Grafting of Oligosaccharides onto Synthetic Polymer Colloids

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A new method to form colloidally stable oligosaccharide-grafted synthetic polymer particles has been developed. The oligosaccharides, of weight-average degree of polymerization ~38, were obtained by enzymatic debranching of amylopectin. Through the use of a cerium(IV)-based redox initiation process, oligosaccharide chains are grafted onto a synthetic polymer colloid comprising electrostatically stabilized poly(methyl methacrylate) or polystyrene latex particles swollen with methyl methacrylate monomer. Ce(IV) creates a radical species on these oligosaccharides, which then propagates, initially with aqueous-phase monomer, then with the methyl methacrylate monomer inside the particles. Ultracentrifugation, NMR, and total starch analyses together prove that the grafting process has occurred, with at least 7.7 wt % starch grafted and a grafting efficiency of 33%. The surfactant used in latex preparation was removed by dialysis, resulting in particles colloidally stabilized with only linear starch as a steric stabilizer. The debranched starch that comprises these oligosaccharides is found to be a remarkably effective colloidal stabilizer, albeit at low electrolyte concentration, stabilizing particles with very sparse surface coverage.

Introduction

Polymer colloids that are stabilized by glycopolymers have many potential applications and also can provide models for the roles of polysaccharides on and in the membranes of living cells. The properties are strongly dependent on the nature of the comonomers used: For instance, biodegradable nanoparticles coated with polysaccharides can be used as biomimetic drug carriers for medical applications. The objective of the present paper is to graft oligosaccharides from debranched starch onto synthetic polymer colloids. Because the colloidal stabilizers of living cells often include polysaccharides, it is also of interest to examine the colloidal stability of cells stabilized only by a steric stabilizer comprising a simple polysaccharide: the linear α-1,4-glucan that results from debranching starch.

A review of work done on the surface modification of nanoparticles with poly- or oligosaccharides has been given by Lemarchand et al.[1] Well-defined glycopolymers have been synthesized by several polymerization techniques (for example, refs 2 and 3). This process enables the preparation of amphipathic copolymers composed of a hydrophobic block and a hydrophilic polysaccharide block, which have the potential to then be used as stabilizers of polymer colloids. Passirani et al.[4] were able to prepare non-biodegradable poly(methyl methacrylate) (PMMA) particles coated with either dextran or heparin. Similarly, Vauthier et al.[5,6] obtained biodegradable poly(isobutylcyanoacrylate) particles coated with dextran or heparin. The polymerization was initiated by polysaccharide radicals produced via the oxidation of the polysaccharide by Ce(IV) ions. This technique produces glycopolymer colloids with versatile properties, which depend on the nature, length, and structure of the two different blocks.

Whole starch is often used as a stabilizer; for example, the common practice of stabilizing emulsions with natural starch relies on the ability of starch to impart some viscosity to the system, thereby impeding the breakdown of the emulsion. For starch to function purely as a (steric or polymeric) stabilizer, further modification is necessary; for example, attaching a hydrophobic group to the starch renders it surface-active.[7] Here we look at a particular way of using starch as a stabilizer, where the starch is chemically grafted to the latex particle.

Particles colloidally stabilized by a grafted water-soluble polymer (functioning as a steric stabilizer) are often referred to as "hairy particles". The present paper uses a new approach to create hairy particles sterically stabilized by oligosaccharides. The new method is as follows. A well-characterized polymer colloid is prepared by conventional emulsion polymerization with an electrostatic stabilizer using standard techniques.[8,9] The resulting latex is then swollen with monomer, as is common for the first step of seeded emulsion polymerizations. Rather than adding an initiator of the type conventionally employed for such seeded emulsion polymerizations, instead oligosaccharides and an oxidizer, Ce(IV), are added in the process presented here. These oligosaccharide radicals are then expected to start to copolymerize with the small amount of monomer present in the water phase. These entities will continue to polymerize with aqueous-phase monomer until the radical end becomes sufficiently hydrophobic to adsorb onto the surface of a latex particle; this is effectively the mechanism by which radical entry into particles occurs in conventional emulsion polymerization.[10,11] The radical end is now in a hydrophobic, monomer-rich environment, and subsequent propagation with monomer will be rapid. The result is a grafted steric stabilizer, formed by a process analogous to what happens in conventional emulsion polymerizations in the presence of a water-soluble monomer such as acrylic acid to form electrosterically stabilized particles.[12,13] The electrostatic stabilizers that colloidally stabilized the parent seed latex are then removed by dialysis, giving

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colloidal particles coated with grafted oligosaccharides, and thus provide the only means of colloidal stabilization.

The oligosaccharide used in the present process comprises short linear starch chains obtained by enzymatically debranching amylopectin,[14] which is that variety of starch that has a very high molecular weight (up to 10^9) and is extremely highly branched, with the individual branches having a relatively low average degree of polymerization (the number-average degree of polymerization $X_n \approx 30$ for typical amylopectins). In the case of the waxy maize starch used herein (Shimuzi mochi Amioca), the weight-average degree of polymerization $\bar{X}_w$ was ~38, with a relatively broad distribution of degrees of polymerization.

Materials and Methods

Chemicals. High-purity water (Milli-Q) was used for all processes. 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (V-50, Aldrich) was recrystallized from 50% w/w water/acetone before use. Maltoheptaose (Aldrich), sodium acetate (Univar), ethanol (95%, Aldrich), sodium hydroxide (Aldrich), acetic acid (Aldrich), cerium(IV) ammonium nitrate (CAN, Aldrich), nitric acid (70%, Riedel-deHaen), and dodecyltrimethylammonium bromide (DTAB, Aldrich) were used as received.

Styrene (Synthetic Resins, stabilized with 4-methoxyphenol inhibitor) was first passed through a column of basic alumina and then distilled under reduced pressure (first and last 10% discarded) before being stored at 0 °C. MMA (Mitsubishi Rayon, stabilized with 4-methoxyphenol inhibitor) was passed through a column of basic alumina and distilled under reduced pressure (first and last 10% discarded) to remove the inhibitor. The purified monomer was stored at 0 °C for no longer than 2 weeks before use. *n*-Butyl acrylate (Aldrich, >99%, stabilized with methyl-hydroquinone) was passed through a deinhibiting column packed with a methyl-hydroquinone inhibitor remover and used immediately.

