Hydrogel Microspheres Formed by Complex Coacervation of Partially MPEG-Grafted Poly(styrene-*alt*-maleic anhydride) with PDADMAC and Cross-Linking with Polyamines

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ABSTRACT: Poly(styrene-*alt*-maleic anhydride) partially grafted with methoxy poly(ethylene glycol) (SMA-*g*-MPEG) was prepared by reacting poly(styrene-*alt*-maleic anhydride) with a substoichiometric amount of MPEG lithium alcoholate. Aqueous solutions of the resulting SMA-*g*-MPEG formed complex coacervates with poly(diallyldimethylammonium chloride) (PDADMAC). These phase-separated liquid polyelectrolyte complexes were subsequently cross-linked by the addition of two different polyamines to prepare cross-linked hydrogel microspheres. Chitosan served as an effective cross-linker at pH 7.0, while polyethylenimine (PEI) was used as cross-linker under basic conditions (pH 10.5). The resulting coacervate microspheres swelled with increasing salinity, which was attributed mainly due to the shielding of the electrostatic association within the polyelectrolyte complex. The morphology of the coacervate microspheres was investigated by environmental scanning electron microscopy.

Introduction

Hydrogel microspheres are being studied for uses in drug delivery, protein separation, and enzyme immobilization.[1,2] They can be prepared by precipitation polymerization or emulsion polymerization,[3−5] though the use of organic solvents in these processes, and the unreacted monomer remaining in the final microspheres, are undesirable for some applications.

In contrast, coacervation is a water-based phase separation process of preformed polymers and hence offers interesting new routes to hydrogel microparticles.[6,7] Coacervation involves the phase separation of an aqueous polymer solution into two immisible liquid phases: a polymer-rich phase (coacervate phase) and a polymer-lean phase (equilibrium phase).[8,9] Coacervation can be induced either by polyelectrolyte complexation (complex coacervation) or by decreasing the polymer−solvent interactions such as by changing the solvent composition or the temperature (simple coacervation).[10] Both coacervation processes have been widely used for protein separations and for encapsulations.[6,7,11,12]

Coacervates can be dispersed as microdroplets in the equilibrium phase. These microdroplets are not colloidally stable and tend to coalesce but can be cross-linked by covalent bonds or by physical gelation.

Until now, natural products, such as gelatin/acacia and chitosan/alginate, were the main materials used for preparing microparticles through coacervation,[13−15] and only a few studies have been carried out using synthetic polymers.[16−18] The gelatin/acacia complex coacervates are typically covalently cross-linked with formaldehyde or glutaraldehyde. In contrast, the ionically cross-linked chitosan/alginate systems, where alginate/calcium gel beads are subsequently coated with the positively charged chitosan, are usually not stable under high salt concentrations.

We describe here a complex coacervate system that combines electrostatic coacervation and covalent cross-linking, based on anhydride-functional polyelectrolytes.

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Maleic anhydride copolymers have been widely studied as surfactants and materials for biomedical applications such as immobilizing proteins.[19,20] In previous studies, we described the use of oil-soluble styrene-maleic anhydride copolymers in interfacial microencapsulation reactions[21,22] and investigated the temperature sensitivity and pH sensitivity of the poly(ethylene glycol)-grafted poly(styrene-*alt*-maleic anhydride) in aqueous solutions.[23]

In this study, we use the methoxy poly(ethylene glycol) partially grafted poly(styrene-*alt*-maleic anhydride) as a reactive polyanion to form complex coacervates with poly(diallyldimethylammonium chloride). The resulting coacervate microdroplets are subsequently cross-linked using both a synthetic and a natural polyamine to form cross-linked hydrogel microspheres. The swelling of the resulting cross-linked coacervate microspheres at different salt levels has been studied.

Experimental Section

Materials. Styrene, maleic anhydride, methoxy poly(ethylene glycol) (MPEG) (M_n = 350), butyllithium (1.60 mol L^{-1} in hexane), poly(diallyldimethylammonium chloride) (PDADMAC) (40 wt % aqueous solution), chitosan, and polyethylenimine (PEI) (branched, M_n = 1800) were purchased from Aldrich. Maleic anhydride was recrystallized in chloroform before use; others were used as received. 2,2′-Azobis(isobutyronitrile) (AIBN) was obtained from American Polymer Standards Laboratories and recrystallized in methanol. Methyl ethyl ketone (MEK), tetrahydrofuran (THF), and anhydrous diethyl ether were obtained from Caledon. THF was dried by refluxing with metallic sodium followed by distillation. The number-average molecular weight and the polydispersity index of chitosan were 1.5×10^4 and 2.5, respectively, measured by aqueous gel permeation chromatography as described below.

