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Poly(styrene) Latex Carrying Cerium(IV)-Initiated Terminally Attached Cleavable Chains: Analysis of Grafted Chains and Model of the Surface Layer

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ABSTRACT: Ce(IV)-initiated grafting of N(2-methoxyethyl-acrylamide) (MEA) to a shell of poly(styrene-*co*-2-hydroxyethyl acrylate) on polystyrene latex has been performed and the molecular weight distribution of the graft determined by size exclusion chromatography (SEC) on grafted chains cleaved from the surface. Cationic poly(styrene) beads were covered with a shell containing poly(styrene-*co*-2-hydroxyethyl acrylate) utilizing an azo initiator. The density of the surface hydroxyl groups was assessed by saponification of the ester bonds followed by conductometric titration of the resulting carboxylic residues. Ce(IV) redox initiation of MEA from the surface hydroxyl groups produced chains terminally attached to the latex surface. MEA consumption kinetics were monitored by HPLC of the supernatant on a reverse phase column. Competing solution polymerization of MEA occurred, probably due to leakage of shell copolymer from the beads. The terminally attached chains were characterized by quantitative SEC following cleavage from the surface by saponification, allowing a model of the tethered surface to be developed.

Introduction

Cerium(IV) salts have been extensively used to initiate graft copolymerization of vinyl monomers on various natural and synthetic polymers.[1−18] One of the applications of this technique is to cover a given support with covalently bound neutral polymer chains in order to permanently reduce interactions with material in solution such as proteins[19,20] or calcium.[21] It seems that no attempts have been yet made to use Ce(IV)-initiated polymerization for modifying the surface of synthetic nonporous polymers in order to improve their biocompatibility. As part of an effort to produce such materials, a core−shell polystyrene latex bearing aldehyde groups has been previously used in our laboratory for grafting an acrylamide derivative.[22] While the grafted chains proved efficient in decreasing nonspecific adsorption of proteins, their size and density on the surface could not be assessed.

Recently a thorough study of polystyrene grafting from modified silica gels carrying covalently attached AIBN-like initiators activated in concentrated styrene/toluene solutions has been described.[23] The structure carrying the initiator was cleavable, allowing the molecular weight and surface concentration of the grafted chains to be determined. It was found that surface chain separations of less than the radius of gyration of the equivalent free chain were obtained and that the released chains exhibited a unimodal molecular weight distribution.

We describe here a method to synthesize a model latex carrying water soluble cleavable terminally attached chains. Our aim is to characterize the grafted chains after separating them from the solid support and to use the results both for optimizing the grafting conditions and for understanding the properties of the surface.

Cationic poly(styrene) latices were first covered with a shell containing poly(styrene-*co*-2-hydroxyethyl acrylate) by a seed copolymerization procedure using an azo initiator. In the second step, grafted chains anchored to the surface were produced by polymerization of *N*-(2-methoxyethyl)acrylamide (MEA), in the presence of Ce(IV) as a redox initiator; the reactions are shown in Figure 1. The parameters of the grafting reaction were varied: surface concentration of hydroxyl starting groups and Ce(IV) and MEA concentration. Analysis of the resulting surface properties provided a model for the grafted layer.

Materials and Methods

1. Materials. Surfactant-free cationic poly(styrene) latex used as the starting material for this study was synthesized in our laboratory according to the procedure described briefly elsewhere.[24] The method involved production of monosized particles in the first step, followed by three seed polymerization steps. In all four stages, the radical polymerization of styrene was initiated using 2,2′-azobis (2-amidinopropane) dihydrochloride. The latex surface charge density (measured by conductometric titration[24]) and size determined by electron microscopy[24] are shown in Table 1.

Once distilled water used was further purified using a Milli-Q Plus water purification system (Millipore Corp., Bedford, MA). Styrene was Aldrich reagent grade material which was purified by vacuum distillation at 40 °C in an argon atmosphere. 2-Hydroxyethyl acrylate (HEA) was Aldrich reagent grade. The first batch of this monomer (used for synthesis of latex H1, see Discussion) was purified by simple vacuum distillation at 90 °C in an argon atmosphere. The second batch of monomer was purified by vacuum fractionation using a 10 cm Vigreux column. The main fraction was collected at 77 °C (vapor temperature). Both styrene and HEA were cooled upon collection during distillation using a dry ice-methanol bath. The purified monomers were stored at −70 °C under argon until future use. 2,2′-Azobis(2-amidinopropane)

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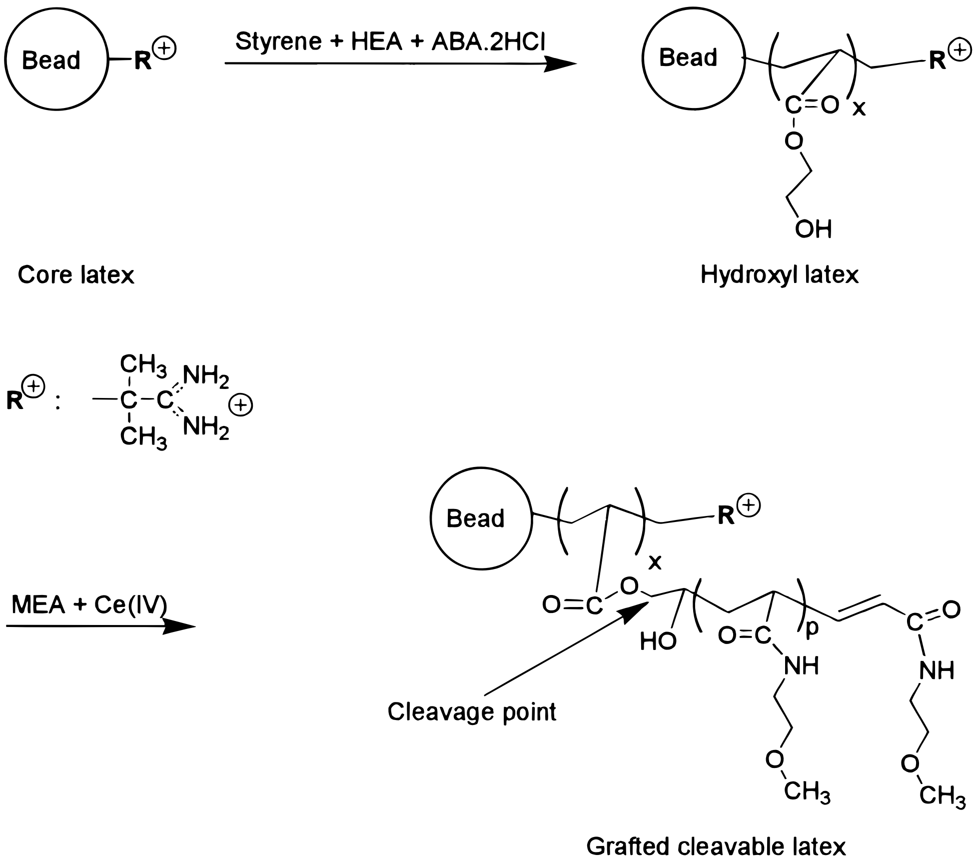

