Formation and Morphology of Methacrylic Polymers and Block Copolymers Tethered on Polymer Microspheres

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ABSTRACT: We report the use of atom transfer radical polymerization (ATRP) to graft off polymeric microspheres. Residual vinyl groups on nonswellable poly(divinylbenzene-80) microspheres were converted into ATRP initiators by hydroboration/oxidation followed by esterification. Subsequent grafting by ATRP of poly(hydroxylethyl methacrylate) (poly(HEMA)) and poly((dimethylamino)ethyl methacrylate) (poly(DMAEMA)) led to hydrophilically modified microspheres. The surface properties of these microspheres, FT-IR spectra, and potentiometric titration confirm the successful modification. In addition, swellable, lightly cross-linked poly(DVB-*co*-HEMA) microspheres were modified in a similar manner by grafting, i.e., methyl methacrylate (MMA) and glycidyl methacrylate (GMA), to form graft block copolymers including poly(MMA-*b*-DMAEMA), poly(MMA-*b*-trimethylammonium ethylmethacrylate (TMAEMA)), and poly(MMA-*b*-GMA). Microphase separation of the grafted amphiphilic block copolymers was studied by scanning electron microscopy. The microspheres modified with poly(MMA-*b*-DMAEMA) and poly(MMA-*b*-TMAEMA) show polyelectrolyte properties.

Introduction

Solid supports have found application in research areas including analytic and synthetic chemistry, biotechnology, and precision engineering. In particular, they are used in analytical and preparative HPLC columns, as polymer support reagents and catalyst supports, and as functional resins for combinatorial synthesis, drug and vaccine delivery, and photonic crystals.[1]

All such applications require solid supports with specific morphologies and surface properties. Polymer support reagents, for example, need to combine proper functionality with sufficient accessibility to reactants. Good compatibility with and swellability in reaction solvents will also enhance the reactivity of the functional groups. For example, Tentagel, a popular polymeric support reagent, has excellent compatibility with both polar and nonpolar solvents, since these supports are comprised of lightly cross-linked polystyrene grafted with a large amount of poly(ethylene glycol).[2] Chromatography stationary phases have to additionally withstand high column back-pressures and maintain high column flow rates. They may need to be modified to have hydrophilic, hydrophobic, or amphiphilic properties and even contain selective binding sites for affinity chromatography. In some cases such as direct analyses of drugs in serum or plasma, the selective modification of interior and exterior surfaces of stationary phase is needed. Tanaka et al. describe the size-selective modification of porous silica to contain both hydrophilic exteriors and hydrophobic interiors.[3] Another porous polymer-based packing material, the highly cross-linked polystyrene called Styrosorb, was recently employed as a restricted-access stationary phase in sample cleanup for HPLC.[4] In this stationary phase, the outer surface is Tris-modified to become hydrophilic, while the unmodified internal surface remains hydrophobic. When these stationary phases were used in the direct analysis of drugs in the presence of polypeptides, the small drug molecules were retained in the hydrophobic micropores, while the polypeptides were excluded from these micropores and did not bind to the hydrophilic exterior either.[5]

This paper deals with the modification of polymer microspheres by grafting polymers and block copolymers from their surface and interior. It also concerns the properties of polymers and block copolymers tethered on polymer microspheres. The resulting monodispersed polymeric microspheres carrying hydrophilic and hydrophobic grafts may lead to useful chromatography stationary phases and polymeric support reagents. The polymer grafted microspheres may overcome the drawback of other porous polymer-based microspheres such as limited mechanical strength. Highly cross-linked nonporous microspheres prepared by precipitation polymerization can withstand column back-pressures of over 10 000 psi.[6] If such microspheres are modified by grafting a dense layer of long polymer chains off their surfaces, the chromatographic separation efficiency would be improved in comparison to porous or swellable microspheres, and the capacity enhanced in comparison to nongrafted, surface functionalized supports. Therefore, the modified particles should have both high mechanical strength and high separation efficiencies. In addition, block copolymer grafted microspheres with hydrophobic blocks attached near the microsphere surface and hydrophilic blocks extended beyond the surface may perform as stationary phase for direct analyses of drugs in serum.

ATRP has been widely used to prepare homopolymers,[7] block copolymers,[8] highly branched polymers,[9] and dendrimers.[10] Recently, it has been used to modify surfaces including planar surfaces,[11] spherical surfaces,[12] and the internal surface of a capillary.[13] Most of these experiments benefited from the excellent properties of ATRP, including (1) a living/controlled polymerization, (2) its tolerance for water[14] and functional groups,[15] and (3) polymerization occurring at designed sites. Surface modification by ATRP mainly includes two steps: introducing ATRP initiators onto the surface and