Preparation of Methoxy Poly(ethylene glycol) Partially Grafted Poly(styrene-*alt*-maleic anhydride) (SMA-*g*-MPEG). Poly(styrene-*alt*-maleic anhydride) (SMA) was prepared by free radical polymerization as previously reported.[23] The number-average molecular weight and polydispersity index of SMA were 1.37×10^4 and 2.2, respectively. In a typical procedure, a solution of MPEG lithium alcoholate, obtained by reacting 2.46 g (7.0×10^{-3} mol) of methoxy poly-

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Table 1. Composition of SMA-*g*-MPEG Copolymers As Estimated from ^{1}H NMR

sample	MPEG/styrene unit (mol/mol)
SMA-*g*-MPEG-55%	0.55
SMA-*g*-MPEG-70%	0.70
SMA-*g*-MPEG-87%	0.87
SMA-*g*-MPEG-97%	0.97

(ethylene glycol) with 4.4 mL of 1.6 mol L^{-1} butyllithium (7.0×10^{-3} mol) in 10 mL of THF, was added to a solution of 2.0 g of SMA in 100 mL of THF. The reaction was carried out at room temperature under a nitrogen atmosphere for 24 h. The grafted copolymer was precipitated into 500 mL of diethyl ether; the product was separated by centrifugation and dried under vacuum at 40 °C overnight. 4.0 g of product was obtained, corresponding to a yield of 90%.

Several SMA-*g*-MPEG copolymers with different MPEG contents were prepared by adjusting the molar ratio of MPEG alcoholate to succinic anhydride groups in the SMA. Their compositions are summarized in Table 1.

SMA-*g*-MPEG copolymers were characterized by FT-IR and ^{1}H NMR. FT-IR spectra were measured on a Bio-RAD FTS-40 spectrometer using KBr pellets. ^{1}H NMR spectra were recorded on a Bruker AC 200, using DMF-d_7 as the solvent.

Chitosan. Chitosan of low molecular weight was prepared by a free radical degradation method following a literature procedure:[24] 5.0 g of chitosan was dissolved in 250 g of 2 wt % acetic acid solution. The solution was heated to 80 °C, and 4.17 g of 30 wt % hydrogen peroxide (BDH) was added. The degradation was carried out under a nitrogen atmosphere for 5 h. The reaction mixture was neutralized to pH 7.0 with 1 N NaOH, centrifuged, and filtered to obtain a clear solution. The final product was obtained by dialysis against distilled water for 1 week, using a Spectrum membrane with a MWCO of 1000. The solution was freeze-dried to give 2.0 g of degraded chitosan (yield 40%).

The molecular weight of the degraded chitosan was estimated by gel permeation chromatography, consisting of a Waters 515 HPLC pump, three Ultrahydrogel columns (0−3K, 0−5K, 2K−300K Da), and a Waters 2414 refractive index detector, with 0.5 mol L^{-1} sodium acetate/0.5 mol L^{-1} acetic acid solution as eluent at a flow rate of 0.8 mL min^{-1} and narrow disperse poly(ethylene glycol) as calibration standards. The M_n and PDI of degraded chitosan were 3.9×10^3 and 1.2, respectively.

The degree of ionization of chitosan at different pH values was determined by potentiometric titration. 20 mL of 2.0 wt % chitosan solution containing 0.05 mol L^{-1} NaCl, acidified to pH 2.0 with 1.0 N hydrochloric acid, was titrated with 0.1 N NaOH. The degree of ionization is defined as $\alpha = \alpha_N + [H^+]/C_p$, where α_N is the degree of neutralization, C_p is the equivalent concentration of chitosan, and $[H^+]$ is the proton concentration and is deduced from the pH of the solution. Titrations were performed on an automatic PC-Titrator (Mandel) at room temperature.

Poly(diallyldimethylammonium chloride) (PDADMAC). The molecular weight of commercial PDADMAC was determined by viscometry in 1.0 mol L^{-1} NaCl solution at 30 °C using an Ubbelohde viscometer. All stock solutions were filtered through a 0.45 μm PTFE membrane filter prior to measurements. The molecular weight of PDADMAC was calculated to be 2.0×10^4, using the Mark−Houwink equation $[\eta] = k[M]^\alpha$, where k is 4.7×10^{-3} and α is 0.83.[25]

Coacervation. Coacervations were conducted in 0.05 mol L^{-1} NaCl solution. The pH value of the solution was adjusted to the desired values by adding 1 N NaOH or HCl.

In a typical procedure, a solution of 2.0 wt % PDADMAC was prepared in 0.05 M NaCl solution at pH 7.0. 2.8 g of this solution was added to 50 g of 0.25 wt % SMA-*g*-MPEG-70% in 0.05 M NaCl solution at pH 7.0 under 600 rpm stirring. The mixture was maintained at pH 7.0 by adding 0.1 N NaOH during this coacervation process. After the coacervate mixture was stirred for 5 min, it was centrifuged at 3000 rpm for 10 min. The coacervate was separated by decanting the transparent supernatant phase and dried to constant weight at 65 °C. 0.070 g of dry coacervate was obtained (yield 38%).