A waxy maize starch, Amioca powder, was obtained from The National Starch and Chemical Company and debranched with isoamylase. Isoamylase (*Pseudomonas* sp.) was obtained from Megazyme and used as supplied: 250 U mL^{-1} stored in 3.2 M ammonium sulfate plus 0.02% sodium azide. Pullulan standards (from Shodex) with narrow polydispersity index and weight-average molar masses ranging from 5900 to 788 000 g mol^{-1} were used to calibrate the size exclusion chromatography (SEC) columns.

A Total Starch Analysis Kit from Megazyme was used following the Total Starch Assay Procedure AOAC method 996.11. The kit consists of thermostable α-amylase (10 mL, 3000 U mL^{-1}), amyloglucosidase (10 mL, 200 U mL^{-1}), glucose oxidase/peroxidase reagent (GOPOD, high-purity), glucose reagent buffer (concentrate), glucose standard solution, and regular maize starch.

Preparation of Debranched Starch. Linear oligosaccharides were prepared by debranching starch using the method described by Batey and Curtin,[15] with the modification developed by Lisle et al.[16] A typical procedure is as follows: A sample of starch (100 mg) was dispersed with ethanol (0.5 mL), gelatinized with sodium hydroxide (2 mL, 0.25 M), and then refluxed at 150 °C for 10 min. The ethanol was allowed to evaporate. Water (4 mL), sodium acetate buffer (pH 4.13, 2 mL), and acetic acid (64 μL) were added to the gelatinized starch. The debranching enzyme, isoamylase (0.1 mL), was added to the mixture and incubated (40 °C, overnight). For SEC analysis, the debranched mixture was then boiled, and then the mixture was desalted (1 h) with mixed bed resin (Biorad, 1 mg) and then centrifuged (10 500*g*, 10 min). The supernatant was retained for analysis.

^{1}H Liquid-State NMR was used to determine the completeness of debranching of the starch by isoamylase. When the α-(1,6) branch point could no longer be detected the debranching was considered to be complete. A sample of the debranched starch was freeze-dried, and then 2 mg was dissolved overnight in 0.5 mL of DMSO-d_6/D_2O (80/20 v/v) at 80 °C. Quantitative ^{1}H NMR spectra were recorded on a Bruker DPX300 at 90 °C at a Larmor frequency of 300.13 MHz. Exactly 64 transients were recorded with a 7.9 μs 90° pulse and a 5 s relaxation delay.[17] The chemical-shift scale was calibrated with a trisilylpropanoic acid internal standard at 0 ppm. The temperature was calibrated with an ethylene glycol standard.[18]

SEC Analysis of Debranched Starch. Molecular weight distributions were determined using a SEC setup comprising a Shimadzu LC-10ATVP pump, a Shimadzu DGU-14AVP degasser, a Shimadzu SIL-10ADVP autoinjector, a Shimadzu CT10ACVP column oven set at 60 °C, Waters Ultrahydrogel 250 and 120 columns fitted with an Ultrahydrogel guard column, and a Wyatt Optilab DSP interferometric refractometer. The system was controlled and data were recorded using Astra software. The eluent used was 0.05 M ammonium acetate (pH 5.2) with 0.05% sodium azide at a flow rate of 0.5 mL min^{-1}. The run time was 80 min. These conditions gave good SEC separation of the oligosaccharides obtained by debranching.[19,20] To obtain the molecular weight distribution, pullulan standards were used for calibration, and molecular weights were obtained by universal calibration with the Mark−Houwink−Sakurada parameters reported for pullulan and linear starch by Castro et al.[21] No corrections were made for band broadening.

Preparation of an Electrostatically Stabilized Poly(methyl methacrylate) Seed Latex. Ce(IV) ions formed a complex with the commoner anionically stabilized latex due to the opposing charges, which then led to coagulation of the system. To overcome this problem, a cationic PMMA latex was prepared using a procedure described in the literature.[22] Water (570 g) and the surfactant (DTAB, 4.57 g) were poured into a Mettler-Toledo RC1e calorimetric reactor, and the solution was heated to 90 °C. The reaction vessel was purged with high-purity nitrogen to remove oxygen. A trace amount of bulk PMMA (1 wt % as compared to the monomer) was dissolved in the MMA monomer (86.52 g) prior to use, and the polymer/monomer solution was then added to the reactor and emulsified by stirring at 450 rpm. The dissolution of a trace amount of polymer in monomer is thought to enhance radical capture efficiency in the early stages of the polymerization leading to a lower polydispersity of the latex particle size distribution.[23−25] After 5 min, the initiator (V-50, 0.288 g dissolved in 30 mL of water) was added. Polymerization was allowed to proceed at 90 °C for 2 h. The latex had a solids content of 10.9 wt % (measured by gravimetry after water evaporation).

The procedure for the synthesis of anionically stabilized polystyrene latex is described in the Supporting Information. The cationically stabilized polystyrene latex was synthesized identically to the procedure described above for PMMA.[22]

Grafting of Oligosaccharides onto the MMA-Swollen PMMA Particles. Preliminary grafting experiments were performed with styrene and *n*-butyl acrylate as swelling monomers. These syntheses are described in the Supporting Information. This leads to the choice of MMA as the swelling monomer. The PMMA latex prepared previously (6.45 g) was diluted with deionized water (3.2 g) and stabilized further with DTAB (0.04 g) before swelling with MMA (0.77 g). The amount of added DTAB was such that it was below the critical micelle concentration (CMC) in the latex to avoid secondary particle nucleation. The CMC in the latex was determined by measuring the surface tension of the latex as a function of DTAB concentration. The suspension of MMA in diluted PMMA latex solution was equilibrated under gentle stirring for 24 h at room temperature. The previously prepared solution of oligosaccharides (0.5 g of debranched starch) was then added under gentle stirring and nitrogen bubbling at 40 °C. After 20 min, 4.0 mL of a solution of CAN (0.1 g) in nitric acid (0.2 M) was fed with a syringe pump over 1 h under vigorous agitation. Nitrogen bubbling was maintained for 5 min, and the reaction mixture was stirred gently overnight. The final latex was dialyzed against Milli-Q water for 7 days with daily changes of the water.