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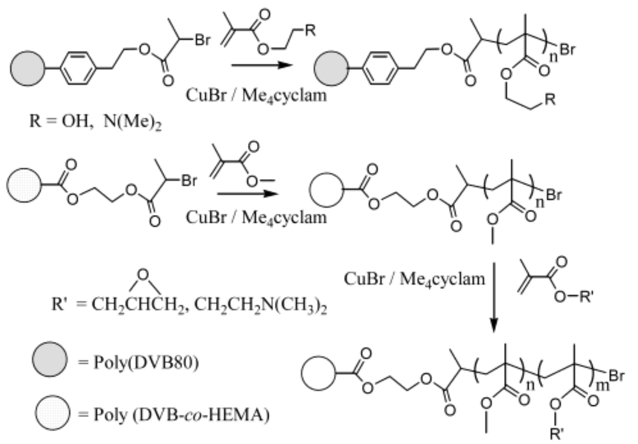
Scheme 1. Surface Modification of Microspheres by ATRP



initiating polymerization from these initiator sites in the presence of an ATRP catalyst. Haddleton's group[16,17] has successfully demonstrated the surface modification of polymer spheres by ATRP. A series of cross-linked polystyrene-based beads including Wang and Merrifield resins as well as primary amino functional resins were converted into ATRP initiators. Subsequently, poly(methyl methacrylate), poly(benzyl methacrylate), and hydrophilic poly(*N*,*N*-dimethylacrylamide) were grafted from those polystyrene-based beads. Patten[18] has reported that 70 nm diameter spherical silica particles were tethered with surface ATRP initiator (2-(4-chloromethylphenyl)ethyl)dimethylethoxysilane, followed by ATRP of styrene at 130 °C in the presence of CuCl/2dNbipy as catalyst. Well-defined polymer–inorganic nanoparticle hybrids formed and the size of the particles increased with conversion, consistent with the molecular weight of the grafted polymer. We recently reported the hydrochlorination of highly cross-linked polystyrene microspheres made from precipitation polymerization to form ATRP initiator particles and the subsequent grafting polymerization of styrene and 4-methylstyrene from their surfaces.[19] The microspheres with hydroxy groups were also used to prepare ATRP initiator microspheres by reaction with α-bromopropionyl bromide.[20] Homopolymers and block copolymers were grafted off these initiator microspheres. This report is a continuation of our efforts at surface modification by ATRP, with emphasis functional grafts based on GMA, TMAEMA and the morphology of the grafted particles (Scheme 1).

Experimental Section

Chemicals. Solvents were purchased from Aldrich and used as received unless otherwise specified. 1,4,8,11-Tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me$_4$cyclam, 98%, Aldrich), 2-bromopropionyl bromide (97%, Aldrich), methyl iodide (99%, Aldrich), sodium borohydride (98%, Aldrich), boron trifluoride diethyl etherate (Aldrich), DVB80 (70–85% divinylbenzene isomers, Fluka, Oakville, Canada), and triethylamine (99%, Aldrich) were used as received. Hydroxyethyl methacrylate (HEMA, 97%), methyl methacrylate (MMA, 99%), glycidyl methacrylate (GMA, 97%), and 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%) were purchased from Aldrich Chemical Co. and purified by distillation under vacuum prior to polymerization. THF (99+%, Aldrich) was refluxed over K–Na alloy and distilled under nitrogen. CuBr was prepared by the reaction of CuBr$_2$ (99%, Aldrich) with dimethyl malonate (99+%, Aldrich).

Syntheses. *Preparation of Hydroxy-Functional Microspheres (Poly(DVB80)-OH).* Microspheres poly(DVB80) were prepared by precipitation polymerization using DVB80 in neat acetonitrile as reported previously.[21] Microspheres poly(DVB80) (5.00 g) were suspended in dry THF (50 mL) in a dry 100 mL round-bottom flask for 2 h, and 0.5 g (13 mmol) of sodium borohydride was added. The suspension was cooled in a cold–water bath, and 1.88 g (13 mmol) of boron trifluoride diethyl etherate dissolved in 5 mL of THF was added dropwise via a syringe to the suspension. The temperature of the reaction suspension was maintained at 20 °C during the addition of BF$_3$ and further for 3 h. At the end of this period, the flask was immersed in an ice bath, and 5 mL of cold water was added slowly to destroy any residual NaBH$_4$.

Hydrogen peroxide (4 mL, 30% in water, 35 mmol) was slowly added to the reaction mixture. During the addition, the pH of the solution was maintained near 8 by adding 3 M sodium hydroxide as needed. The final solution were filtered through a 0.5 μm membrane filter and washed with water (pH = 8) followed by methanol. The resultant microspheres were dried under vacuum at room temperature for 24 h. The yield of the hydroxy microspheres was 5.07 g.

Preparation of Initiator Microspheres (Poly(DVB80-Br)). Hydroxy microspheres, poly(DVB80-OH) (2.00 g), were suspended for 2 h in 20 mL of THF containing 0.4 g (4.0 mmol) of triethylamine in a 50 mL two-necked round-bottom flask, followed by dropwise addition of 0.8 g (3.7 mmol) of 2-bromopropionyl bromide to the suspension, which was cooled with an ice–water bath. The mixture was stirred at room temperature overnight. The initiator particles were filtered and washed with THF and methanol thoroughly. The yield of the resultant particles was 2.04 g after drying at room temperature under vacuum for 24 h.

Conversion of Microspheres Poly(DVB-co-HEMA) (H_1) *into Initiator Microspheres* (H_1*-Br).* Precursor microspheres (H_1) comprised of DVB80 and HEMA (molar ratio of 17.5/82.5) were prepared by precipitation polymerization as reported previously.[22] The precursor microspheres H_1 (2.0 g) were dispersed in 20 mL of dry THF for 2 h in a dry flask, and 0.95 g (9.39 mmol) of triethylamine was added followed by dropwise addition of 2.0 g (9.26 mmol) of 2-bromopropionyl bromide to the mixture. The mixture was stirred at room temperature overnight. The initiator microspheres were filtered and washed with THF and methanol thoroughly. The yield of the resultant particles was 3.08 g after drying at room temperature under vacuum for 24 h.