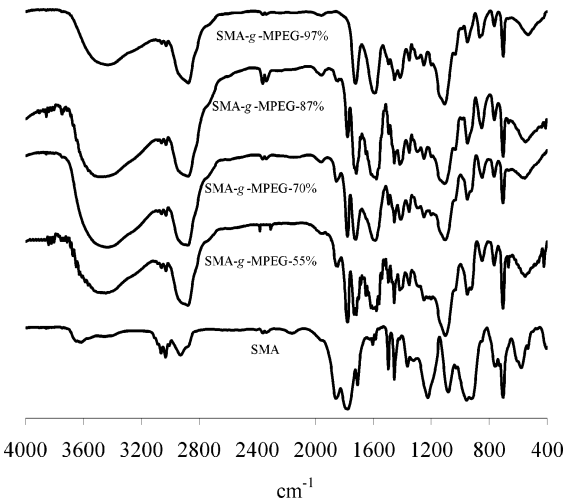

Figure 1. IR spectra of SMA and SMA-*g*-MPEG copolymers.

Preparation of Hydrogel Microspheres. Microspheres were prepared using the same coacervation process as described above. After coacervation and while still stirring, 4.2 g of 2.0 wt % chitosan in 0.05 mol L^{-1} NaCl solution at pH 7.0 was added to cross-link the coacervate droplets into microspheres. The cross-linking reaction was continued for 3 h. Microspheres were isolated by centrifugation at 500 rpm for 10 min and were washed with 0.05 M NaCl solution to remove unreacted materials. They are stored in 0.05 M NaCl solution for further studies.

Characterization of Hydrogel Microspheres. Optical images of particles were recorded using a scale-calibrated Olympus BH-2 microscope equipped with a Kodak DC 120 Zoom digital camera. The mean diameter of the microspheres was estimated by analyzing about 200 particles using UTHSCSA Image Tool software.

Morphologies of microspheres were examined using a Phillips ElectroScan 2020 environmental scanning electron microscope (ESEM). For the sample preparation, the microspheres were dehydrated using a series of water/acetone mixtures with increasing acetone contents. ESEM samples were prepared by applying a drop of microparticles in acetone suspension to a glass-covered ESEM stub, drying under vacuum, and sputter-coating with about 5 nm thick layer of gold.

Equilibrium Swelling Studies. The microspheres were incubated in 0.05, 0.1, 0.2, 0.5, and 1.0 M NaCl solutions at pH 7.0 in order to study their swelling response to changes of NaCl concentrations. The microsphere diameters were monitored by optical microscopy, by taking samples at different times and measuring the size of the swollen particles until they reached equilibrium. The average diameters of the swollen particles were estimated using UTHASCA Image Tool software, as described above.

Results and Discussion

Preparation and Characterization of SMA-*g*-MPEG. Several partially methoxy poly(ethylene glycol)-grafted poly(styrene-*alt*-maleic anhydride) copolymers (SMA-*g*-MPEG) were prepared by reacting substoichiometric amounts of MPEG lithium alcoholate with poly(styrene-*alt*-maleic anhydride) (SMA). This grafting reaction is quantitative. Figure 1 shows FT-IR spectra of both SMA and SMA-*g*-MPEG. The spectrum of SMA displays characteristic anhydride peaks at 1780 and 1850 cm^{-1}. In spectra of partially grafted copolymers (SMA-*g*-MPEG-55%, -70%, and -87%), the anhydride absorptions are still present but much weaker. In

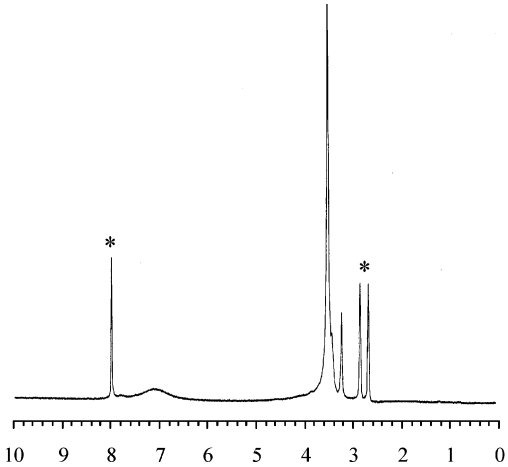

Figure 2. NMR spectrum of SMA-*g*-MPEG-70% copolymer dissolved in DMF-d_7. The residue DMF solvent peaks are at around 2.7, 2.9, and 8.0 ppm.

addition, these spectra show absorptions characteristic for ester carbonyl at 1730 cm^{-1}, ether at 1106 cm^{-1}, and carboxylic acid at 1605 cm^{-1}. In the spectrum of fully grafted copolymer (SMA-*g*-MPEG-97%), the anhydride peaks have disappeared.