Figure 1. Reaction scheme for the synthesis of poly(syrene) latex carrying cleavable grafted chains.

Table 1. Properties of Seed Latices (Radius and Charge Density)

batch no.	r,[a] cm ± s.d.	area/charge[b] Å^2/group
11	$3.7 \times 10^{-5} \pm 3.7\%$; $n = 160$	250.2
14G1233	$1.35 \times 10^{-4} \pm 31\%$; $n = 135$	153.9
14G1223	$1.38 \times 10^{-4} \pm 32\%$; $n = 194$	181.9

[a] Mean radius of the beads, from size distribution histogram (image analysis performed on scanning electron micrographs[24]). [b] Area corresponding to each initiator residue on the surface (conductometric titration).

dihydrochloride (ABA·2HCl) was supplied by Wako Pure Chemical Industries Ltd. (Osaka, Japan) and was used without further purification. Cerium(IV) ammonium nitrate was Aldrich reagent grade. The 2-methoxyethyl acrylamide (MEA) was kindly supplied by Merck (Darmstadt, Germany) and was used without further purification.

2. Methods

2.1. Synthetic Methods. 2.1.1. Copolymer Shell Preparation. The apparatus consisted of a three-necked flask (250 or 500 dm^3 capacity), equipped with overhead stirrer, condenser and argon inlet with stopcock (argon outlet was through the top of condenser). Argon flow was controlled by a needle valve and stirring rate by a tachometer. The flask was maintained at a constant temperature by immersion to the neck in a thermostated bath. Surfactant-free poly(styrene) latex was used as seed. An equimolar mixture of styrene and HEA was used to produce a shell around the poly(styrene) core. Detailed recipes are given in Table 2. All the concentrations are reported with respect to the total volume of the reaction mixture.

Typically seed latex was charged in the flask, which was then evacuated and flushed with argon eight times. The temperature was raised to 50 °C, under gentle argon flow (one bubble/s) and stirring at 350 rpm. Styrene was added and the seed allowed to swell for 15 min and then HEA monomer dissolved in 10 mL of water was introduced, followed after 5 min by initiator solution in 10 mL of water. The reaction was continued at 50 °C, under argon flow and with stirring at 350 rpm, for 5 h. The reaction mixture was then cooled to room temperature and filtered through glass wool to remove large aggregates. The product was dialyzed against distilled water for one week in a 10 L tank changing the water every day. Further cleaning was done by centrifugation and supernatant replacement (three times). The latex suspension was then weighed and the solid content determined by freeze-drying. All latex suspensions, after cleaning, were stored at 4 °C in polypropylene tubes until future use.

2.1.2. Ce(IV)-Initiated MEA Polymerization. The apparatus consisted of a three-necked cylindrical flask (50 mL) equipped with magnetic stirrer, argon inlet and outlet with stopcocks, and rubber stopper. Detailed recipes are given in Table 3; all concentrations were calculated with respect to the total volume of the reaction mixture.

Typically a latex suspension and MEA monomer were introduced to the reactor, which was then degassed and flushed with argon twice. The appropriate amount of cerium(IV) ammonium nitrate dissolved in 10 mM nitric acid solution (the volume was adjusted to give a final concentration of 1.82 mM nitric acid in the reaction mixture) was added through the rubber stopper from a syringe, previously filled with argon. The contents of the flask were mixed by hand, then stirred for 1 h at 40 °C. The reaction was continued for a given time at room temperature, under stirring and gentle argon flow. At

Table 2. Recipes for Copolymerization of Styrene/HEA on the Seed Latex

batch no.	seed latex	solid concn, %	10^3[HEA], mol/g of latex	10^3[styrene], mol/g of latex	10^4[ABA·2HCl], mol/L
H1	11	2.97	2	2	9.22
H2	14G1233	3.33	2	2	9.22
H3	14G1223	3.33	1.5	1.5	9.22

Table 3. Recipes for Ce(IV)-Initiated MEA Grafting Experiments

batch no.	[Ce(IV)], mol/L	10^3[OH] mol/L	[MEA], mol/L	reaction time, h	solid content, %
H2GR1	5.14×10^{-2}	6.68	6.93×10^{-1}	68	24.36
H2GR2	1.03×10^{-2}	6.68	6.93×10^{-1}	41	24.36
H3GR1	3.07×10^{-3}	3.07	1.1	18	18.18
H3GR2	3.07×10^{-3}	3.07	6.48×10^{-1}	18	18.18
H3GR3	1×10^{-2}	3.07	6.48×10^{-1}	18	18.18
H3GR4	4.61×10^{-3}	3.07	6.48×10^{-1}	18	18.18
H3GR5	1×10^{-2}	3.07	1.30	18	18.18

the end of the reaction, a sample of the latex suspension was removed for monomer content analysis. The rest of the product was suspended in 250 mL water and cleaned either by filtration (Millipore membrane, 1.25 μm) or by centrifugation. It was washed with 25 mL of 0.1 M sodium sulfite solution in 0.1 M acetic acid (freshly prepared) and then with 50 mL of 0.03 M ethylenediaminetetraacetic acid trisodium salt hydrate (EDTA) and rewashed with water. The latex was resuspended in 25 mL of water and stored in polypropylene tubes at 4 °C.

