MAA-*g*-EG) nanospheres were produced by the precipitation polymerization of MAA and PEGMA without the addition of stabilizers. Clearly, the stability of the P(MAA-*g*-EG) nanosphere dispersion was due to the electrostatic repulsion between the P(MAA-*g*-EG) nanospheres. Additional stabilization between the P(MAA-*g*-EG) nanospheres occurred due to the grafted PEG chains that provided steric stabilization. Electrostatic repulsion between particles is usually higher in water and polar organic solvents due to the increased surface charges of the particles.

Figure 3 shows the effect of polymerization time on the average diameter of the P(MAA-*g*-EG) nanospheres prepared at the four different MAA/EG molar feed ratios. The polymerization was carried out at the monomer concentration of 0.008 g/mL for the nanospheres prepared at MAA/EG molar feed ratios of 1:1, 2:1, and 1:2. A monomer concentration of 0.0125 g/mL in water was used to produce P(MAA-*g*-EG) nanospheres produced at a MAA/EG molar feed ratio of 4:1. The average diameter of the P(MAA-*g*-EG) nanospheres increased with increasing polymerization times. The increase in average diameter was concluded to be due to the increasing conversion of the polymerization at longer polymerization times. At longer polymerization times, more of the unreacted monomer was absorbed in the primary particles of P(MAA-*g*-EG) resulting in growth by polymerization within the particles.

The effect of cross-linking concentration during the precipitation polymerization of MAA and PEGMA was studied in order to understand their effect on average diameter of the resulting P(MAA-*g*-EG) nanospheres. Figure 4 presents the results obtained from this study. The P(MAA-*g*-EG) nanospheres were prepared at a monomer concentration in water of 0.008 g/mL. The results showed that the average diameter increased slightly with the increasing cross-linker concentration for the P(MAA-*g*-EG) nanospheres prepared at MAA/EG molar feed ratios of 1:1, 2:1, and 4:1. The average diameter of the P(MAA-*g*-EG) nanospheres produced from the 4:1 MAA/EG molar feed ratio doubled in size from a cross-linker concentration of 0.75−5 mol %. However, the size distribution increased with increasing cross-linker concentration. The opposite behavior was observed in the P(MAA-*g*-EG) nanospheres produced from the 1:2 MAA/EG molar feed ratio. As the cross-linker concentration increased, the average diameter of the P(MAA-*g*-EG) nanospheres produced from the 1:2 MAA/EG molar feed ratio decreased. A decrease in particle size when more cross-linker is incorporated in the polymer particle is typically expected of highly cross-linked particles. However, the contrasting behavior between the P(MAA-*g*-EG) nanospheres produced from the 4:1 and 1:2 MAA/EG molar feed ratios was possibly due to differences in particle growth.

The observed increase in average diameter of the P(MAA-*g*-EG) nanospheres produced from 4:1 MAA/EG molar feed ratio was probably due to aggregation of the nanospheres. Aggregation and some precipitate were observed at the bottom of the reaction flask after precipitation polymerization of MAA/EG molar feed ratios of 1:1, 2:1, and 4:1 at higher cross-linker concentrations. This observation was indicative of the possibility of aggregation during the polymerization. Therefore,