The amount of MPEG in SMA-*g*-MPEG can be estimated by ^1H NMR spectroscopy (Figure 2). The ^1H NMR spectrum of SMA-*g*-MPEG reveals a broad aromatic resonance at $\delta = 7.2$ ppm and peaks at $\delta = 3.56$ and 3.24 ppm, corresponding to the ethylene oxide and methoxy units of the MPEG grafts, respectively. The extent of MPEG reaction with anhydride units in SMA is estimated from the ratio of peak areas for methoxy protons (3H) vs phenyl protons (5H). The results are shown in Table 1.

The primary objective for this research is to use SMA-*g*-MPEG copolymers as reactive polyanions to prepare coacervate microspheres. The SMA-*g*-MPEG backbone contains both carboxylic acid and anhydride groups. The carboxylic acid forms a complex with polycations to induce the coacervation, and the anhydride group can be used to subsequently cross-link the dispersed complex coacervate droplets to prepare microspheres. SMA-*g*-MPEG-55% is only water-soluble under basic conditions, while the content of anhydride groups in SMA-*g*-MPEG-87% is too low for cross-linking reactions. Hence, SMA-*g*-MPEG-70% is chosen for further studies to prepare coacervate hydrogel microspheres.

Complexation of SMA-*g*-MPEG-70% with Reactive Polyamines. In initial experiments, we attempted to cross-link the complex coacervate in situ, by complexing the SMA-MPEG-70% with reactive polycations based on the partially protonated polyamines, chitosan and PEI. These complex coacervations were carried out at pH 7.0 to minimize the hydrolysis of the anhydride groups in SMA-*g*-MPEG-70%. At pH 7.0, the degree of ionization of PEI is about 50%,[26] while that of chitosan is approximately 30% (Figure 3).

We found that the addition of a dilute PEI solution at pH 7.0 to the SMA-*g*-MPEG-70% solution resulted not in coacervation, but rather in bulk precipitation of the cross-linked polymer. Similarly, addition of chitosan produced a mixture of some spherical coacervate particles besides much gel precipitate (Figure 4).

Clearly, coacervation and cross-linking compete in these reactions. Significant cross-linking occurring prior to or during the coacervation would lead to macroscopic

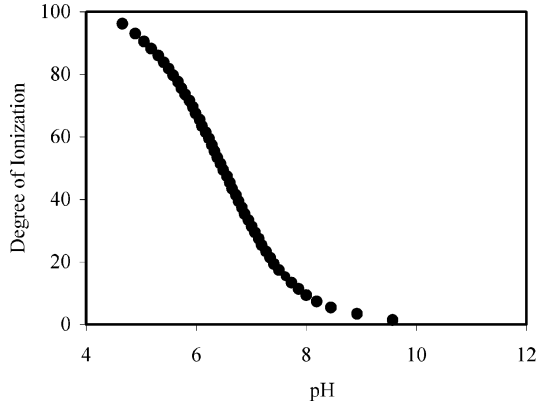

Figure 3. Degree of ionization of chitosan vs pH.

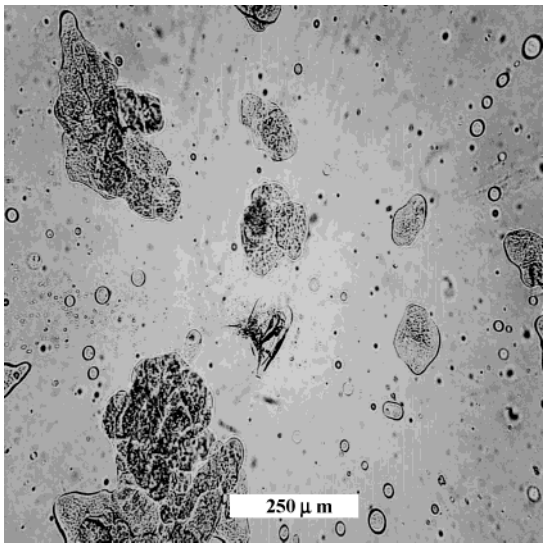

Figure 4. Optical microscope image of uncontrolled phase separation and cross-linking upon reacting SMA-*g*-MPEG-70% with chitosan at pH 7.0 in 0.05 mol L^{-1} NaCl solution.

gelation. Apparently, chitosan and especially PEI react rapidly with the anhydride groups, preventing the formation of distinct coacervate droplets and hence microspheres. In principle, working at a lower pH could reduce the concentration of free amine and slow the cross-linking reaction. However, an acidic pH would catalyze anhydride hydrolysis[20] and hence reduce the effective cross-linking achievable.

We describe here another, two-step strategy to form cross-linked complex coacervates from SMA-*g*-MPEG. Specifically, poly(diallyldimethylammonium chloride) (PDADMAC) is used to form an initial complex coacervate with SMA-*g*-MPEG, which is subsequently cross-linked by adding chitosan or PEI.