2.2. Analysis Methods. 2.2.1. Acrylic Acid Content in Distilled HEA. One of the impurities present in commercial 2-hydroxyethyl acrylate monomer is acrylic acid. Because it was found (see Discussion of batch #H1) that inadequate purification of the monomer can alter the properties of the product, a method of analysis was developed to assess the acrylic acid content of the purified monomer. The experimental procedure was as follows: 0.1−0.2 g of purified HEA was weighed and diluted to 10 mL with water. The solution was purged with argon for 5 min and then placed in a stirred conductometric cell. The titration with sodium hydroxide solution proceeded under argon flow.

2.2.2. Analysis of Monomer (HEA or MEA) Consumption during Reaction. The amount of unreacted monomer was determined by HPLC analysis (performed on a Merck-Hitachi, Inert Version system, E. Merck, Darmstadt, Germany; Monitor L4200, Merck-Hitachi) of the supernatant separated from the reaction mixture by centrifugation and filtration. A 50 × 10 mm Superformance column (E. Merck, Darmstadt, Germany) packed with Merck Licrospher 60 RP-Select B beads (Merck, Darmstadt, Germany) was used for this analysis (3 mL/min; mobile phases—A was 0.1% TFA in water and B was 50% by volume acetonitrile in A; monitor at 240 nm; 20 μL sample). The sample was run on the column using a 50 min gradient program (100% A for 5 min; gradient B from 0 to 100% for 35 min.; 100% B for 5 min.; 100% A for 10 min.). Calibration runs were performed using known amounts of monomer dissolved in solvent A. The conversion was calculated with respect to the initial concentration.

2.2.3. Hydroxyl Group Content of the Copolymer Shell. The technique involved saponification of the ester bonds in HEA residues, followed by conversion of sodium carboxylate groups left on the latex into carboxylic acid upon acidification. The chemical reactions are shown in Figure 2. Conductometric titration was then used to asses the concentration of the acid groups.

A series of experiments was done on latex #H1 in order to determine the minimum concentration of sodium hydroxide required for complete cleavage of the ester bonds; Table 4 contains the experimental conditions used. Batches #H2 and #H3 were cleaved under exposure to 0.67 M sodium hydroxide. The treatment of the latex after saponification was the same in the analysis of all three batches. The detailed procedure is as follows.

A 2 M sodium hydroxide solution was prepared. A sample of latex suspension of known solid content was weighed in a glass vial (approximately 2 g of suspension containing about 0.2 g of solid particles), and then 1 mL of 2.0 M sodium hydroxide solution was added. The mixture was left to react overnight tumbling in a rotating rack at room temperature. The latex was then washed four to six times in the centrifuge with water until the pH of the supernatant was neutral. The pellet was then resuspended in 2 mL of 0.1 M hydrochloric acid and washed again two to four times with water to neutral pH. After the final wash, the volume was adjusted to 10 mL with water. The suspension was purged with argon for 5 min and transferred to the conductometric cell. The titration proceeded as described in section 2.2.1.

2.2.4. Cleavage of Grafted Chains. The chemistry for cleavage of the grafted chains from the latices was the same as described in section 2.2.3. It was performed on approximately 10 g of latex suspension of known solid content (about 1 g of solids). An exact amount of 2.0 M sodium hydroxide solution was added to give 0.67 M final concentration in the mixture. Under these conditions the mono-N-substituted propionamide side chain is stable to hydrolysis.[25] After the cleavage reaction was completed (36 h; completion was controlled by titration of the beads and comparing the result with that given by the bare beads), the latex was treated exactly as described in the previous section. The supernatant and the first wash were mixed, neutralized with hydrochloric acid and concentrated by evaporation (40 °C, Rotovap). The concentrated solution containing the released grafted chains was then weighed and analyzed by size exclusion chromatography.

2.2.5. Size Exclusion Chromatography of Grafted Chains. A 300 × 10 mm column packed with BIO-SEC EMD tentacle type gel (Merck, Darmstadt) was used to analyze the grafted polymers. Aliquots of 200 μL of the concentrated solutions obtained by cleaving the grafts from latex were run on the column in 0.2 M sodium chloride at 0.5 mL/min, monitoring at 220 nm. The characteristics of the column were as follows: void volume (V_0) 7.18 mL (determined with DNA) and pore volume $V_p = 13.05$ mL ($V_p = V_{NaNO_3} - V_0$), where V_{NaNO_3} was the volume at which 0.02 M $NaNO_3$ eluted. To convert the partition coefficients of the grafted polymer to molecular weights, a set of commercial poly(acrylamide) standards (Polysciences, Inc.) was used. The

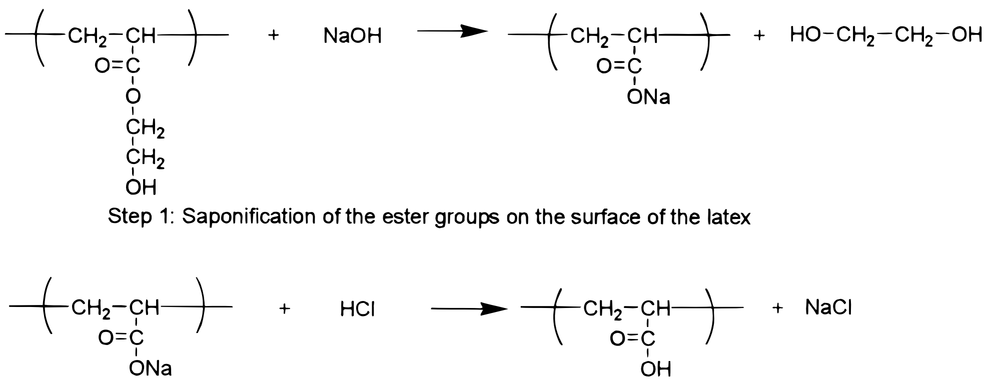

Figure 2. Analysis of hydroxyl groups in the copolymer shell.