Grafting Poly(HEMA) or Poly(DMAEMA) from Microspheres Poly(DVB80-Br) by ATRP. Initiator microspheres poly(DVB80-Br) (2.00 g) and HEMA (2.0 g, 20 mmol) were added in 8 mL of THF in a 25 mL round-bottom flask purged with THF saturated nitrogen for 1 h, and then a degassed 5 mL THF solution of CuBr (39 mg, 0.27 mmol)/Me$_4$cyclam (69 mg, 0.27 mmol) was added via a cannula. The mixture was stirred with a magnetic bar at room temperature under nitrogen for 10 h. The resulting microspheres were centrifuged and redispersed at least three times in THF and then repeatedly in methanol. The yield of poly(HEMA) grafted microspheres was 2.26 g.

When monomer DMAEMA was used to grafting poly(DMAEMA) from the same initiator microspheres, all other conditions were kept the same except for using 2.0 g of DMAEMA instead of HEMA. The yield of poly(DMAEMA) grafted microsphere was 2.30 g.

Grafting Poly(MMA) from Microspheres H_1*-Br by ATRP.* Initiator micospheres (H_1-Br, 1.20 g) and methyl methacrylate (8.0 g, 80 mmol) were added in 32 mL of THF in a 100 mL round-bottom flask purged with THF saturated nitrogen for 1 h, and then a degassed 4 mL acetonitrile solution of CuBr (103 mg, 0.72 mmol)/Me$_4$cyclam (184 mg, 0.72 mmol) was added via a cannula. The mixture was stirred with a magnetic bar at room temperature under nitrogen for 15 h. The resulting blue particles were centrifuged and redispersed at least three times in THF and then repeatedly in methanol until the particles became almost white. The yield of poly(MMA) grafted particles was 4.80 g.

Other weight ratios (1:3 and 1:1) of initiator microspheres to MMA were used to prepare microspheres with different grafted poly(MMA) loading, under the same conditions. The yields of these two other grafted microspheres were 3.72 and 2.88 g, respectively. These three different grafted microspheres have poly(MMA) loadings of 0.75, 0.68, and 0.58 g/g microspheres, respectively.

Grafting Poly(DMAEMA) from Microspheres H_1-g-polyMMA-Br by ATRP. The microspheres H_1-*g*-polyMMA-Br (0.50 g, poly(MMA) loading 0.75 g/g microspheres) were suspended in 5 mL of THF containing 2.0 g of DMAEMA and purged by passing THF-saturated nitrogen for 30 min. Predegassed CH_3CN solution (2 mL) containing CuBr (35 mg, 244 μmol)/Me_4-cyclam (62.6 mg, 244 μmol) was added to the suspension through a cannula. The mixture was stirred with a magnetic stir bar at room temperature under nitrogen, and the reaction took place for 10 h. The resulting particles were centrifuged and redispersed with THF at least three times and with NH_4OH/methanol (1/20 by volume) many times until the particles became almost white. The particles were finally washed with ether and dried at room temperature under vacuum for 24 h. The yield of the resultant microspheres was 2.06 g.

Two other poly(MMA) grafted microspheres with poly(MMA) loadings of 0.68 and 0.58 g/g microspheres produced 2.14 and 2.10 g of poly(MMA-*b*-DMAEMA) grafted microspheres, respectively, at the same reaction condition.

Grafting Poly(GMA) from Microspheres H_1-g-polyMMA-Br by ATRP. The microspheres H_1-*g*-polyMMA-Br were prepared by a procedure was identical to that used for DMAEMA grafting, except that 0.50 g of particles with a poly(MMA) loading of 0.68 g/g microspheres was used. As well, the final particles were only centrifuged and resuspended three times using THF, to give 2.40 g of the resultant microspheres, H_1-*g*-poly(MMA-*b*-GMA).

Conversion of Poly(MMA-b-DMAEMA) Grafted Microspheres into Poly(MMA-b-TMAEMA) Grafted Microspheres. Poly(MMA-*b*-DMAEMA) grafted microspheres (1.0 g, described above) were suspended in acetonitrile (10 mL), and 1.0 g of iodomethane was added to the mixture. The mixture was stirred at room temperature for 15 h. The resulting microspheres were centrifuged and redispersed with acetonitrile at least three times. The yield of the microspheres was 1.52 g.

Particle Size Analysis. The particle sizes and size distributions were measured using a 256-channel Coulter Multisizer interfaced with a computer. A 30 μm aperture tube was chosen to accommodate the particles size range of 1−10 μm. A small amount of particles, dispersed in acetone or in water, was added to 25 mL of Coulter Isoton II electrolyte solution and stirred for 1 min with a ministirrer attached to the instrument. The Coulter Multisizer measurements were confirmed using a Philips ElectroScan 2020 environmental scanning electron microscope (ESEM). The samples for ESEM measurement were prepared by dispersing the particles in THF and casting a drop of the particle suspension on a piece of glass glued onto a microscopy stub. The samples were dried under vacuum for 2 h and sputter-coated with 5 nm gold.