Complex Coacervation between SMA-*g*-MPEG and PDADMAC. Figure 5 shows a typical optical microscopy image of complex coacervates of SMA-*g*-MPEG-70% and PDADMAC. The coacervate droplets are not colloidally stable and will coalesce after several minutes without stirring.

The dependence of the coacervate yield on the ratio of PDADMAC to SMA-*g*-MPEG-70% is described in Figure 6. The coacervation takes place over a range of PDADMAC/SMA-*g*-MPEG-70% ratios, and the coacervate yield goes through a maximum upon increasing the PDADMAC/SMA-*g*-MPEG-70% ratio.

Complex coacervation is believed to take place in two steps: spontaneous electrostatic aggregation of op-

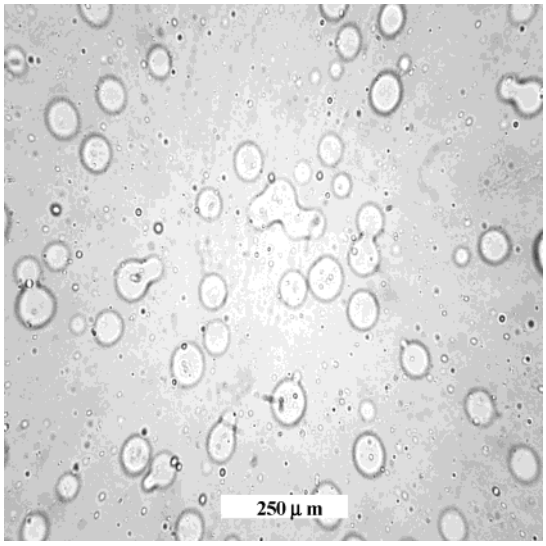

Figure 5. Optical microscopy image of the SMA-*g*-MPEG-70%/PDADMAC complex coacervate.

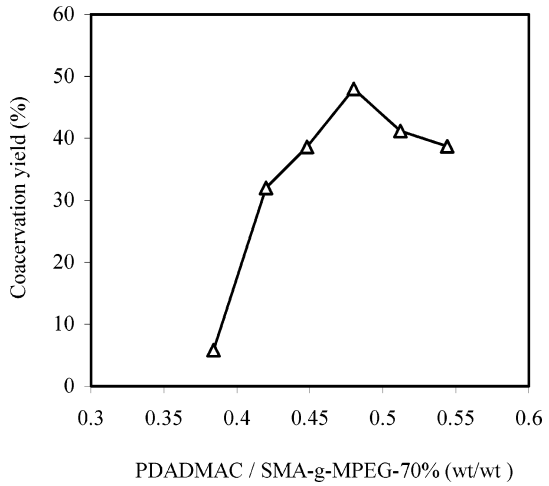

Figure 6. Effect of PDADMAC to SMA-*g*-MPEG-70% ratio on coacervation yield. pH of 7.0 in 0.05 mol L^{-1} NaCl solution. SMA-*g*-MPEG, 0.5 wt %; PDADMAC, 2.0 wt %.

positely charged polyelectrolytes to form primary aggregates and then the rearrangement of these primary aggregates to form the coacervate phase.[27] The coacervation yield is affected both by the properties of the two oppositely charged polymers and by solution conditions such as concentration, ionic strength, and the charge neutralization of the aggregate.[8,28,29] Usually, maximum coacervate yields occur at the electrostatic neutrality point of complex polyelectrolytes.

Formation of Microspheres by Cross-Linking SMA-*g*-MPEG/PDADMAC Coacervates with Chitosan. The results of cross-linking SMA-*g*-MPEG-70%/PDADMAC coacervates with chitosan are listed in Table 2. The mass ratios of PDADMAC to SMA-*g*-MPEG-70% used correspond to the coacervation yield shown in Figure 6. Under these conditions, the coacervation yield is only about 6% (Figure 6) when the mass ratio of PDADMAC to SMA-*g*-MPEG-70% is 0.38. Most of the polymer remains in the solution, and the coacervate droplets are finely dispersed. When chitosan is added in this coacervate mixture, gelation occurs due to the reaction of chitosan with the SMA-*g*-MPEG-70% in the solution. The ESEM microphotograph (Figure 7a) reveals some cross-linked coacervate microspheres within the precipitate matrix. The coacervation yield increases with increasing the mass ratio of PDADMAC/SAM-*g*-MPEG-70% to 0.42 and 0.45. When chitosan is added at such conditions, cross-linked coacervate microspheres are obtained. The ESEM images of these microspheres are shown in Figure 7b,c.