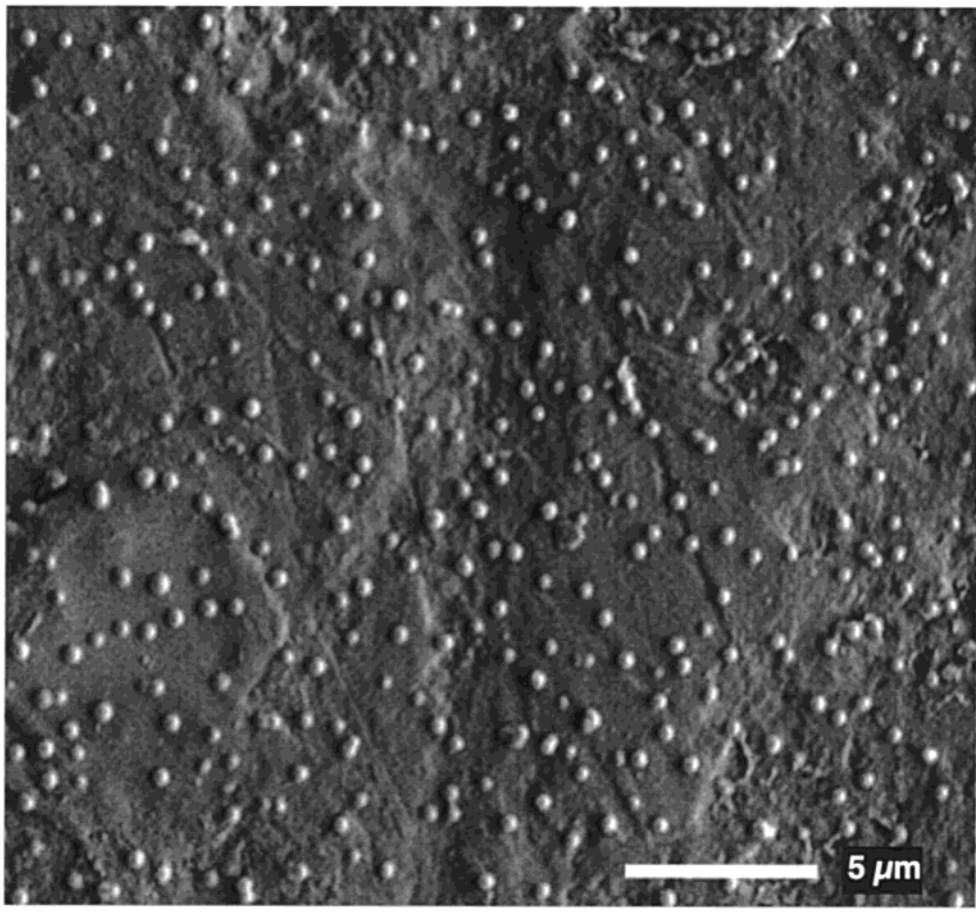

Figure 5. Cryogenic SEM image of P(MAA-*g*-EG) nanospheres prepared from a MAA/EG molar feed ratio of 1:1. Magnification = 3000×. Scale bar length = 5 μm.

it was concluded that the increase in particle size was due to aggregation of smaller particles.

Morphology. Cryogenic scanning electron microscopy was used to obtain images of the P(MAA-*g*-EG) nanospheres and to confirmed the shape of the P(MAA-*g*-EG) nanospheres produced by the precipitation polymerization method. The images were taken of P(MAA-*g*-EG) nanospheres after the washing process but before freeze-drying. The P(MAA-*g*-EG) nanospheres were prepared by precipitation polymerization of MAA and PEGMA at a the 1:1 MAA/EG molar feed ratio using a monomer concentration of 0.008 g/mL, with a cross-linker concentration of 0.75 mol % and an initiator concentration of 0.5 wt % polymerizing for 15 min. Figures 5 and 6 are images obtained by cryogenic SEM of the P(MAA-*g*-EG) nanospheres. The images were taken at magnifications of 3000× and 5000×, respectively, at an accelerating voltage of 5 kV.

The images show that spherical particles of P(MAA-*g*-EG) were produced during the precipitation polymerization of MAA and PEGMA. The size of the P(MAA-*g*-EG) nanospheres appeared to have diameters of approximately 0.5 μm. The P(MAA-*g*-EG) nanospheres also appear to be uniform in shape and size, and no significant aggregation was observed.

Equilibrium Swelling Studies. Equilibrium swelling studies of the P(MAA-*g*-EG) nanospheres were performed in order to characterize their swelling behavior in response to changes in the pH of the swelling medium. The effect of parameters such as MAA/EG molar feed used during the precipitation/dispersion polymerization and the degree of cross-linking of the P(MAA-*g*-EG) nanospheres were investigated.