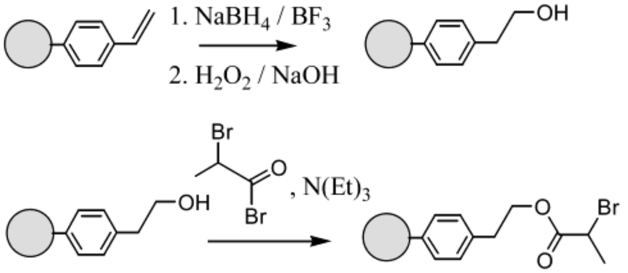
TEM Analysis. The internal structure of the grafted particles was studied using a JEOL 1200EX transmission electron microscope. Here, the samples were embedded in Spurr's epoxy resin and microtomed to generate 40−60 nm thick slices.

FT-IR Analysis. Fourier transform infrared analysis was performed on a Bio-Rad FTS-40 FT-IR spectrometer. All samples were prepared as pellets using spectroscopic grade KBr in a Carver press at 15 000 psi. The spectra were scanned over the range 4000−400 cm^{-1} in the transmission mode.

Results and Discussion

Preparation of Initiator Microspheres PDVB80-Br. Highly cross-linked polystyrene microspheres made by precipitation polymerization contain a considerable amount of residual vinyl groups.[23] Hydroboration/oxidation was used to hydroxylate these vinyl groups at the β-position, followed by esterification with bromopropionyl bromide to produce ATRP initiators (Scheme 2).

Scheme 2. Formation of Initiator Microspheres from Highly Cross-Linked Polystyrene



The starting poly(DVB80) microspheres were very hydrophobic, while the hydroxyl functional microspheres were polar enough to be dispersed in water with little aggregation. Earlier, we reported that hydrochlorination of similar poly(DVB80) microspheres may proceed about 100 nm into the particle.[19] The hydroxy groups in the present microspheres should have a comparable functional group depth profile, since both modifications are based on reaction of residual vinyl groups with fairly small reagents. In the hydroboration/oxidation the reactants are $(BH_3)_2$ formed by in situ reaction of $NaBH_4$ and BF_3 in the first step and H_2O_2 in the second step, respectively. These molecules are a little larger than HCl and hence may penetrate a little less far into the microspheres. The hydroxy loading in the modified microspheres may therefore be less than the 0.9 mmol/g found for hydrochlorination. The sizes of microspheres before and after the hydration were measured and found not to show any change, similar to the case of the hydrochlorinated microspheres.

After incorporating the ATRP initiators, the surface properties changed back to hydrophobic, and the microspheres were not suspendable in water any more. Both hydration and formation of initiators on the microspheres were monitored by FT-IR (Figure 1). Hydration led to an increase of the characteristic hydroxy band at 3454 cm^{-1}.[24] Meanwhile, the intensity of the band at 992 cm^{-1} corresponding to the vinyl group completely disappeared. After introducing the initiator functionality, the characteristic ester carbonyl stretch at 1725 cm^{-1} appeared in the initiator microspheres and the hydroxy band at 3454 cm^{-1} decreased back to about the same intensity as in the starting microspheres. The results indicated the formation of the ester groups and the disappearance of the (accessible) hydoxy groups. In the spectrum of the initiator microspheres, another characteristic band at 1373 cm^{-1} is assigned to the stretching of the methyl groups in the α-position with regard to the bromine.

Grafted Poly(HEMA) and Poly(DMAEMA) on the Microspheres Poly(DVB80). The grafting polymerization of HEMA from the initiator microspheres was catalyzed by Cu(I)Br/Me_4Cyclam at room temperature. Although solution polymerizations catalyzed by this system exhibit poor control over molecular weight,[25] grafting polyMMA from microspheres H_1-Br while using the same catalyst system showed the ability to prepare block copolymer (see below). That result indicated that a significant portion of propagating sites was still active. In the current grafting polymerization of HEMA, the polymerization was carried out for 10 h, and the size of the microspheres increased from 3.04 to 3.14 μm as measured using the Coulter Multisizer.

Figure 2 shows the diameters of the microspheres before and after grafting with poly(HEMA). The diam-

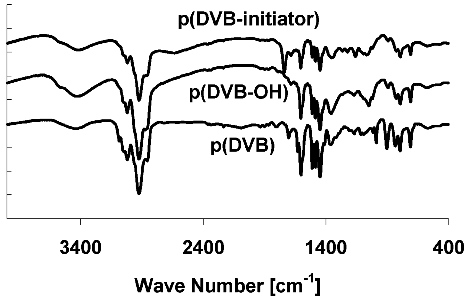


Figure 1. FT-IR spectra of p(DVB), p(DVB-OH), and initiator microspheres p(DVB-initiator).