Table 4. Saponification Conditions for Latex H1

sample no.	latex suspension,[a] g	solid latex, g	[NaOH],[b] mol/L	reaction time, h
1	1.9798	0.2435	0.083	18
2	2.2135	0.2734	0.167	18
3	2.1780	0.2690	0.333	18
4	2.0634	0.2515	0.67	18
5	1.9631	0.2424	1	72

[a] To this amount was added sodium hydroxide solution to give a final volume during saponification of 3 mL for all samples. [b] Concentration reported in the final volume during saponification (3 mL).

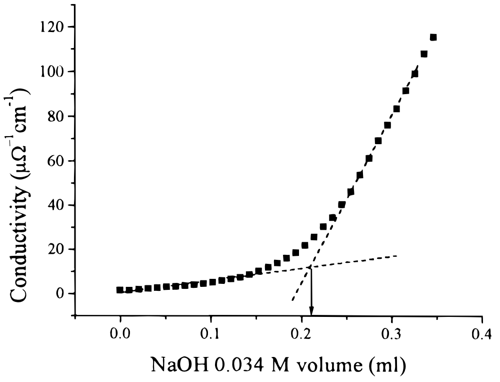

Figure 3. Titration curve of cleaved latex.

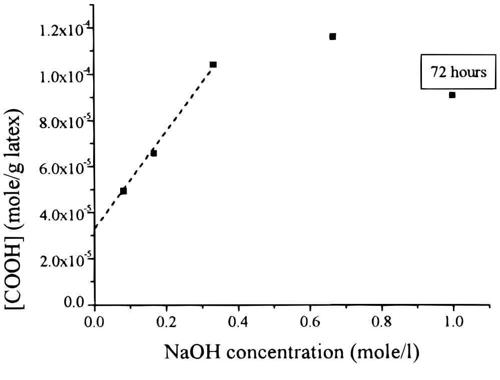

Figure 4. Carboxylic acid content found on cleaved latex as a function of sodium hydroxide concentration during saponification of the ester bond (optimization of the cleavage method done on latex #H1).

calibration equation obtained as described above, was

$$\log(M_n) = 5.072 + 2.246K_D \qquad (1)$$

where M_n= number average molecular weight; K_D= partition coefficient calculated from the elution volume, V_e, of the sample, so $K_D = (V_e - V_0)/V_p$.

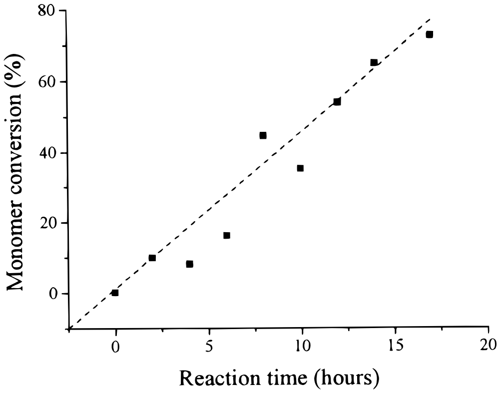

Figure 5. MEA conversion during grafting.

Equation 1 contains the correction factor of 129/71 to account for the ratio between the molecular weight of MEA and acrylamide. It can be used to obtain a good approximation of the average numerical molecular weight of the grafts assuming that the methoxyethyl side chains do not change the mean end-to-end distance of the poly(MEA) molecules in the column compared to poly(acrylamide). For freely coiling solutes of similar architecture, this assumption is reasonable.[26]

To produce a quantitative estimate of the terminally attached chain concentration a calibration of the optical density of the polymer was performed as follows.

The grafted polymer solution resulting from cleavage of latex H3GR4, which was made in a large batch for this purpose, was purified to remove low molecular weight components (salt and monomer) using a 600 × 26 mm Sephadex G25 (Pharmacia, Uppsala, Sweden) size exclusion gel column run at 3 mL/min in water, monitored at 220 nm, with a 1 mL sample. The first fraction, which eluted around 12 min, contained high molecular weight components (this was checked by running the eluate, after concentration by evaporation, on the BIO-SEC column and comparing the profile with that run before purification). The eluates from three runs on the Sephadex column were combined, concentrated approximately 5-fold by evaporation, and a portion weighed (1.9578 g) and freeze-dried to determine the solid content (4.8×10^{-3}g), which corresponded to 2.45×10^{-3} g/mL in the pooled eluate. A 200 μL aliquot of this solution containing 4.9×10^{-4} g of polymer was injected twice in the BIO-SEC column and, monitored at 220 nm, gave an average area of 29.7×10^6 μV s (28.29 and 31.10) in the polymer region, at a flow rate of 0.5 mL/min. The effect of potential terminal double bonds, formed by cerium termination or by disproportionation,[27] on the optical density at 220 nm was checked by investigating a high molecular weight poly(MEA) sample synthesized by hydrogen peroxyde initiation. A solution of this polymer was prepared (containing 1.47×10^{-3} g/mL). A 200 μL aliquot of this solution, when injected in the BIO-SEC column, gave an average area of 14.66×10^6 μV s (14.12 and 15.20 μV s in two trials) at a flow rate of 0.5 mL/min. Because of the high molecular weight of the sample (its elution volume was 7.23 mL, almost equal to the void volume of the column), the effect of any terminal double bond chromophore on the optical density of the solution would be minimal in this measurement. Compared to the calibration done using the polymer cleaved from batch H3GR4, the area of the polymer peak per gram was 20% lower. It is concluded that the method of calibration described above can be used to estimate the amount of polymer cleaved from the beads to within an uncertainty of at most 20%.

The areas of the polymer peaks were converted to absolute mass of grafted material with the formula

$$GW = (4.9 \times 10^{-4}\ \mathrm{PA} \times S)/(0.2w),\ \text{g of grafted polymer/g of latex} \quad (2)$$

where PA = total area of the polymer peaks, μV s; S = amount of grafted polymer solution obtained after cleavage and evaporation, g; and w = the amount of solid latex exposed to cleavage, g. It was assumed that the density of the grafted polymer solution was 1 g/cm^3.