Freeze-dried P(MAA-*g*-EG) nanospheres were resuspended in a buffer solution according to the procedure described earlier. Figure 7 shows the equilibrium swelling behavior of the P(MAA-*g*-EG) nanospheres prepared from MAA/EG molar feed ratios of 1:1, 2:1, and 4:1. At low pH values of 3.3 and 4.0, the nanospheres were in a collapsed state due to hydrogen bonding between the carboxylic acid groups of PMAA and the ether groups of the PEG chains. At high pH values of 7.5, the equilibrium swelling volume of the P(MAA-*g*-EG) nanospheres produced from the 1:1 MAA/EG molar feed ratio was approximately 100 times that of the equilibrium swelling volume of the nanospheres at a pH value of 3.3. The equilibrium swelling volumes of the P(MAA-*g*-EG) nanospheres produced from the 2:1 and 4:1 MAA/EG molar feed ratio were approximately 200 and 400 times their equilibrium volume at a pH value of 3.3. Swelling of the nanospheres occurred due to electrostatic repulsion within the network caused by the ionization of the carboxylic acid groups. At high pH values, the carboxylic groups were ionized and the hydrogen bonds formed between PMAA and PEG at low pH values are broken.

Differences in the degree of swelling between the nanospheres produced from the different MAA/EG molar feed ratios were attributed to the extent of ionization within the nanospheres. According to the reactivity ratios determined by Smith and Klier [18] for the precipitation/dispersion polymerization of PEGMA and MAA, the polymer that is formed has the same overall composition as the feed. The P(MAA-*g*-EG) nanospheres prepared from a MAA/EG molar feed ratio of 4:1 is rich in PMAA. Therefore, the P(MAA-*g*-EG)

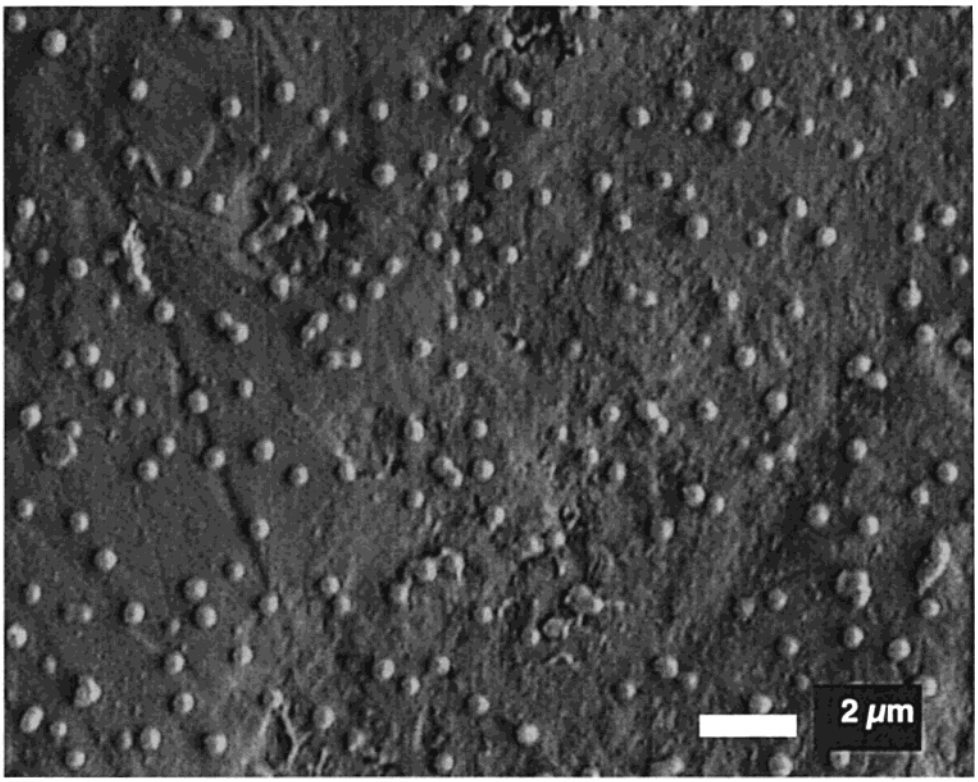

Figure 6. Cryogenic SEM image of P(MAA-*g*-EG) nanospheres prepared from a MAA/EG molar feed ratio of 1:1. Magnification = 5000×. Scale bar length = 2 μm.