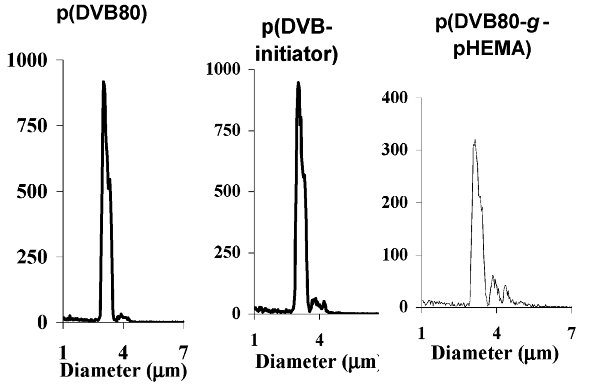


Figure 2. Size and size distribution of poly(divinylbenzene-80) microspheres before and after grafting with HEMA.

eters of the starting and initiator microspheres show no notable difference, while the grafted microspheres have increased in diameter. As well, Figure 2 shows two small peaks on the right-hand side of the major peaks, corresponding to particle doublets and triplets formed by temporary association of particles during the Coulter measurement. The abundance of these doublets and triplets is highest for the grafted particles, which can be attributed to interactions between the poly(HEMA) grafts in the aqueous medium. ESEM confirmed the absence of permanent dimeric or higher aggregates in these samples, when deposited from THF.

The grafted microspheres showed some aggregation in water and only dispersed well in acidic aqueous media. We believe that the grafted poly(HEMA) chains form interparticle bonds that break down under acidic conditions, likely due to slight protonation of the particles.

Grafting of poly(DMAEMA) from the same initiator microspheres was carried out in a similar fashion. The resulting grafted microspheres were easily dispersible in water. The amount of grafted poly(DMAEMA) was measured by potentiometric titration, and an amine loading of 0.81 mmol/g was found, which is consistent with the particle weight increase of 15%.

The FT-IR spectra of the microspheres grafted with poly(HEMA) and poly(DMAEMA) are shown in Figure 3. The microspheres grafted with poly(HEMA) show two bands at 3442 and 1732 cm^{-1}, corresponding to hydroxy and ester groups, respectively. These two signals are increased significantly over the nongrafted precursor (Figure 1). In comparison, grafting the microspheres with poly(DMAEMA) leads to a weaker band at 3400 cm^{-1} and a stronger band at 1740 cm^{-1}, as expected.

Grafting Block Copolymer Poly(MMA-*b*-DMAEMA) off Poly(DBV80-*co*-HEMA) Microspheres. Block copolymers in solution and in matrices can adopt many different morphologies.[26] There have been several reports of block copolymer formation by grafting off polymeric initiators.[27] However, little work on block copolymer formation under confined conditions, such as grafted off solid surfaces, has been reported.[28] One of the most significant features of living radical polymerization is its ability to produce block copolymers. The swellable initiator microspheres, H_1-Br, were previously used to prepare microspheres grafted with block copolymer of poly(MMA-*co*-DMAEMA).[20] Here we graft block copolymer with different block lengths from the initiator microspheres and study the morphology of the tethered block copolymers formed. The length of the polymer blocks could be controlled by varying polymerization times and monomer loadings. Under the same reaction conditions, the weight of the grafted particles increases linearly with both polymerization time and initial monomer loading.

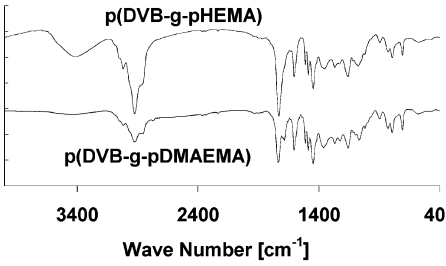


Figure 3. IR spectra of poly(divinylbenzene-80) microspheres grafted with HEMA and DMAEMA.

The microspheres, H_1Br, were grafted with poly(MMA) using ATRP at monomer loadings (monomer/initiator microsphere) of 2.0, 3.0, and 6.7 g/g to produce new initiator microspheres H_1-*g*-poly(MMA)-Br, having weight ratios of grafted poly(MMA) to the original microspheres of 1.4, 2.1, and 3.0 g/g. This corresponds to poly(MMA) loadings of 0.58, 0.68, and 0.75 g/g grafted microspheres. According to the mechanism of ATRP, these polymer-grafted microspheres should have a 2-bromoisobutyrate group at the end of each living polymer chain, which can be used for the second polymerization. The second polymerization of (*N,N*-dimethylamino)ethyl methacrylate from the poly(MMA) grafted microspheres in the presence of CuBr/Me_4-Cyclam generated the second block poly(DMAEMA). Three types of microspheres comprised of different weight ratios of core to first block poly(MMA) to second block poly(DMAEMA) were prepared. The ratios for the three types of microspheres were 1.0:1.4:7.7; 1.0:2.1:10.2; and 1.0:3.0:12.5, respectively. Although the catalyst of CuBr/Me_4Cyclam used in the grafting polymerization has shown a poor control in solution polymerization, it has demonstrated a better control in the preparation of block copolymer from preformed macroinitiator.[29] This implies that the limited diffusion of propagating radicals attached to a preformed polymer or to a surface may reduce the radical combination rate compared to solution conditions and could produce well-defined polymers and block copolymers. This is supported here by the observed linear particle weight increase with polymerization time and the ability to generate block copolymer. Therefore, the weight gain ratios in each type of microsphere should reflect the length ratios of the two blocks. This will be tested in future work using cleavable grafts.