Particle Size Analysis. Determination of particle size distributions was carried out by hydrodynamic chromatography (HDC) using a Polymer Laboratories particle size distribution analyzer (PSDA) and dynamic light scattering on a Malvern high-performance particle sizer

(HPPS). The average particle size of the cationic samples was determined by dynamic light scattering on a Malvern Instruments zetasizer.

Zeta Potential Analysis. Determination of the ζ potential of the colloids was carried out using a Malvern Zetasizer Nano Series operating at an angle of 177° coupled with an MPT-2 titrator. This employs the M3-PALS technique, which is a combination of laser Doppler velocimetry and phase analysis light scattering (PALS) to measure the particle electrophoretic mobility (velocity of a particle in a unit electric field). The ζ potential is then related to the electrophoretic mobility by the Henry equation.

Transmission Electron Microscopy Analysis. Information on particle morphologies was obtained by using a Biofilter Philips CM120 transmission electron microscope at the Electron Microscope Unit, Sydney University, with a CCD camera and an accelerating voltage of 120 kV. Dilute dispersions of polymer particles (about 0.05 wt %) were embedded on a carbon-coated copper grid. After being dried under vacuum, the grid containing the polymer particles was placed in the transmission electron microscope. The length scale was calibrated with a standard sample of polystyrene latex particles.

Surface Tension Measurements. The surface tension of the PMMA seed latex was obtained using a standard ring measurement on a KSV Sigma 70 surface tensiometer. The sample, PMMA latex (40 mL), was added to the vessel. The standard ring was first heated to red hot on a Bunsen burner before being attached to the balance unit. The surface tension of the latex was measured as successive amounts of the surfactant (DTAB) were added until no change in the surface tension was observed.

Solid-State ^{13}C NMR. Characterization methods requiring complete solubility of all components are inapplicable for amphiphilic copolymers, as the copolymers are not completely soluble in any solvent.[26] ^{13}C solid-state NMR was therefore chosen to quantify the amount of grafting. ^{13}C NMR spectra from a homogeneous copolymer gel swollen in either perdeuterated DMSO or a mixture of perdeuterated DMSO and perdeuterated pyridine were used by Gurruchaga et al.[27] to prove grafting for their acrylate graft copolymers without the need for complete solubility.

In the present study, solid-state ^{13}C NMR was used to confirm that grafting had occurred. The latex obtained from the grafting experiment was ultracentrifuged at 90 000 rpm and 25 °C for 30 min. The centrifugate, which should contain the water-insoluble fraction in the form of oligosaccharide-*g*-PMMA/polystyrene, was analyzed by solid-state ^{13}C NMR on a Bruker Avance 300 wide-bore spectrometer (Bruker Biospin Pty Ltd.), operating at 75 MHz for ^{13}C, using a 4 mm magic-angle spinning (MAS) probe (Bruker Biospin Pty Ltd.). The spectrometer was operated with XWIN-NMR software (Bruker Biospin GmbH). A 4 mm zirconia rotor with a KelF cap was used and spun at 3.0 kHz MAS. A ^{13}C single-pulse excitation (SPE) spectrum was recorded at ca. 50 °C with a 5.0 μs 90° pulse and continuous wave decoupling (optimized on adamantane) and externally calibrated with respect to the resonance of adamantane at 29.5 ppm. The parameters for the acquisition of the data were as follows: 9306 transients were recorded, the duration of the free induction decay was 0.16 s, the delay before a scan was 10 s, and a total delay of 10.16 s between scans was applied.

Liquid-State ^{1}H NMR. Exactly 2 mg of the solids from drying the supernatant obtained from ultracentrifugation of the oligosaccharide-*g*-PMMA/polystyrene latex was dissolved in 0.5 mL of DMSO-d_6 at 80 °C overnight. Quantitative ^{1}H NMR spectra were recorded on a Bruker DPX300 at 90 °C at a Larmor frequency of 300.13 MHz. Exactly 64 transients were recorded with a 7.9 μs 90° pulse and a 6 s relaxation delay.

Dialysis of the Latexes. After being grafted, the latexes were dialyzed to remove the electrostatic stabilizer used in seed synthesis. MEMBRA-CEL dialysis membrane MD 25-14 (Viskase) with a molecular weight cut off of 14 000 Da was conditioned by boiling in deionized water for 5 h prior to use. The conditioned membranes were filled with the latex samples, which were then placed in beakers filled with deionized water under gentle stirring at room temperature with daily water exchanges. Conductivities of the serum phase from dialysis became constant after 10 days of dialysis (with the water changed daily), showing that all of the labile electrostatic stabilizer had been removed.

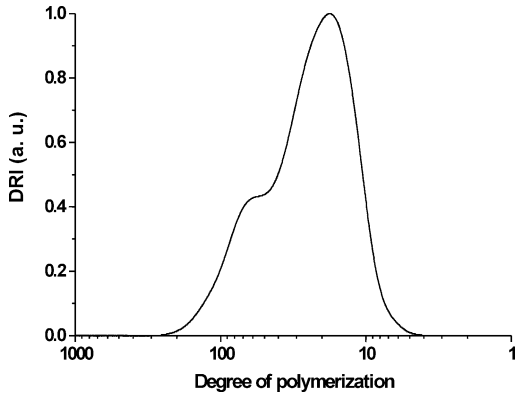


Figure 1. SEC molecular weight distribution of debranched starch. DRI is the raw signal from a differential refractive index detector.