2.2.6. Electrophoretic Mobility Measurements. The electrophoretic mobilities of latex particles were determined in a Rank Mark I apparatus with a cylindrical chamber at 25 °C, in 10 mM sodium chloride (pH = 7). All measurements were made with 40 V applied and an electric field strength of approximately 3.6 V/cm. The electrical length and field strength were calculated from the currents measured with KCl solutions in the chamber.[28] The reported results are based on a minimum of two separate mobility determinations, each of which consisted of measuring the velocity of 10 particles, alternating the direction of the applied field after each timing. The mobility was calculated from the averaged velocities, the applied voltage and the chamber electrical length, from

$$u = vl_E/V \quad (3)$$

where u = elecrophoretic mobility, m^2/V s; v = averaged velocity, m/s; l_E= the electrical length of the chamber, m; and V= applied voltage.

Results and Discussion

1. Copolymer Shell Preparation and Characterization. The goal of the first step in the latex surface modification was to introduce a useful concentration of hydroxyl groups—ideally uniformly distributed and solidly anchored to the bead—in order to provide efficient initiation of grafting in the second step. This had to be done without reducing the charge and stability of the beads. To be easily analyzed before and after grafting, the HEA residues in the shell had to be entirely accessible from the water phase, which meant that a very small amount was needed, ideally covering less than a monolayer on the surface of the latex.

Conductometric titration is considered[29] to be a suitable method for analyzing latices containing carboxylic groups on the surface. In Figure 3, a titration curve of saponified core/shell latex is shown. To relate the result of the titration quantitatively to the HEA content of the copolymer shell, the cleavage conditions had to be optimized in order to ensure that all the ester bonds had been hydrolyzed. Batch #H1 was used for this purpose, in the set of experiments performed under the conditions in Table 4. In Figure 4, the carboxylic acid content of the cleaved latex is plotted as a function of the concentration of the sodium hydroxide used for saponification. The number of the cleaved groups increased proportionally with the base concentration until saturation occurred; that is, all groups were cleaved. The last point on the graph would be expected to be higher, but the time for saponification was longer than for all the other points, which might have caused secondary reactions to occur. We also encountered an experimental difficulty in the titration of the sample because the latex aggregated upon the addition of the base and remained that way during the titration. Hence, the number of carboxylic groups could have been underestimated.

Table 5. Electrophoretic Mobility of Latex before and after the Copolymerization Step

synthesis stage		mobility × 10^8,[a] m^2/v-s
seed latex:	#11	+3.82
	#14G1233	+4.52
	#14G1223	+5.21
latex covered with copolymer shell:	#H1	−1.19
	#H2	+0.55
	#H3	+0.49
cleaved latex:	#H1CL	−2.87
	#H2CL	−3.83
	#H3CL	−4.29

[a] Measured in 10 mM sodium chloride, pH = 7.

The intercept of the straight line defined at low concentrations in Figure 4 is 3×10^{-5} mol/g of latex, which means that the beads contained carboxylic groups or other weak acid groups before cleavage. This result is consistent with the eletrophoretic mobility, shown in Table 5. The bare latex (seed #11) was positively charged, it became negatively charged upon shell preparation (H1), and it had a higher negative charge (higher negative mobility) after cleavage due to the new COOH groups created by saponification). The net negative surface charge observed on latex H1 after addition of the copolymer shell, despite the cationic initiator used with the already positively charged beads, was due to an acrylic acid impurity present in the HEA monomer. The analysis sheet accompanying the monomer indicated that it contained 1% w/w acrylic acid. Titration of the monomer after purification by simple distillation showed the presence of 1.69×10^{-4} mol of acid/g of monomer, or 1.2 wt %. Assuming that all of it was distributed on the surface of the beads, their carboxylic acid content would have been approximately 3.9×10^{-5} mol/g, which is close to the value of the intercept. These results demonstrate that simple distillation of monomer was not sufficient to remove the acrylic acid impurity from the HEA.

A fractionation procedure therefore was used for a second batch of monomer. Conductometric titration of the product showed that no weak acid was present. This monomer was used to prepare batches H2 and H3. As shown in Table 5, these beads had positive surface charges following addition of the shell. The lower mobility of the bead covered with copolymer shell, compared to the one of the corresponding seed, is likely

Table 6. Characterization of Latices after Seed Copolymerization Step

batch no.	HEA conversion, % (HPLC)	reacted HEA,[a] mol/g of seed	OH content (conductometric titration), mol/g latex	area per OH group, Å^2/group
H1	NA	NA	1.1×10^{-4} (8×10^{-5})[b]	11.6
H2	38	7.6×10^{-4}	2.74×10^{-5}	12.9
H3	38	5.7×10^{-4}	1.69×10^{-5}	20.1

[a] Calculated under the assumption that all reacted HEA is located on the beads (using data from second column and the amount of seed used). [a] The number in the bracket is calculated by subtracting the COOH content due to acrylic acid in the monomer (intercept in Figure 4).

due to masking of the surface charge or enhanced electroosmotic drag due to hydrophilic surface groups.[30]

In Table 6, data from HPLC analysis of the supernatant and from the conductometric titrations are presented. A mass balance of the consumed HEA, comparing data in the third column with the actual amount found on the beads by titration (fourth column), shows that only approximately 3% of the reacted monomer was contained in the shell on the latex.

This finding is consistent with the theory of seed polymerization[31] in surfactant-free systems. Free radical polymerization starts in solution, and the oligomers grow until they reach the critical length at which they are not soluble in water. At this point they either coagulate with each other to form new nuclei or they coagulate with the seed, which is swollen with monomer, and continue polymerization at the surface. When the monomer is not very soluble in water (e.g. styrene), the critical length is very small (3−4 monomer units). On the other hand, if the monomer and its polymer are soluble in water (e.g. HEA), polymerization in solution independent of the seed will take place. When copolymerization of a mixture of two monomers is attempted, both their solubility and the reactivity ratios in the copolymerization have to be considered to predict their behavior. We are not aware of any reports describing the copolymerization of styrene with 2-hydroxyethyl acrylate. However, reactivity ratios for styrene/4-hydroxybutyl acrylate have been reported [32] and we used them to predict the behavior of our system: $R_{\mathrm{sty}} = 0.72$; $R_{\mathrm{HEA}} = 0.73$. This means that the rate constant for the reaction of a styrene terminated radical with a HEA molecule (when an alternating sequence results) is only slightly higher than the rate constant of the reaction in which the same radical captures a styrene molecule (when a homopolymer sequence results). The same statement is valid for a HEA terminated radical.