Each of the block copolymer grafted microspheres shows a significant size increase from the starting

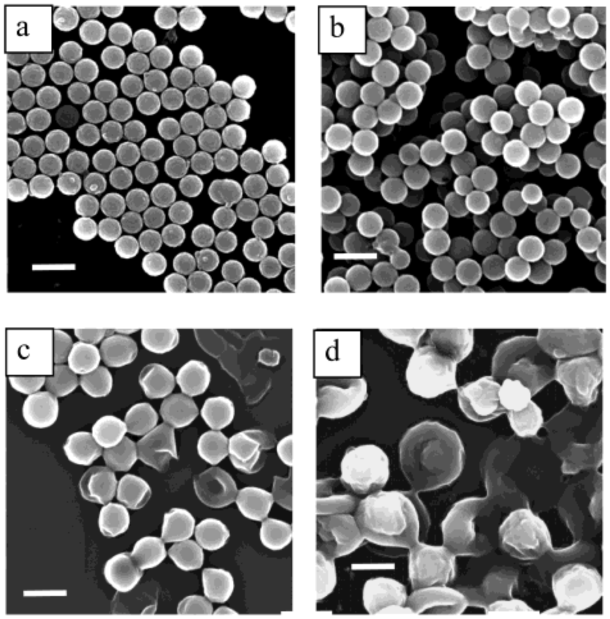
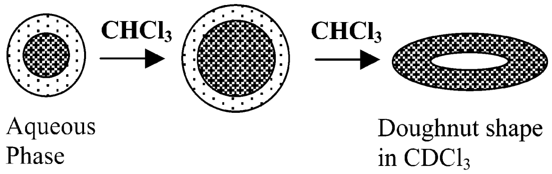


Figure 4. ESEM images of grafted microspheres with different grafted block lengths. The weight ratio of the first (PMMA) block to the second (PDMAEMA) block: (a) 1:0, (b) 1.4:7.7, (c) 2.1:10.2, and (d) 3.0:12.5. The scale bar is 5 μm.

Scheme 3. Morphologies of Amphiphilic Block Copolymer in Different Solvents



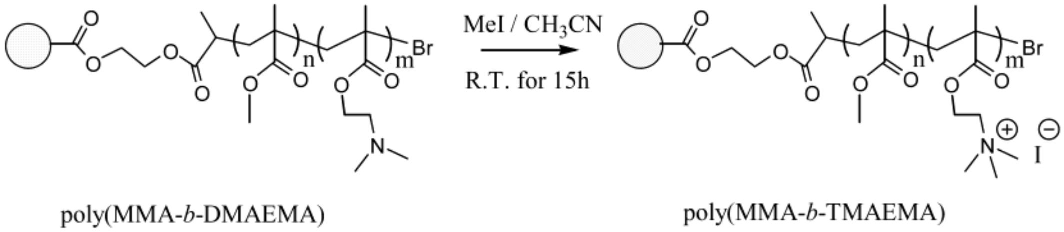
microspheres. There are differences in diameter and morphologies between these three microspheres (Figure 4). Interestingly, the microspheres with different lengths of block copolymer show much different morphologies. The microspheres (Figure 4a,b) without and with the shortest first block poly(MMA) show spherical morphology. With the length of the two blocks increasing, some of the microspheres changed their shape from spherical to doughnut shape. The population of the microspheres with this changed morphology also increased with the overall graft length (Figure 4c,d). A tentative explanation of this phenomenon is that the grafted amphiphilic block copolymers underwent phase separation, especially at higher graft length, and that the two phases reversed between external layer and internal layer. Chen[30] has reported that amphiphilic poly(St-*co*-HEMA) latex changes its morphology upon the addition of styrene or toluene as shown in Scheme 3. Initially, the hydrophilic poly(HEMA) faces outside and the hydrophobic polystyrene stays inside the latex. When a nonpolar solvent such as styrene or toluene is added in the form of vapor, the polystyrene core swells until finally the morphology changes when the solvency of the environment becomes favorable to polystyrene. The block copolymer grafted microspheres may show analogous phase separations, during both reaction and workup in differentiating solvents, except for the restriction that the cross-linked core places on their rearrangement. Changing the solvent used to deposit the microspheres onto ESEM stubs did not change the populations of the two morphologies significantly, suggesting that the structure of the present particles may be more strongly affected by the grafting rather than by posttreatment with differentiating solvents.

Formation of Poly(MMA-*b*-TMAEMA) Grafted off the Microspheres Poly(DBV80-*co*-HEMA). The grafted block copolymer, poly(MMA-*b*-DMAEMA), was further modified by methylation with methyl iodide to form a ionic block copolymer, poly(MMA-*b*-TMAEMA) (Scheme 4). The methylation of the microspheres lead to charged microspheres that are much more polar and even easier to disperse in water. The degree of methylation was measured by potentiometric titration of the remaining tertiary amine and found to be quantitative. This result also confirmed that the tertiary amines of poly(DMAEMA) grafted onto these swellable microspheres are easily accessible. Both DMAEMA and TMAEMA grafted microspheres are polyelectrolytes. The tertiary amines in poly(DMAEMA) are strong bases, which can be protonated by water to form a swellable polycationic particle. An interesting experiment of size measurement by a Coulter Multisizer has shown that the sizes of the microspheres grafted with poly(MMA-*b*-DMAEMA) and poly(MMA-*b*-TMAEMA) were much smaller than expected. According to the principle of the Coulter Multisizer, the size of polymer microspheres is measured by determining the electric resistance between two electrodes placed at each side of an aperture. The size (volume) of a microsphere correlates to the increase of electric resistance due to the volume of electrolyte solution that is replaced by a microsphere passing through the aperture. If microspheres are swollen in the aqueous isotonic solution used in the Coulter Multisizer, the increase of the electric resistance by microsphere passage will be smaller than that of normal microspheres. For microspheres modified with polyelectrolyte, the apparent size of the microsphere may hence be smaller than that of the microspheres without modification.