Quantification of Grafted Starch. The dialyzed latex was ultracentrifuged at 90 000 rpm and 25 °C for 30 min. The centrifugate, which should contain the oligosaccharide-*g*-PMMA, was dried in an oven at 60 °C for 24 h. The amount of starch in 100 mg of the dry centrifugate was obtained with a standard assay procedure (AA/AMG) using the Total Starch Analysis Kit (Megazyme).

Results and Discussion

Debranched Starch The SEC molecular weight distribution of the debranched starch oligosaccharides is presented in Figure 1. All measurements were performed directly after the end of the debranching procedure to ensure that retrogradation of the oligosaccharide chains was minimized.[28] The detailed shape of this debranched chain-length distribution in amylopectin, with its characteristic shoulder, has been explained in detail by Castro et al. in terms of the enzymatic processes involved in starch biosynthesis.[21] For our purposes, the weight-average molecular weight of the oligosaccharides from debranching was used in all calculations, $\bar{M}_w = 6150$ g mol^{-1}, i.e. a weight-average degree of polymerization ($\bar{X}_w$) of 38 anhydroglucose units. The resulting oligosaccharides were used in seeded polymerization onto preformed polymer particles. ^{1}H NMR spectroscopy was used to check that debranching was complete.[29] An overlay of the ^{1}H NMR spectra for the native and debranched Amioca starch is shown in Figure 2. Complete debranching is demonstrated by the disappearance of the signal corresponding to α-(1,6) linkages at 4.8 ppm[30] as well as an increase in signals corresponding to α and β reducing ends at 5.15 and 4.4 ppm, respectively.[30] The signal at 1.9 ppm present in the spectrum of debranched starch is from the acetic acid[31] introduced in the medium for the debranching step.

PMMA Seed Latex. Preliminary work using an anionically stabilized polystyrene latex as a seed resulted in coagulation of the system on addition of the Ce(IV) solution (Supporting Information). The loss of stability was probably a result of the destabilizing effect of the Ce^{4+} cation with the anionic latex before any starch was added to the system. A cationically stabilized polystyrene latex was found to circumvent this problem (Supporting Information). Further improvement in the system was obtained when PMMA was used instead of polystyrene as the cationically stabilized seed latex, as discussed in detail above. The particle size of the PMMA seed is shown in Table 1.

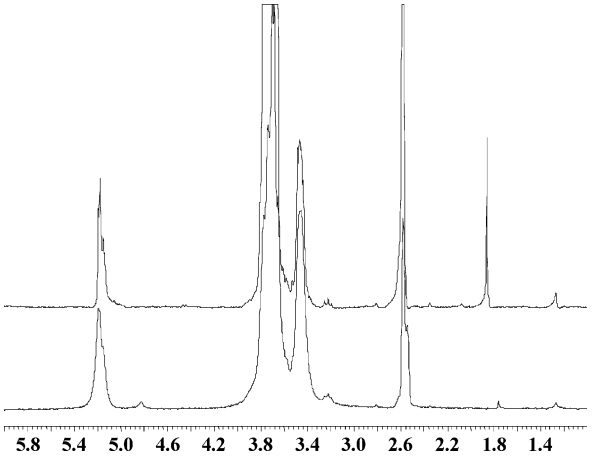


Figure 2. ^{1}H NMR spectra overlay for the native Amioca starch (solid line) and the debranched Amioca starch (broken line).

Table 1. Particle Size Distribution of the Latexes by HDC and HPPS

latex	weight-average diameter (nm)	weight-average width of distribution (nm)
cationic polystyrene seed	62.5	8.9
oligosaccharide-*g*-polystyrene/PMMA	64.5	11.5
cationic PMMA seed	87.5	11.8
	99.2[a]	21.7
oligosaccharide-*g*-PMMA	90	12.6
	102.6[a]	14.8

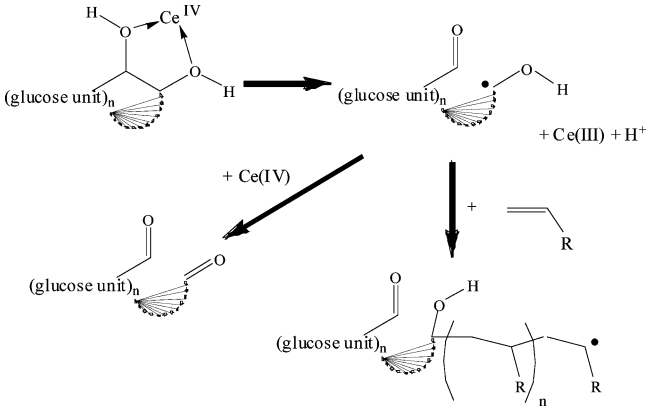
[a] Values determined by HPPS as the *z*-average.

Second-Stage Polymerization of Seed. Preliminary grafting experiments were performed with styrene and *n*-butyl acrylate. In the system where the anionically stabilized polystyrene seed latex was swollen with styrene, the conversion was about 48%. ^{1}H NMR of the supernatant in DMSO-d_6 showed a significant amount of polystyrene oligomers, Figure S1 in the Supporting Information. This was unexpected, since polystyrene is hydrophobic and therefore should precipitate from the aqueous phase. It was observed that the addition of Ce^{4+} to the monomer-swollen monomer latex without the oligosaccharides resulted in coagulation of the anionic latex but no coagulation of the cationic latex, as expected from double-layer theory. The water-soluble polystyrene oligomers are thought to be a result of the slow propagation of styrene monomer in the aqueous phase grafted onto an oligosaccharide chain, leading to the propagating radical being terminated by another Ce(IV) or another propagating radical. In choosing a monomer that will minimize aqueous-phase termination, it is necessary to minimize the residence time of the growing radicals in the aqueous phase. This is encapsulated in the expression for initiator efficiency f_{entry}[10]

$$f_{entry} \approx \left\{ \frac{2\sqrt{k_d[I]k_{t,aq}}}{k_{p,aq}[M]_{aq}} + 1 \right\}^{1-z} \quad (1)$$

where z is the critical degree of polymerization for entry of a radical into a particle, k_d is the initiator dissociation rate coefficient, [I] is the initiator concentration, $[M]_{aq}$ is the monomer concentration in the aqueous phase, and $k_{p,aq}$ and $k_{t,aq}$ are the rate coefficients for propagation and termination in the aqueous phase (which for hydrophobic monomers can be assumed to be close to that in pure monomer,[32] denoted k_p). The monomer must have a high k_p to ensure high initiator efficiency. Thus *n*-butyl acrylate was chosen to replace styrene because of its high propagation rate coefficient.[33,34] In the system swollen with *n*-butyl acrylate, there was a significant amount of coagulation during the swelling stage (this problem can be solvent using a cationic seed), but the characterization of the latex was also complicated by the observation of three phases upon ultracentrifugation. The phase on the top of the centrifugation tube was unstable; it disintegrated and made the supernatant, which was clear after centrifugation, become cloudy when the supernatant was extracted with a syringe, making quantification of the process impossible.