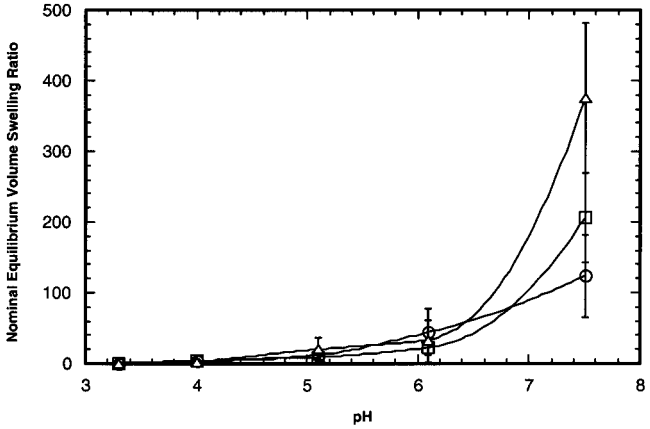

Figure 7. Effect of pH on the equilibrium swelling ratio (Vs/Vc) of P(MAA-*g*-EG) nanospheres prepared from MAA/EG molar feed ratios of (○) 1:1, (□) 2:1, and (△) 4:1 molar ratios.

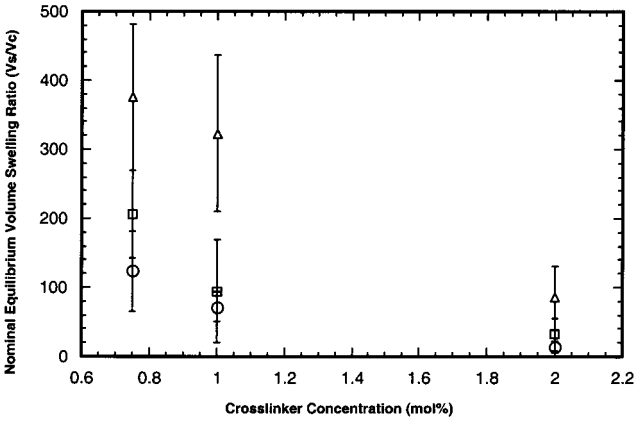

Figure 8. The equilibrium swelling ratio of the P(MAA-*g*-EG) nanospheres prepared at varying cross-linking concentrations from MAA/EG molar feed ratios of (○) 1:1, (□) 2:1, and (△) 4:1.

nanospheres prepared from the MAA/EG molar feed ratio have more ionizable carboxylic acid groups. The increased ionization in the P(MAA-*g*-EG) nanospheres increased the electrostatic repulsion within the network. Therefore, higher swelling occurred in the P(MAA-*g*-EG) nanospheres prepared from the 4:1 MAA/EG molar feed ratio.

The effect on the nominal equilibrium volume swelling ratio of the P(MAA-*g*-EG) nanospheres produced at increasing reaction cross-linking concentrations is shown in Figure 8. The nominal equilibrium swelling volume of the P(MAA-*g*-EG) nanospheres produced from 1:1, 2:1, 4:1 MAA/EG molar feed ratios decreased with increasing cross-linking concentration. As observed previously, the P(MAA-*g*-EG) nanospheres produced from the 4:1 MAA/EG molar ratio swelled to a higher degree in comparison to the P(MAA-*g*-EG) nanospheres produced at the other MAA/EG molar feed ratios. Swelling studies were attempted on P(MAA-*g*-EG) nanospheres produced at a cross-linker concentration of 5 wt %, but the nanospheres produced at this cross-linker concentration did not resuspend in buffer solutions.

The differences in nominal equilibrium swelling ratios of the P(MAA-*g*-EG) nanospheres produced from precipitation polymerization of MAA and PEGMA at various cross-linking concentrations provided information about the degree of cross-linking within the P(MAA-*g*-EG) nanospheres. It can be concluded that more cross-linking agent is incorporated in the P(MAA-*g*-EG) nanospheres when the cross-linking concentration during the precipitation polymerization is increased.

Mechanism of Particle Formation

From the above experiments, the following mechanism of particle formation of the P(MAA-*g*-EG) nano-