Table 2. Effect of Polymer Ratios on Cross-Linking of SMA-*g*-MPEG-70%/PDADMAC Coacervates with Chitosan[a]

sample	PDADMAC/ SMA-*g*-MPEG (w/w)	chitosan/ PDADMAC (w/w)	appearance[b]	mean diam of particles[c] (μm)
SDC-1	0.38	1.5	precipitate	
SDC-2	0.42	1.5	microspheres	38
SDC-3	0.45	1.5	microspheres	40
SDC-4	0.48	1.5	no cross-linking	
SDC-5	0.51	1.5	no cross-linking	
SDC-6	0.54	1.5	no cross-linking	
SDC-7	0.45	1.0	no cross-linking	
SDC-8	0.45	2.0	microspheres	43

[a] The coacervation and cross-linking reaction were carried out at pH 7.0 in 0.05 mol L^{-1} NaCl solution. The concentrations of SMA-*g*-MPEG-70%, PDADMAC, and chitosan were 0.5, 2.0, and 2.0 wt %, respectively. [b] Observed by optical microscopy. [c] In 0.05 mol L^{-1} NaCl solution.

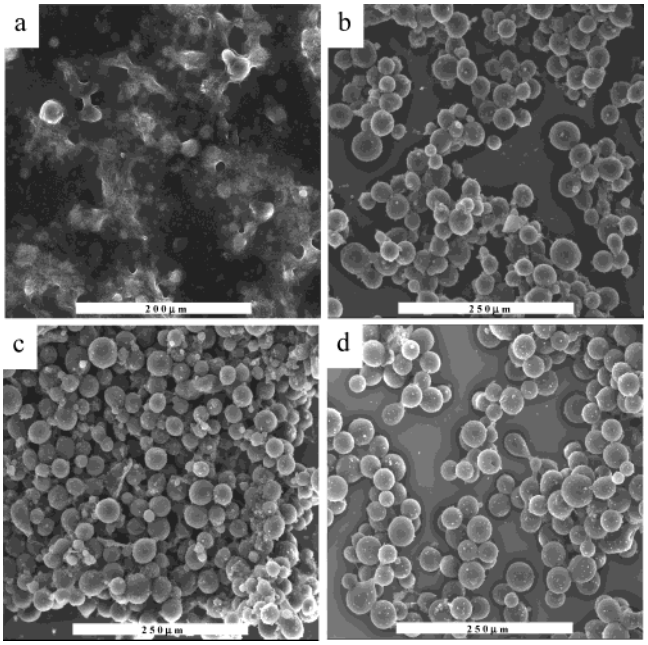

Figure 7. ESEM micrographs of microspheres formed by cross-linking SMA-*g*-MPEG-70%/PDADMAC coacervates with chitosan at pH 7.0 in 0.05 mol L^{-1} NaCl solution: (a) SDC-1, (b) SDC-2, (c) SDC-3, (d) SDC-8.

The coacervation has the highest yield at the mass ratio of 0.48 of PDADMAC/SMA-*g*-MPEG-70% under the studied conditions. However, no cross-linked microspheres are obtained when chitosan is added to this coacervate mixture (Table 2).

As described above, coacervation has its maximum yield at the electrostatic neutralization point of the complexed polyelectrolytes. Before the coacervation reaches the maximum yield point, the SMA-*g*-MPEG-70%/PDADMAC coacervates should be partially negatively charged. When chitosan, which is about 30% ionized at pH 7.0, is added, it will penetrate into the coacervate droplets by electrostatic association and subsequently react with the SMA-*g*-MPEG-70% to cross-link coacervates into microspheres. On the other hand,

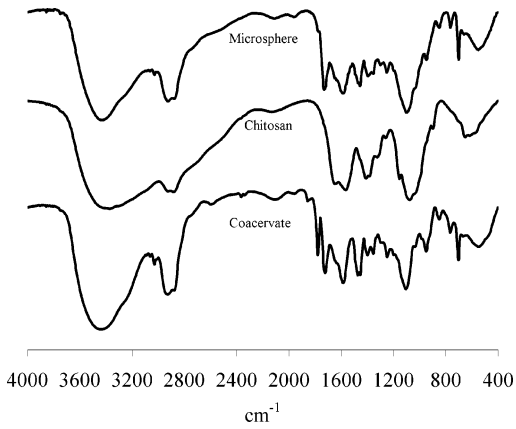

Figure 8. IR spectra of SMA-*g*-MPEG-70%/PDADMAC coacervates, chitosan, and cross-linked microspheres (SDC-3).

at the point of maximum coacervate yield, the coacervate is charge-neutral, Hence, chitosan is no longer electrostatically attracted into the coacervate, and no cross-linking takes place. These results were confirmed by further increasing the mass ratio of PDADMAC/SMA-*g*-MPEG-70% to 0.51 and 0.54. These coacervates are now positively charged, and again no cross-linking takes places when chitosan is added (Table 2).

The amount of chitosan added affects the cross-linking density of the microspheres (SDC-3, SDC-7, and SDC-8 in Table 2). At a mass ratio of chitosan to PDADMAC of 1.0, no stable microsphere is observed, indicating that the concentration of chitosan is too low to diffuse into and cross-link the coacervates efficiently. Increasing the addition amount of chitosan, cross-linked coacervate microspheres are obtained. ESEM images of these microspheres are shown in Figure 7c,d.