The probability of finding significant concentrations of oligomeric radicals with homopolymeric sequences of either of the two monomers will be moderately high. Those containing more styrene units will become insoluble faster and will coagulate with the seed, while the more hydrophilic oligomers will tend to grow in solution. Alternating monomer strings will also be reasonably water soluble, so only those oligomers with significant regions of homooligostyrene would be expected to incorporate into the seed surface, where addition of styrene partitioned into the bead or the HEA from the aqueous phase will take place. Because of the high water solubility of HEA, insoluble oligomers will be relatively rare, qualitatively explaining the low HEA incorporation observed. As the surface concentration of hydroxyl groups required for subsequent grafting was small, we considered the amount incorporated as sufficient and did not attempt to optimize the conditions further.

Other factors which influenced this step were the specific area of the seed (more groups were incorporated per gram latex when the seed was smaller, due to the higher surface area available) and the concentration of HEA monomer (lower HEA concentration produced lower incorporation).

2. Ce(IV)-Initiated MEA Polymerization. 2.1. Kinetics of Grafting Reaction. The reaction is a redox-initiated polymerization which proceeds at the vinyl bond of the MEA molecule. In the initiation step, the rate determining process is the disproportionation of the Ce(IV) complex with the primary alcohol. The reducing agent forms a radical in this initial step. The more stable this radical is, the slower will be the polymerization. According to the literature [6] the rate of polymerization decreases in the following order: 1,2-glycol > aldehyde > primary alcohol > secondary > tertiary alcohol. The termination reaction by Ce(IV) is prevalent at high ceric ion concentrations, relative to termination in which two polymeric chains are involved (recombination or disproportionation).[33] If all the alcohol radicals produced are consumed in the initiation of polymer chains and mutual termination is neglected, the rates of initiation (R_{i}), propagation (R_{p}), and termination (R_{t}) are described by the following equations:[27,33]

$$R_{\mathrm{i}} = Kk_{\mathrm{d}}[\mathrm{Ce}^{4+}][\mathrm{alcohol}] \qquad (4)$$

$$R_{\mathrm{p}} = k_{\mathrm{p}}[\mathrm{M}][\mathrm{M}^{\bullet}] \qquad (5)$$

$$R_{\mathrm{t}} = k_{\mathrm{t1}}[\mathrm{Ce}^{4+}][\mathrm{M}^{\bullet}] \qquad (6)$$

Here M is the monomer, k_{d}, k_{p}, and k_{t1} are the rate constants for disproportionation of the Ce(IV) complex, polymerization, and monomolecular termination, respectively, and K is the equilibrium constant for the Ce(IV)/alcohol complex formation. If the steady-state approximation is made ($R_{\mathrm{i}} = R_{\mathrm{t}}$), the polymerization rate is given by

$$R_{\mathrm{p}} = (Kk_{\mathrm{p}}k_{\mathrm{d}}/k_{\mathrm{t}})[\mathrm{M}][\mathrm{alcohol}] \qquad (7)$$

In Figure 5, data illustrating the conversion of MEA vs reaction time are plotted. Monomer conversion was determined by HPLC analysis of the supernatant during the grafting reaction (experiment H3GR1).

The linearity is consistent with data in the literature.[34] The conversion obviously would plateau at longer times but the experiment was stopped before monomer exhaustion, due to the high viscosity of the reaction mixture. The high viscosity probably contributed to scattering of the data points because difficulties with stirring allowed local variations in concentration. From the first two concentrations, the initial rate of polymerization was calculated to be 8.9×10^{-4} mol L^{-1} min^{-1} ± 10%. This value is 1 order of magnitude less than that found in the literature,[34] for polymerization of acrylamide using Ce(IV)/L-cysteine initiation. The difference may be due to the relatively limited mobility of the hydroxyl groups anchored to the latex.

2.2. Efficiency of the Grafting Reaction. In Table 7, data on monomer conversion following completion of grafting are compared with the mass of grafted chains

Table 7. Mass Balance of MEA during Grafting Experiments

batch no.	MEA conversion, % (HPLC)	poly(MEA)/g of latex[a]	grafted chains,[b] 10^4 g/g of latex			% reacted MEA in grafted chains[c]
			polymer	short chains	tot.	
H2GR1	20	0.068	∼12.3	∼2.3	∼14.6	2.1
H2GR2	43.5	0.159	∼8.1	∼1.0	∼9.1	0.6
H3GR1	72	0.562	17.8	1.7	19.5	0.3
H3GR2	50	0.153	2.6	1.5	4.1	0.3
H3GR3	29	0.133	8.3	3.7	12.0	0.9
H3GR5	31	0.285	9.8	3.4	13.2	0.5

[a] Calculated under the assumption that all polymerized MEA was distributed on the beads (using MEA conversion data and the amount of seed in each recipe). [b] Calculated with the formula in eq 2, using the area of each peak on the chromatogram of grafted polymer solution. [c] Percentage of polymerized MEA associated with the grafted chains.

cleaved from the beads. Data referring to batches H2GR1 and H2GR2 are approximate (±30%) because only the approximate volume of the grafted polymer solution was known (for all the other batches, the grafted polymer solution was weighed). The last column shows the percentage of the total monomer polymerized associated with the grafted chains. The mass balance calculations indicate that two kinds of polymer resulted: polymer in solution and grafted polymer on the beads. The percentage of MEA found covalently attached to the surface was only 0.3−2.1% of the total amount of polymerized MEA. This is consistent with the observation that a high viscosity supernatant resulted during grafting reactions H3GR1 and H3GR2, implying the presence of high molecular weight polymer in the liquid phase. Four reactions could lead to this event.

(1) The presence of Ce(IV) ions could have initiated bulk polymerization of MEA monomer, independent of the presence of the hydroxyl groups on the latex. If this was the case, the supernatant should have been viscous in all the experiments, not in only some of them. Also, while homopolymerization in the presence of Ce(IV) without a reducing agent has been reported for acrylonitrile, acrylamide is not known to react this way.[5] Since MEA is an acrylamide derivative, this reaction is unlikely. Finally, in previous experiments done in our laboratory utilizing Ce(IV)-initiated polymerization of MEA on aldehyde derivatized latex,[22,24] highly viscous supernatants were never observed.