Scheme 1. Radical Initiation on the Reducing End of a Starch Chain by Cerium(IV) and Subsequent Propagation to a Vinylic Monomer to Form a Block Copolymer (Right) or Termination by Cerium(IV) (Left) in the Aqueous Phase



The grafting experiments with styrene and *n*-butyl acrylate as monomers onto a cationically stabilized polystyrene seed gave products with coagulum of about 20 wt % as compared to monomer and polymer. There are competing effects with regard to the water solubility of the monomer; it must be sufficiently soluble so that its aqueous-phase concentration will give a sufficiently high rate of propagation ($k_p[M_{aq}]$) but sufficiently hydrophobic so that surface activity of the block copolymer will be obtained with fewer monomer units. MMA could be acceptable for the present purpose. It has a relatively high k_p[35] and an intermediate water solubility (0.15 M at 50 °C[36]) and therefore is expected to have a relatively high entry efficiency.[10,11,37] With MMA as the monomer the coagulum was about 1.5 wt % as compared to monomer and polymer instead of 20% with styrene and *n*-butyl acrylate. Styrene and *n*-butyl acrylate were therefore not used further as monomers; the MMA monomer was used to swell the seed latex in all subsequent experiments, for which the results are discussed in the next section. Following second-stage polymerization, the electrostatic stabilizer (DTAB) used in seed preparation and swelling stages was removed by dialysis.

It has been demonstrated that Ce(IV) complexes with diols, cleaving the carbon−carbon bond between the hydroxyls in polysaccharides to yield an aldehyde or ketone and a free radical (Scheme 1).[38−40] Ce(IV) mainly creates radical sites on oligosaccharides.[41] The hemiacetal group at the reducing end of a cellulosic chain has been shown to be much more reactive toward Ce(IV) than the midchain glycol groups.[42−44] It is therefore likely that a radical is relatively easily created at the reducing end of most of the oligosaccharide chains. Moreover, as the oligosaccharides and the cerium were added in equimolar amounts, the formation of only one radical per oligosaccharide is favored. The oligosaccharide radical then propagates with MMA in the aqueous phase until it becomes sufficiently surface-active to irreversibly enter a monomer-swollen particle, analo-

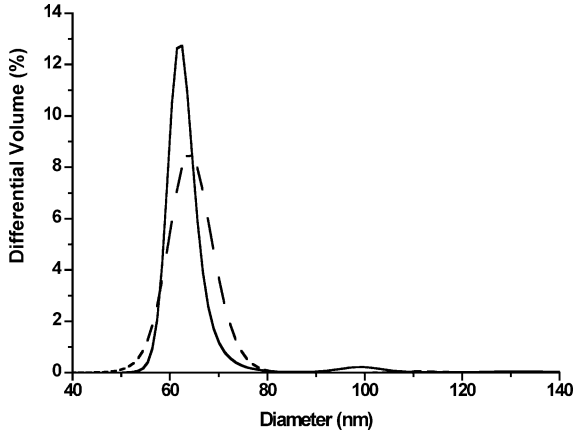


Figure 3. Hydrodynamic chromatography analysis of the cationic polystyrene seed latex (solid line) and the second-stage oligosaccharide-*g*-PMMA/polystyrene latex (dashed line).

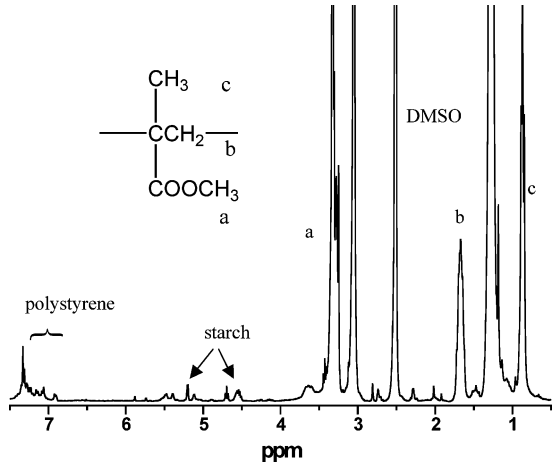


Figure 4. ^{1}H NMR spectrum of the supernatant of an oligosaccharide grafted onto an MMA swollen polystyrene latex.

gous to the radical entry process in conventional emulsion polymerization.[10,11] The polymerization would then be expected to continue within the swollen particles, forming a diblock poly(anhydroglucose-*block*-MMA) with the PMMA block anchored to the particle. This procedure should thus optimize grafting of the oligosaccharides to the surfaces of the particles.

Characterization of the Oligosaccharide-*g*-PMMA/Polystyrene Latex. The particle size distribution of the oligosaccharide-grafted latex was obtained using HDC. HDC suggested a monomodal population for the oligosaccharide-*g*-PMMA/polystyrene latex, as shown in Figure 3. Although HDC may not be accurate for cationic latexes, it is reasonable to assume that the monomodality is not an artifact. However, the apparent small increase in particle size and broadness of the distribution may or may not be an artifact of the use of HDC for a cationic latex. The presence of the MMA oligomers in the supernatant due to aqueous-phase termination was confirmed by liquid-state ^{1}H NMR of the supernatant in DMSO-d_6, as seen in Figure 4.