Figure 5 shows the size and size distribution of microspheres with and without the polyelectrolyte modification as measured by the Coulter Multisizer. The apparent microsphere sizes as measured by the Coulter Multisizer in Figure 5 are 2.69, 3.80, and <1.0 μm for H_1-*g*-poly(MMA), H_1-*g*-poly(MMA)-*b*-poly(DMAEMA), and H_1-*g*-poly(MMA)-*b*-poly(TMAEMA), respectively. In fact, H_1-*g*-poly(MMA)-*b*-poly(DMAEMA) and H_1-*g*-poly(MMA)-*b*-poly(TMAEMA) should be much larger than H_1-*g*-poly(MMA) since a large amount of second block polymer has been added.

This is confirmed by the ESEM images shown in Figure 6. The size order of these microspheres is approximately H_1-*g*-poly(MMA)-*b*-poly(TMAEMA) $\cong$ H_1-*g*-poly(MMA)-*b*-poly(DMAEMA) $\gg$ H_1-*g*-poly(MMA). The morphologies of these microspheres show significant differences as well. H_1-*g*-poly(MMA) microspheres were spherical as expected (Figure 6A). The H_1-*g*-poly(MMA)-*b*-poly(DMAEMA) microspheres showed morphologies affected by phase separation of the block copolymer as mentioned above (Figure 6B). The microspheres H_1-*g*-poly(MMA)-*b*-poly(TMAEMA) were found to have broken shells and oval shapes (Figure 6C). Some small microspheres, about 1.2−1.4 μm in diameter as measured in the image, in the center of the image should be original cores separated from their shells. All methacrylate grafted microspheres proved sensitive to e-beam radiation damage during longer-term or high-resolution SEM study, likely due to depolymerization.[31]

Scheme 4. Methylation of Tertiary Amine To Form Quaternary Ammonium Cations in the Block Copolymer Grafted Microspheres



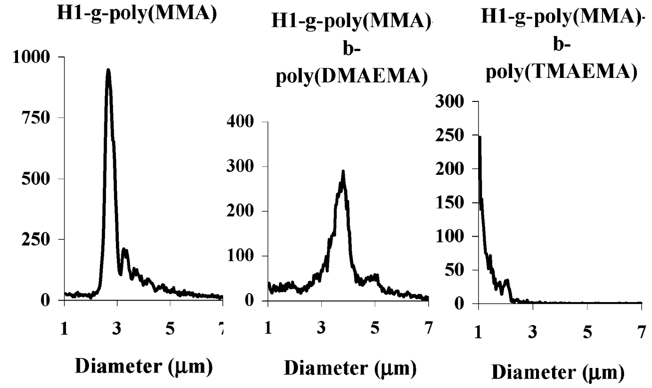


Figure 5. Size and size distribution of the microspheres grafted with and without polyelectrolyte.

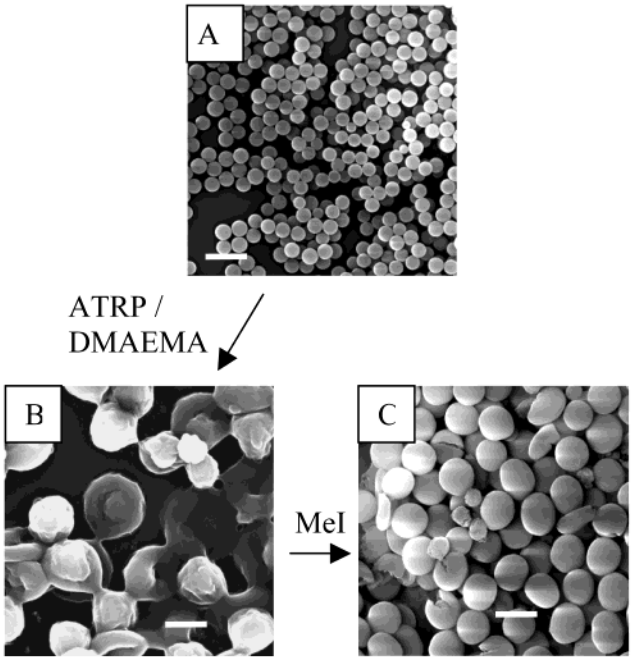


Figure 6. ESEM images of the polymer grafted microspheres. The scale bar is 5 μm.