(2) Very reactive 1,2-glycol units physically adsorbed on the surface of the latex could have acted as activators for solution polymerization. As the glycol unit splits upon oxidation with Ce(IV), forming a free radical and an aldehyde [6,35] two initiating centers result. The ethylene glycol would have to have been present as an impurity in the HEA monomer used to produce the shell or it could have been produced by hydrolysis of HEA residues during grafting (by cleavage of the ester bond). As the monomer used for shell copolymerization was purified by fractionation, it is very unlikely that traces of ethylene glycol would still be present (the boiling point of ethylene glycol at 18 mmHg is 100 °C; the main fraction during HEA purification came over at 77 °C, at approximately 18 mmHg). Also, the copolymer shell preparation was done at neutral pH and MEA grafting at pH 2.7. Splitting the ester bonds under either condition is very improbable since it was found that highly basic conditions and long reaction times were necessary to hydrolyze the ester bonds in HEA residues.

(3) Chain transfer reactions in principle could have promoted solution polymerization of MEA. As the monomer chain transfer constants are generally small, however (i.e. of the order of 10^{-5} for acrylamide[27]), this was unlikely to be the main cause of the low grafting efficiency. Also, chain transfer should have caused low density of the grafting chains when using aldehyde as the reducing group as well. The high density of the grafted chains found on aldehyde latex[24] suggests transfer was not responsible for the solution polymerization observed.

(4) Chains with an appropriate content of hydroxyl groups desorbed from the beads during grafting could have promoted solution polymerization. The existence of such chains on the surface of the latex is consistent with the values of the copolymerization constants for styrene/HEA. Statistically, it is reasonable to expect that some adsorbed copolymers will contain relatively hydrophobic regions anchoring more hydrophilic tails. The conformation of such species adsorbed on a solid surface is known; see Chapter 6 of ref 36. The styrene-rich part will form an anchor layer, while the HEA-rich parts, being in a good solvent, will extend into solution as free dangling loops or tails. As the grafting of MEA proceeds, these HEA-rich regions have a higher probability of being the first ones to react, as the hydroxyl groups which initiate polymerization will be more mobile in this region. The newly formed poly(MEA) grafted regions will increase the size of the hydrophilic part of the chains, ultimately leading to their desorption in some cases. In solution, they can continue to react and form higher molecular weight species.

This last possibility is the most likely to have occurred and caused the poor grafting efficiency. Considering the synthesis conditions for all the grafting experiments, batches H3GR1 and H3GR2 were prepared using comparatively small Ce(IV) concentrations (3×10^{-3} to 4.6×10^{-3} mol/L), so the termination rate would be relatively low. This explains the high molecular weight of the polymer obtained in solution, resulting in high supernatant viscosities. In all the other preparations, higher Ce(IV) concentrations likely caused lower molecular weight products to be formed in solution.

An alternate explanation for poor grafting efficiency has recently been suggested by Okieimen et al.,[3,7,8] who also found high levels of homopolymer formation during grafting of various acrylates on modified cellulose or starch. They explain the result by assuming that most of the grafts are the result of interaction of homopolymer radicals with radicals on the support. In this model it is crucial, if a high frequency of grafting is to be achieved, that these sites are still reactive by the time the radicals formed in solution reach them. In the referenced work, addition of low molecular weight reducing agents such as 2-propanol or thiourea increased the efficiency of grafting by promoting fast homopolymerization. Also, incremental addition of the monomer and the initiator enhanced the yield of the grafting reaction by producing active sites on the

Table 8. Characterization of the Chains Grafted on the Beads

batch no.	grafted chains,[a] mol/g	size distribution[a] of grafted polymer		chain[b] separation, Å	radius of gyration,[c] Å
		mole %	DP		
H2GR1	~6.3×10^{-9}	~1	240	~236	45.9
	~3.7×10^{-7}	~57.2	24	~31	14.5
	~2.7×10^{-7}	~42.8	6	~36	7
H2GR2	~2.4×10^{-9}	~0.6	261	~383	47.9
	~3.1×10^{-7}	~73.7	18	~34	12.6
	~1.08×10^{-7}	~25.7	7	~57	7
H3GR1	4.3×10^{-8}	11.9	247	89	46.6
	1.5×10^{-7}	41.3	21	48	13.6
	1.7×10^{-7}	46.8	8	45	8
H3GR2	1.40×10^{-7}	48.3	14	49	11
	1.5×10^{-7}	51.7	8	47	8
H3GR3	1.5×10^{-8}	3.7	288	150	50.3
	1.1×10^{-7}	27.2	19	56	12.9
	2.8×10^{-7}	69.1	10	34	9
H3GR5	1.6×10^{-8}	3.4	251	146	47.0
	1.6×10^{-7}	33.6	21	46	13.6
	3.0×10^{-7}	63.0	9	34	9

[a] Determined by SEC analysis of the cleaved tentacles. [b] Calculated with the formula $D = [(\mathrm{SA/g})/(GN_A)]^{1/2}$, Å, where SA/g = surface area per gram solid beads, Å^2, G = amount of grafted chains cleaved from 1 g of latex, mol/g of latex; N_A= Avogadro's number, mol^{-1}; and $G = \mathrm{GW}/M_n$, where GW = mass of grafted polymer per gram latex, calculated with eq 2, and M_n= number average molecular weight of grafted chains, calculated with eq 1 using the elution volume of each fraction. [c] Estimated, according to[36] $R_g = aM_n^{1/2}$ (linear polymers in near θ solvent), where $a = 261 \times 10^{-4}$ nm (PMMA).

support later in the process. Further experiments are needed in order to decide if the described mechanism is relevant to grafting on latex, however.

It is concluded that bulk MEA polymerization forming soluble polymers occurred in all the experiments to a much greater extent than the actual grafting reaction on the beads. Hence, no straightforward assessment of the reaction conditions necessary to optimize MEA conversion can be made from the present results.