The proof that the starch was indeed grafted was obtained using solid-state ^{13}C NMR. In Figure 5, a resonance peak at 62.8 ppm due to carbon number 6 in the glucose unit was observed.[45] The complete chemical-shift assignment is given in the Supporting Information. The data are not quantitative, as the relaxation delay was not optimized, but do indicate significant grafting. Analysis of the supernatant with ^{1}H NMR in DMSO-d_6 showed a significant amount of the water-soluble PMMA oligomer fraction and a small amount of starch (Figure 4). Since

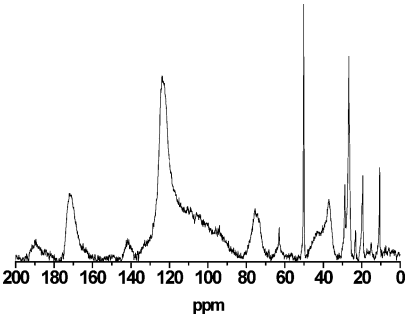


Figure 5. Solid-state ^{13}C NMR spectrum for the precipitate of an oligosaccharide-grafted polystyrene/PMMA copolymer.

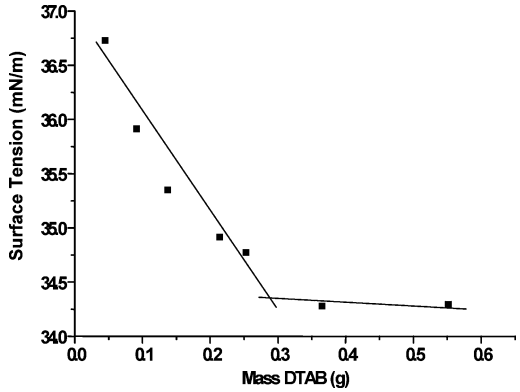


Figure 6. Surface tension measurement for a cationic PMMA latex (40 mL) with increasing additional DTAB. The linear fits on two different ranges are shown as solid lines.

starch is soluble in DMSO,[46,47] this confirms that most of the starch precipitated with the particles during ultracentrifugation.

Extensive dialysis of the seed and grafted latexes was performed to remove electrostatic stabilizers. It was expected that on removal of the electrostatic stabilizer the oligosaccharide-*g*-PMMA/polystyrene latex would remain stable, sterically stabilized by the grafted oligosaccharide chains, whereas the seed latex, stabilized only electrostatically, would coagulate.-Two latexes were synthesized with theoretically different surface coverages; the one with higher surface coverage (32 nm^2 per oligosaccharide chain) was stable after dialysis whereas the one with lower coverage (91 nm^2 per oligosaccharide chain) coagulated. The synthesis at the higher surface coverage is discussed below.

Characterization of the Oligosaccharide-*g*-PMMA. On swelling of the seed latex with monomer, precipitation of polymer was observed after some time. This loss of stability was presumed to be due to insufficient surfactant for adequate coverage of the swollen polymer particles. More DTAB surfactant was added to stabilize the monomer-swollen latex particles, the amount of surfactant being chosen to be just below the latex CMC to avoid secondary nucleation. The CMC was determined by measuring the surface tension of the PMMA seed latex as a function of the amount of DTAB added (Figure 6). The latex obtained using the optimized conditions, i.e., cationic PMMA swollen with MMA in the presence of additional DTAB and the increased starch concentration to give a surface area of 32 nm^2 per oligosaccharide chain, was extensively characterized. The *z*-average diameter of the oligosaccharide-*g*-PMMA latex particles, 103 nm, was slightly larger than that of the starting seed of 99 nm (Table 1). (The *z*-average, commonly used in light scattering size measurements, is significantly higher for polydisperse distributions than, say, a volume average.) Particle size distribution curves for the PMMA seed and the oligosac-

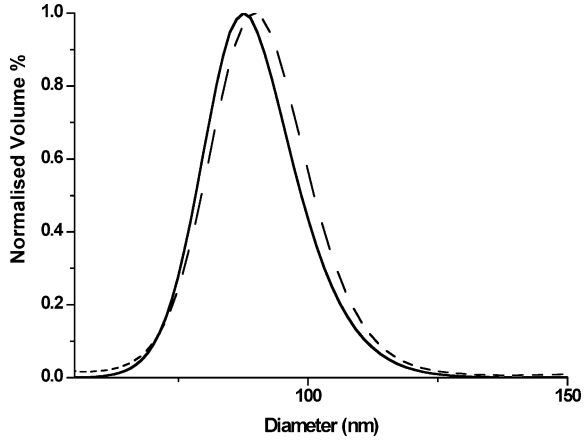


Figure 7. HDC particle size distribution for the PMMA seed latex (solid line) and the oligosaccharide-*g*-PMMA latex (dashed line).

charide-*g*-PMMA latexes measured with HDC as shown in Figure 7 also show an apparent slight increase in particle size after the grafting experiment, but as discussed above this could be an artifact. The latex particles were also examined using TEM, as shown in Figure 8. Unlike the PMMA seed latex, the oligosaccharide-*g*-PMMA latex particles do not merge under the electron beam; the grafted particles in the TEM images are more spherical and better defined than those of the PMMA seed latex particles.

The oligosaccharide-*g*-PMMA latex was dialyzed for over a week, and the latex was still stable afterward. The degree of grafting was determined by enzymatic digestion of the centrifugate from the dialyzed latex to glucose using the Total Starch Analysis Kit. UV−vis spectrometry was used to quantify the amount of glucose. The amount of starch that was grafted was found to be at least 7.7 wt % to total solids, giving a grafting efficiency of at least 33% based on the amount of the initial starch used in the formulation. This measurement may underestimate the amount of grafted starch because of possible limitations of the Total Starch Analysis Kit on starch grafted onto latex particles. When the Total Starch Analysis Kit was applied to pure natural starch, a recovery of 94% starch was achieved; this recovery is taken into account in the calculation of the grafting efficiency, but for grafted starch the recovery may be lower in the presence of synthetic polymer. Some starch may be embedded inside the particles and is not taken into account at all in the Total Starch Analysis Kit. Previously, solid-state ^{13}C NMR on a centrifugate of the grafted latex showed the presence of starch, Figure 5. Since ultracentrifugation may result in the breaking of grafted starch chains and also precipitation of adsorbed starch, the measurement with solid-state ^{13}C NMR after ultracentrifugation cannot be relied upon to be quantitative. Dialysis is able to remove adsorbed starch and should be sufficiently gentle so as not to break grafted oligosaccharide chains. Thus the dialysis of the grafted latex followed by subjecting the centrifugate to the Total Starch Analysis Kit can be regarded as the best quantitative analytical method. The observed latex stability is thought to be from an increased particle surface coverage by the oligosaccharide chains.