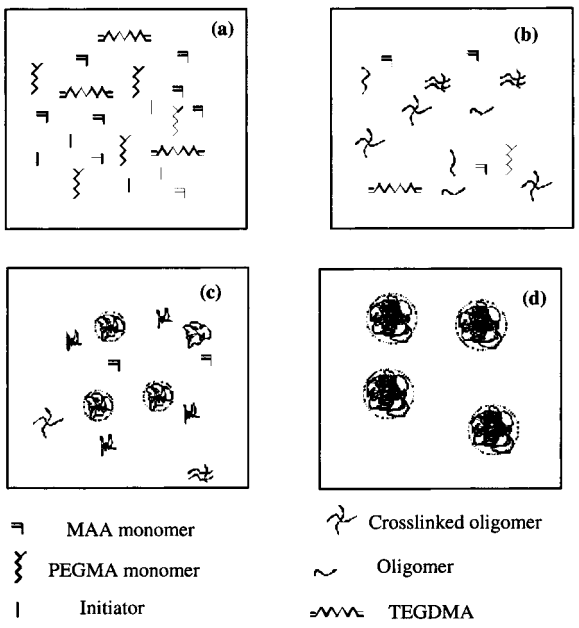

Figure 9. Proposed mechanism of particle formation during the precipitation polymerization of MAA and PEGMA cross-linked with TEGDMA.

spheres by UV-initiated free radical precipitation polymerization of MAA, PEGMA, and TEGDMA in water has been formulated (see Figure 9).

The reaction begins as a homogeneous mixture of MAA, PEGMA, TEGDMA, and photoinitiator dissolved in water (a). Upon exposure to UV light, the photoinitiator decomposes to free radicals that react with the monomers to form monomer radicals. The monomer radicals continue to propagate, forming growing PMAA−PEG oligomer chains with reactive end groups (b). As the PMAA−PEG oligomer chains grow, the cross-linker is incorporated and the PMAA−PEG oligomer chains begin to cross-link. Thus, precipitation is also initiated. As the oligomers cross-link, they begin to aggregate. Aggregation takes place partially due to the hydrogen-bonded complexes between the carboxylic acid groups of the PMAA and the ether oxygens on the PEG chains, and partially due to the cross-linking. The oligomer chains will continue to aggregate until a critical size is reached where they are stable and insoluble in water. At this point, particle nuclei are formed (c). Subsequent particle growth occurs by polymerization within the particle through absorption of monomer and oligomer radicals. Particle growth stops when all of the growing chains are terminated or until all of the monomers are consumed (d).

Conclusions

Nanospheres of poly(methacrylic acid-*g*-ethylene glycol) (P(MAA-*g*-EG)) were synthesized in water by a UV-initiated free radical dispersion polymerization using tetraethylene glycol dimethacrylate as the cross-linking agent. P(MAA-*g*-EG) nanospheres were prepared from MAA/EG molar feed ratios of 1:1, 2:1, 4:1, and 1:2. The particle sizes of the P(MAA-*g*-EG) nanospheres were evaluated using photon correlation spectroscopy (PCS), and the morphology was assessed using cryogenic scanning electron microscopy and transmission electron microscopy.

Particle size was found to be controlled mainly by the monomer concentration in water. Stable dispersions of P(MAA-*g*-EG) nanospheres were produced during the precipitation/dispersion polymerization up to a monomer concentration of approximately 0.016 g/mL. Results showed that P(MAA-*g*-EG) nanospheres of relatively narrow size distribution were produced up to monomer concentration of 0.016 g/mL. Depending on the monomer concentration, P(MAA-*g*-EG) nanospheres can be produced to have particle sizes ranging from approximately 150 nm to 650 nm.

Increasing the cross-linker concentration in the feed decreased the particle size of the P(MAA-*g*-EG) nanospheres produced from the 1:2 MAA/EG molar feed ratio. A slight increase in particle size was observed for the nanospheres produced from the 4:1 MAA/EG molar feed ratio, but no effect on the resulting particle size was observed for the P(MAA-*g*-EG) nanospheres produced from the 1:1 and 2:1 MAA/EG molar feed ratios.

The equilibrium swelling behavior of the P(MAA-*g*-EG) nanospheres was investigated in buffer solutions with pH values ranging from 3.3 to 7.5. The P(MAA-*g*-EG) nanospheres were shown to undergo a pH-sensitive volume phase transition. The P(MAA-*g*-EG) nanospheres prepared from the 4:1 MAA/EG molar feed ratio exhibited the highest increase in volume in comparison to the nanospheres produced from the 1:1 and 2:1 MAA/EG molar feed ratios. The equilibrium swelling ratio of the P(MAA-*g*-EG) nanospheres decreased when the cross-linking agent concentration was increased during polymerization, an indication of increased cross-linking incorporation during polymerization.

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