Figure 8 shows IR spectra of SMA-*g*-MPEG-70%/PDADMAC coacervate and coacervate microspheres cross-linked with chitosan. The spectrum of the coacervate displays characteristic anhydride peaks at 1780 and 1850 cm^{-1}. In the spectrum of cross-linked microspheres, these characteristic absorptions have disappeared.

Formation of Microspheres by Cross-Linking SMA-*g*-MPEG/PDADMAC Coacervates with PEI. When PEI is used as the cross-linker at pH 7.0, macroscopic precipitation takes place as soon as the PEI is added into SMA-*g*-MPEG-70%/PDADMAC coacervate mixture under all circumstances.

Compared to chitosan, PEI has both a high charge density and high amine content at pH 7.0.[26] Furthermore, the PEI chain is more flexible. PEI is able to access the coacervate more easily than chitosan. During the accessing process, PEI may substitute PDADMAC from SMA-*g*-MPEG-70%/PDADMAC coacervates. Meanwhile, the cross-linking reaction between amines and anhydrides takes place very quickly, leading to the similar situation as PEI complexing directly with SMA-*g*-MPEG and gel precipitations.

One solution to prevent the PEI from breaking up SMA-*g*-MPEG-70%/PDADMAC coacervates during the cross-linking process is to reduce the charge density of PEI, making it function only as a cross-linker. We find that PEI is an effective cross-linker for cross-linking coacervates under basic conditions, such as pH 10.5, where PEI is less than 5% ionized.[26]

The results of cross-linking SMA-*g*-MPEG-70%/PDADMAC coacervates with PEI at pH 10.5 are listed in Table 3. ESEM images of these cross-linked microspheres are shown in Figure 9.

Table 3. Effects of Polymer Ratios on the Morphology of PEI Cross-Linked Coacervate Microspheres of SMA-*g*-MPEG-70%/PDADMAC[a]

sample	PDADMAC/SMA-*g*-MPEG (w/w)	appearance[b]	mean diam of particle[c] (μm)
SDE-1	0.45	agglomerated microspheres	
SDE-2	0.51	agglomerated microspheres	
SDE-3	0.58	agglomerated microspheres	
SDE-4	0.70	microspheres	38
SDE-5	0.78	microspheres	36

[a] The coacervation and cross-linking reaction were taken at pH 10.5 in 0.05 mol L^{-1} NaCl solution. 1.0 g of 2.0 wt % PEI was added after coacervation. The concentrations of SMA-*g*-MPEG-70% and PDADMAC were 0.5 wt % and 2.0 wt %. [b] Observed by optical microscopy. [c] In 0.05 mol L^{-1} NaCl solution.

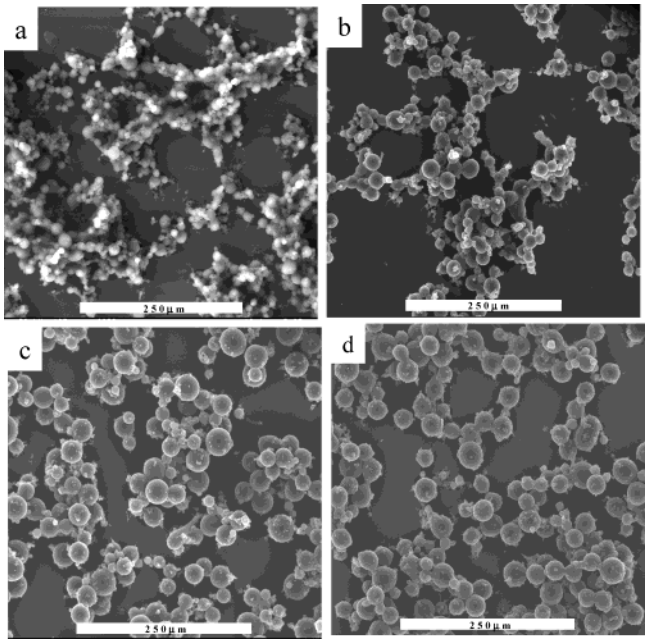

Figure 9. ESEM micrographs of microspheres formed by cross-linking SMA-*g*-MPEG-70%/PDADMAC coacervates with PEI at pH 10.5 in 0.05 mol L^{-1} NaCl solution: (a) SDE-1, (b) SDE-2, (c) SDE-4, (d) SDE-5.