The theoretical number of chains per particle for the syntheses described in the Supporting Information was 200, whereas the higher-coverage synthesis described in this paper with the cationic PMMA seed that led to a stable latex was 820 oligosaccharide chains per particle. If grafting efficiency were 100%, then there would be 820 oligosaccharide chains per particle; hence the experimental grafting efficiency is 33% with the actual

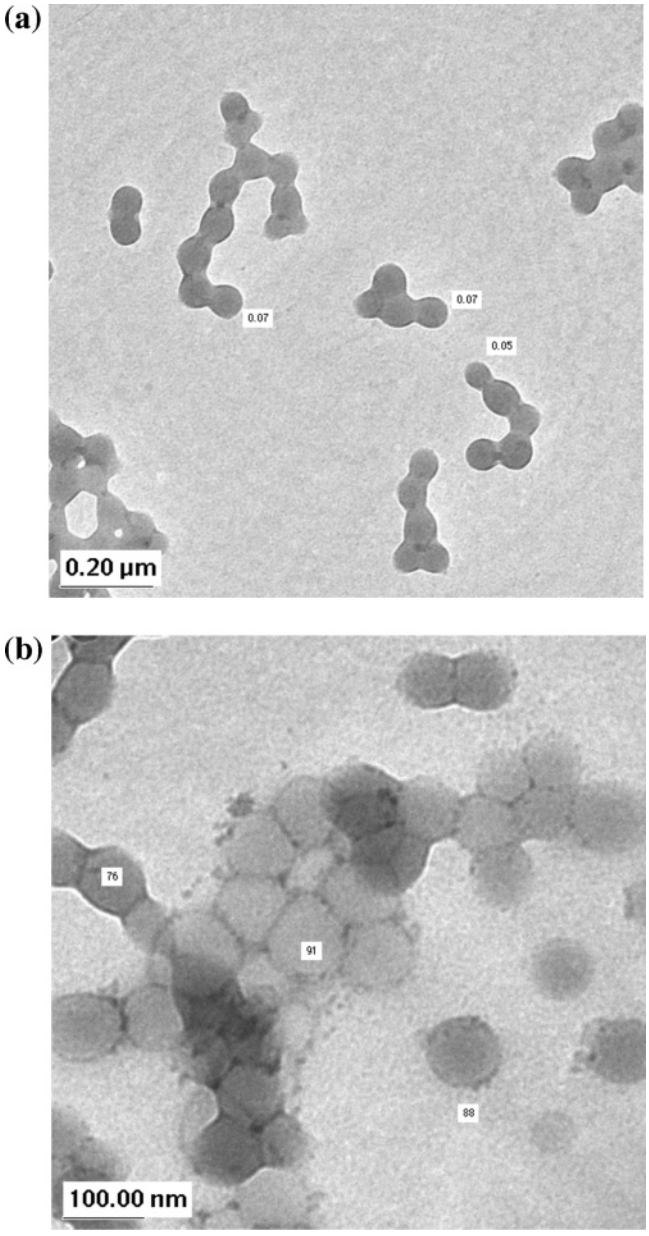


Figure 8. TEM micrographs of (a) PMMA seed latex and (b) oligosaccharide-*g*-PMMA latex (not dialyzed).

number of chains per particle being 270. The number of oligosaccharide chains per particle was calculated by dividing the number of oligosaccharide chains by the number of particles. The number of particles per unit volume, N_p, is calculated as the ratio of the total polymer mass to the mass of one particle

$$N_p = \frac{m_p^0}{\frac{4}{3}\pi r_u^3 d_p} \quad (2)$$

where m_p^0 is the initial mass of polymer (as the dry weight of seed latex) per unit volume of the aqueous phase, r_u is the weight-average unswollen particle radius, and d_p is the polymer density.

The number of oligosaccharide chains given above was determined from the number-average molecular weight ($\bar{M}_n$ = 2500 g mol^{-1}) obtained from the SEC molecular weight distribution. This $\bar{M}_n$ value may be inaccurate because (i) the number-average molecular weight suffers from low accuracy for most samples exhibiting a broad molecular weight distribu-

Table 2. Number-Average Molecular Weights and Degrees of Polymerization from Molecular Weight Distributions from Capillary Electrophoresis Data Obtained for Five Types of Debranched Starch[49] [a]

starch type	$\bar{M}_n$	$\bar{X}_n$	surface area per oligosaccharide chain (nm^2) assuming the present Amioca system had the same $\bar{X}_n$
potato	4200	26	163
barley	3278	20	127
rice	2806	17	109
maize	2786	17	108
wheat	2350	14	91
Amioca[b]	2500	15	97

[a] These data were used to estimate the range of surface area per oligosaccharide chain on each latex particle for the present debranched Amioca sample. [b] Values were obtained using SEC data for the debranched Amioca starch used in the grafting experiments.

tion[48] and (ii) aqueous SEC of starch suffers from incomplete recovery.[29] Capillary electrophoresis (CE) is a technique that can used to determine absolute molecular weight data but was unavailable for the present study. To obtain an estimate of the area occupied per chain, the number-average molecular weight data obtained from the SEC for the Amioca starch used here was compared to that of various starch types obtained with CE[49] assuming that the debranched Amioca sample had the same $\bar{X}_n$ as that of each of the debranched starches for which CE data are available. The results are shown in Table 2. The theoretical surface area per oligosaccharide chain using the CE data shown in Table 2 was determined with the assumption that the grafting efficiency with all of the starch types would be 33% under grafting conditions similar to those used for the Amioca starch. The surface area per oligosaccharide chain for the Amioca starch lies within the range of those assuming the same $\bar{X}_n$ as the rice, maize, and wheat starches (103 ± 10 nm^2).