2.3. Characterization of the Terminally Attached Chains. In Table 8, a characterization of the grafted chains is given. The results combine the data obtained by size exclusion chromatography of the cleaved polymer with the absolute amount calculated from the area of the peaks to give an estimate of the area per molecule and the average chain separation between terminally attached chains on the bead. All the calculations assume uniform distribution of the grafted chains on the latex surface. In the last column, the approximate radius of gyration of the polymer graft is calculated (assuming that the radius of gyration of the graft is the same as for the macromolecule in solution). As all the values calculated for twice the radius of gyration are smaller than the values of the chain separation, we conclude that the grafted chains take on a mushroom configurationl see Chapter 8 in ref 36.

In all the grafting experiments, two categories of terminally attached chains were obtained: polymeric grafted chains (with DP between 18 and 360) and short grafted chains (with DP between 7 and 10). A possible explanation for this distribution of molecular weights is that the mechanism of polymerization is strongly influenced by the local concentration of hydroxyl groups on the surface. As these groups start initiating, those which are sufficiently close to each other can mutually terminate by combination or disproportionation and account for the short graft population. Groups which are further apart or are separated by terminated oligomers initiate chains which continue to grow. At some point, some of the very long chains will restrict monomer diffusion toward shorter growing chains located under them, i.e., within a radius of gyration of the longer grafted chains. This could result in the two populations of chains observed. The fact that the grafted polymer cleaved from the beads containing the highest surface concentration of hydroxyl groups in the shell (batch H2) contained mostly short chains (99 mol % on H2GR1 and 99.4 mol % on H2GR2), independent of the Ce(IV) concentration, is consistent with the above mechanism.

The results show that higher Ce(IV) concentrations improved the grafting efficiency over the concentration utilized. The overall coverage with grafted chains was higher on batch H2GR1 compared to H2GR2, which was synthesized at lower Ce(IV) concentration. The same result was obtained for batch H3GR3 when compared with batch H3GR2 (grafted under the same conditions, except for lower Ce(IV) concentration for batch H3GR2). This finding is consistent with the mechanism of initiation and with results reported for grafting on cellulose.[8] According to these studies,[8] the grafting efficiency would level off or decrease at higher Ce(IV) concentrations, due to the prevalence of termination over the propagation reaction. This range was not investigated in the present study.

Batches H3GR1 and H3GR2 were synthesized under the same conditions, the only difference being a higher monomer concentration for H3GR1. The oligomeric graft content was approximately the same. The coverage with polymeric grafted chains was higher at higher monomer concentration, however. A similar result was obtained by comparing batches H3GR3 and H3GR5, the second one being synthesized using a higher monomer concentration. Evidently, higher monomer concentrations increased the amount of product by mass action and enhanced the yield of the grafting reaction. This is in agreement with previous studies of Ce(IV)-initiated

Table 9. Characterization of the Surface of the Grafted Beads[a]

batch no.	bead area, 10^{-9} Å^2	a_0,[b] 10^{-8} Å^2	a_1,[b] 10^{-8} Å^2	a_2,[b] 10^{-8} Å^2	a_{OH},[c] 10^{-9} Å^2	mushroom coverage, %	hydrophil. coverage, %
H2GR1	2.3	2.7	15.7	2.7	3.4	93.2	244.7
H2GR2	2.3	1.1	9.8	1.1	3.5	53.5	207.3
H3GR1	2.4	2.4	6.2	21	2.3	122.1	218.7
H3GR2	2.4	2.2	3.9	0	2.4	25.0	122.5
H3GR3	2.4	5.2	4.1	8.5	2.4	73.4	170.9
H3GR5	2.4	5.4	6.6	7.9	2.3	82.3	179.0

[a] a_x is the area covered by a specific group on one bead (the subscript "x" is assigned as 0 for chains with DP < 10, 1 for short grafted chains, 2 for long grafted chains, and OH for unreacted hydroxyl groups). Mushroom coverage is the percentage of the bead area covered by tethers (a_0, a_1, and a_2). Hydrophilic coverage is the percentage of the bead area covered by all groups on the surface (grafted chains and unreacted hydroxyl groups). [b] Area covered by one grafted chain was modeled as a circle of radius R_g. [c] Area covered by one unreacted hydroxyl group was considered[39] 20 Å^2.

grafting on starch[1] and poly(vinyl alcohol).[37]

In conclusion, the composition of the terminally attached chains was affected by the copolymer shell composition, and the Ce(IV) and monomer concentration. More investigation is required in order to understand the grafting conditions sufficiently to tailor the grafted chains concentration and molecular weight distribution.

2.4. Model of the Surface. In Table 9, a model for the surface of the grafted beads is presented. The area covered by grafted chains was modeled as a circle of radius R_g, as it is done for an unconstrained mushroom; see Chapter 8 in ref 36. The goal was to calculate the percentage of the area of the bead that was covered.

Two levels of coverage are considered in the table: hydrophilic coverage, which is calculated as the sum of the area per species for all groups (including unreacted HEA), and mushroom coverage, which is the percentage of the area covered by oligomeric or polymeric grafted chains only. The unreacted hydroxyl groups were included in the calculation because they are hydrophilic, and according to the electrophoretic measurements done on the latex covered with the shell, they contribute to shielding the positive charge on the surface of the seed.

The results show that, for all the batches, the hydrophobic poly(styrene) surface of the bead was covered on average with hydrophilic groups (all values in the last column of Table 8 are higher than 100%). This statement is consistent with the electrophoretic mobility of the grafted beads, which was zero for all batches. That is, the effect of the positive charge initially present on the seed was completely shielded.

The mushroom coverage was between 25 and 120%. Given the size distribution of the grafted chains and the model for the distribution outlined above, we expect the surface to look like a "lawn" of hydroxyl groups with interspersed "shrubs" (oligomers) and "trees" (polymers) extending above it about 50 Å away from the surface (approximately equal to R_g for the largest grafted chains). This type of distribution differs significantly from that reported for polystyrene grown from silica gel in concentrated styrene/toluene solution, where a unimodal distribution was found.[23] The role these species play when the surface is placed in contact with protein solutions is discussed elsewhere.[38]

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