At basic conditions, even though the hydrolysis of anhydride groups happens during the coacervation and the cross-linking processes, the remaining anhydride groups seem sufficient to be cross-linked by the PEI chains and form stable coacervate microspheres. Morphologies of these cross-linked coacervate microspheres are dependent on the PDADMAC/SMA-*g*-MPEG-70% ratios used. Microspheres aggregate extensively at relatively low mass ratios. As shown in Figure 9a,b, these microspheres are clustered and appearing to be jointed or welded. Colloidally stable microspheres are obtained at relatively high mass ratios of PDADMAC/SMA-*g*-MPEG-70% (Figure 9c,d). Although definitive proof is lacking, it seems very likely that the coacervates have excess anionic charges due to the hydrolysis of anhydride groups. These excess anionic charges play an important role in the accessing of PEI into the coacervates. At low mass ratio of PDADMAC/SMA-*g*-MPEG-70%, the charge association between PEI and SMA-*g*-MPEG-70% is still relatively strong, causing the PEI to penetrate the coacervates very quickly. A PEI chain may penetrate into several coacervates at the same

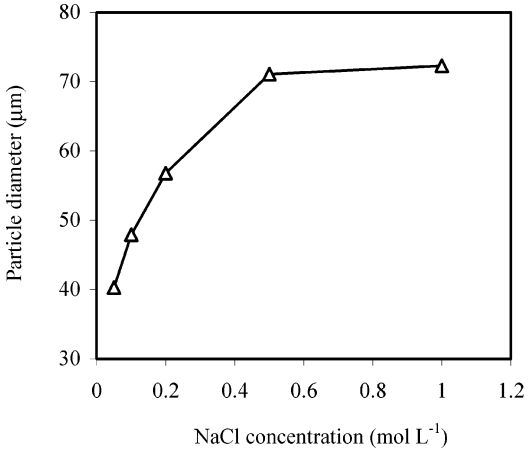

Figure 10. Dependence of swelling properties of the coacervate microspheres upon NaCl concentrations. SDC-3 coacervate microspheres were used.

time, and hence bridge microspheres together, leading to agglomerations. While at high mass ratio of PDADMAC/SMA-*g*-MPEG-70% PEI penetrates into the coacervates and cross-links them smoothly, agglomeration does not take place. As shown in the ESEM image (Figure 9), particles obtained at low mass ratios of PDADMAC/SMA-*g*-MPEG-70% have smaller sizes than those obtained at high mass ratios.

Independent Swelling Properties of Coacervate Microspheres. The rapid response of hydrogel microspheres to pH, temperature, and salinity has attracted much interest in recent years.[30−34] These hydrogel microspheres contain either anionic polymers, such as poly(acrylic acid), or cationic polymers, such as poly((dimethylamino)ethyl methacrylate). The swelling of the hydrogel microsphere at different pH and salinity is mainly due to the osmotic pressure, which results from the net difference in concentration of mobile ions between the interior of the microsphere and the exterior bathing solution.[32,33] As a result, the microspheres will shrink with increasing salt concentrations.

Up to now, few studies have studied the salt-dependent swelling of microspheres containing both anionic and cationic polymers. Some research has targeted the swelling properties of polyampholyte hydrogels.[35,36] Here, we present the salt-dependent swelling of complex coacervate microspheres.

Figure 10 describes the sizes of coacervate microspheres at various NaCl solutions. In contrast to the anionic or cationic polyelectrolyte hydrogel microspheres, the coacervate microspheres swell with the increase of salt concentrations. The particle sizes first increase and then reach a roughly constant value at the studied salt concentrations.

At low salt concentrations, the polymer chains in complex coacervate microspheres are oppositely charged polyelectrolytes. With the increase of salt concentrations in the bathing medium, the salt ion penetrates into coacervate microspheres and partially shields intermolecular ionic bonds. The polymer chains expand with the breakage of ionic cross-linking points, leading to the swelling of coacervate microspheres. Hence, the swelling of complex coacervate microspheres upon increasing the salinity is essentially based on the breakage of electrostatic interactions instead of osmotic pressures.

At high NaCl concentrations (above 0.5 mol L^{-1}), most of the charge of polyelectrolytes in coacervate microspheres is screened, and the particle sizes remain roughly constant with further increasing salt concentrations.

Conclusion

Because of the rapid cross-linking reaction between anhydride groups and polyamines, directly complexing poly(ethylene glycol) partially grafted poly(styrene-*alt*-maleic anhydride) with charged polyamines results not in coacervate microspheres but in gel precipitates. Hydrogel microspheres have been successfully prepared by complex coacervations of poly(ethylene glycol) partially grafted poly(styrene-*alt*-maleic anhydride) with poly(diallyldimethylammonium chloride), followed by cross-linking with chitosan or polyethylenimine. Chitosan serves as an effective cross-linker at pH 7.0, while polyethylenimine (PEI) is used as cross-linker under basic conditions (pH 10.5). The difference of cross-linking conditions is mainly due to the structure of polyamines, which affects their accessing abilities to coacervates.

Coacervate microspheres swell with increasing salinity in the bath medium. This swelling is mainly due to the interruption of electrostatic associations of complexed polyelectrolytes with the addition of salts.

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