Assuming the experimental grafting efficiency for both the polystyrene (PS) and the PMMA particles to be 33%, then the surface areas per oligosaccharide chain become 275 and 97 nm^2, respectively. These two oligosaccharide-grafted particles were synthesized with starting amounts of oligosaccharide that would have given surface areas of 91 and 32 nm^2, respectively, if grafting efficiency had been 100%. The area of 97 nm^2 per oligosaccharide chain is 4−8 times higher than values in the literature when polyethylene oxide (PEO) macromonomer is used as a steric stabilizer.[50,51] Specifically, Kawaguchi et al. describe a critical point at which sterically stabilized particles are formed and coalescence between similar sized particles stops.[50] At this point the maximum surface area (S_{crit}) covered by undeformed polymer coils is calculated as the maximum surface area occupied by a single stabilizer chain, which in their work is 12.6 nm^2. At 100% conversion the surface area per chain is drastically reduced to 0.59 nm^2. In the work of Wu et al. on PMMA and PS latexes stabilized by PEO macromonomer, stable latexes were obtained with the PEO macromonomer covering 25 nm^2 with 0.2 and 0.1 wt % PEO grafted onto particles.[51]

To ascertain that there were no other contributory stabilizers in the system in this work, the ζ potential of the dialyzed grafted latex was measured in deionized water and found to be 7.2 mV. This amount of charge is too low to contribute to the stability of the latex and hence it can be concluded that the observed stability is due to the ability of the oligosaccharide chains to act as steric stabilizers. This result shows that sufficiently high particle surface area coverage by the oligosaccharides is able to render the particles colloidally stable through steric stabilization. When the dialyzed latex was diluted in salt solutions with a sodium chloride concentration range of 0.1−3 M, the solutions became cloudy and coagulated over time.

In an ionic environment, the neutral oligosaccharide chains change conformation, which we postulate is the cause for the loss in stability. Theories of colloidal stability of sterically stabilized particles, such as that given by Vincent and coworkers,[52] require (through the Flory−Huggins χ parameter) a knowledge of the (hairy-layer) oligomer/water interaction and how this changes with ionic strength. There is no quantitative information on this in the literature; although there are interesting observations that amylopectin increases in size with higher ionic strengths,[53] that could well be due to effects of ionic strength on the conformations of the branches as much as on the conformations of the component oligomeric branches. Colloidal stability studies with particles stabilized by monodisperse polysaccharides may provide an answer to this interesting question in the future.

Conclusions

A new method has been successfully developed that allows grafting of debranched natural starch onto monomer-swollen electrostatically stabilized PMMA particles. The process involves the in situ grafting by a redox-initiated radical polymerization process between water-soluble linear oligosaccharide chains and electrostatically stabilized seed latex particles swollen with an appropriate hydrophobic synthetic monomer, chosen to minimize undesirable events such as inefficient grafting. The oligosaccharide that was used to form grafted latexes was obtained by enzymatically debranching amylopectin, yielding a broad distribution of linear oligosaccharides of $\bar{X}_w \approx 38$. The initiating radicals are created on oligosaccharide chains by Ce(IV). These water-soluble chains then undergo polymerization with the small amount of monomer present in the water phase until the radical end becomes sufficiently hydrophobic to enter the monomer-swollen polymer particles. This results in rapid polymerization within the monomer-swollen particles to form poly(anhydroglucose−*b*−synthetic monomer) block copolymers, with the polymeric hydrophobic component irreversibly buried in the particle. MMA proved to be an appropriate monomer for the grafting experiments (more suitable than styrene and butyl acrylate) because it has a relatively high k_p and intermediate water solubility, which allowed a rapid aqueous-phase propagation promoting high entry efficiency into the particles and thus ensuring that aqueous-phase termination is minimized, whence a high proportion of initiated chains actually become grafted to the particle.

The MMA-based system was analyzed as follows. The polymer colloids formed in the supposed grafting process precipitated under ultracentrifugation, giving a distinct separation between the supernatant phase and the centrifugate. Solid-state ^{13}C NMR and analysis of the amount of starch present in the centrifugate proved that significant grafting of the oligosaccharides onto the synthetic polymer seed latex particles had indeed occurred. After dialysis to remove the ionic surfactant used to make the seed latex, the latexes resulting from various syntheses were examined for colloidal stability. Both the seed and the oligosaccharide-grafted latexes with low coverage per oligosaccharide (275 nm^2 of surface area per chain) coagulated after 7 days of dialysis, while the latex with higher surface coverage (97 nm^2) was stable for about a week after 7 days of dialysis. It is surmised that the improved stability is due to increased

coverage of the latex particle surface by the linear oligosaccharide chains.

The area per oligosaccharide stabilizer, 97 nm^2 per oligosaccharide chain, is very sparse compared to those of synthetic steric stabilizers. This result would make debranched starch a very efficient stabilizer in terms of the number of starch molecules required to stabilize a particle, though it should also be noted that its effectiveness is markedly reduced in the presence of strong electrolyte. Since polysaccharides (from different sizes and structures) are one of the colloidal stabilizers used in biological cells, this high stabilizing efficiency in the case of linear oligosaccharides at least can be seen to be advantageous from an evolutionary point of view, because it frees up considerable space on a cell surface for the large number of other components on the surface needed for cell viability (ion pumps, cholesterol, etc.).

The procedures developed here enable biomimetic polymer colloids to be synthesized with potential applications in biomedicine, including as model colloids at low electrolyte concentrations, which share some of the same colloidal stabilization mechanisms as cells.

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Supporting Information Available. Additional methods as well as results and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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