Grafted Block Copolymer Poly(MMA-*b*-GMA) on the Microspheres Poly(DBV80-*co*-HEMA). Poly(glycidyl methacrylate), poly(GMA), as a second block polymer was grafted from the microspheres grafted with a first block of poly(MMA), using a similar procedure as described above. The grafting polymerization of GMA was found to be very effective, and the conversion of monomer was much higher than that of other monomers. The initiator microspheres, H_1-*g*-poly(MMA)-Br (0.5 g, poly(MMA) loading 0.68 g/g microspheres), were reacted with 2.0 g of GMA in the presence of CuBr/Me_4-Cyclam for 15 h at room temperature, resulting in 2.4 g of the grafted microspheres, H_1-*g*-poly(MMA-*b*-GMA).

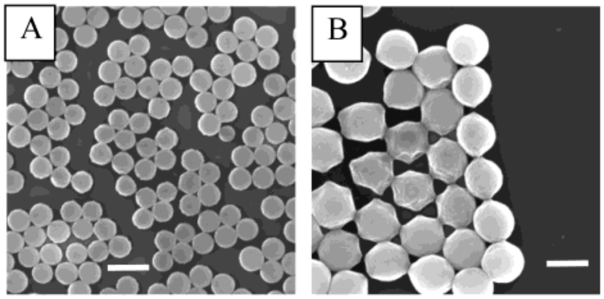


Figure 7. Damaged microspheres of H_1-*g*-poly(MMA)-*b*-poly(TMAEMA). The scale bar is 5 μm.

Parts A and B of Figure 7 show the ESEM images of H_1-*g*-poly(MMA)-Br and the poly(MMA-*b*-GMA) grafted microspheres, respectively. The block copolymer, poly(MMA-*b*-GMA), grafted microspheres were large and had relatively smooth surfaces (Figure 7B). The microspheres in the center of the image had been subjected to higher e-beam intensities from high-resolution observation. Some of these microspheres show intact but shrunken surfaces. This is attributed to cross-linking of the GMA layer and decomposition of the poly(MMA) blocks due to the electron beam.

The microspheres were reacted with butylamine without catalyst at room temperature for 20 h to determine the reactivity of the glycidyl groups on the microspheres. No weight gain was observed after recovery. In a parallel experiment, the microspheres were reacted with 10% sulfuric acid under the same conditions, and a 7.0% weight gain was observed, corresponding to a 70% acid-catalyzed ring-opening of the glycidyl groups. Investigation into the reactivity of the grafted poly(GMA) will be done in the future.

The internal structure of these grafted microspheres was studied by TEM (Figure 8). The small dark core visible in on particle represents the poly(DVB-*co*-HEMA) starting microsphere, and the larger outer rings are block copolymer poly(MMA-*b*-GMA). These large rings were apparently swollen by the Spurr's epoxy resin used for embedding. The apparent presence of epoxy in the center of the particles is likely due to their donut shape. The surface of the microspheres (outer perimeter of the large ring) appears denser than the interior of the large ring, perhaps due to a surface reaction with the epoxy resin. This question is being pursued using scanning transmission X-ray microspectroscopy (STXM).

Conclusion

Grafting polymer and block copolymer by ATRP from cross-linked polymeric microspheres was successfully demonstrated. Residual vinyl groups on hard poly(DVB80) microspheres were converted into ATRP initiators by hydroboration/oxidation reaction followed by

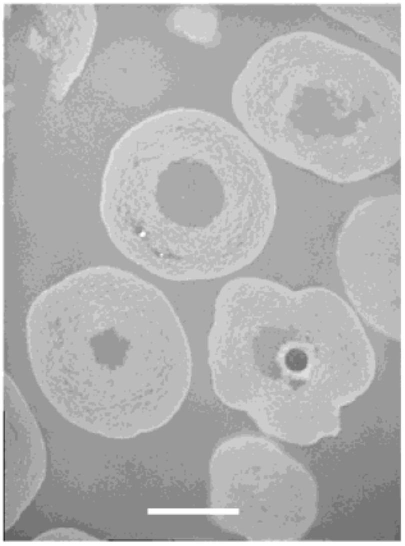


Figure 8. TEM image of the section of the poly(MMA-*b*-GMA) grafted microspheres. The scale bar is 5 μm.

esterification. The surface modification on the hard poly(DVB80) microspheres by grafting poly(HEMA) and poly(DMAEMA) via ATRP has generated hydrophilic microspheres, which may be useful as chromatographic stationary phases. Swellable lightly cross-linked poly(DVB-*co*-HEMA) microspheres were modified with various block copolymers, including poly(MMA-*b*-DMAEMA) with different block lengths as well as poly(MMA-*b*-TMAEMA) and poly(MMA-*b*-GMA). The phase separation of the amphiphilic block copolymer occurring on the microspheres was studied using scanning electron microscopy.

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(23) The bromination test by the addition of bromine to microspheres suspension in THF showed the considerable amount of bromine consumed. Nevertheless, the amount of surface vinyl groups could not to determines since bromine was continuously consumed by diffusion in the microspheres. A control test showed the microspheres made from suspension polymerization has much less residual vinyl groups.

(24) The hydroxy peak at 3454 cm^{-1} found in the starting microspheres may be attributed to the oxidation of some vinyl groups during the formation of the microspheres. Water was carefully kept away from the starting microspheres by using anhydrous solvents for preparation and workup, and the isolated microspheres were dried under vacuum for 24 h. The oxidation of the vinyl group was not caused by storage since a fresh sample also showed the hydroxy peak.

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