Surface Imprinting of Cholesterol on Submicrometer Core−Shell Emulsion Particles

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ABSTRACT: Submicrometer surface-imprinted particles were prepared by a two-stage aqueous emulsion polymerization with a poly(divinylbenzene) shell over a cross-linked poly(styrene) core. The second stage polymerization was performed in the presence of a polymerizable surfactant (pyridinium 12-(4-vinylbenzyloxycarbonyl)dodecanesulfate, **PS**) and pyridinium 12-(cholesteryloxycarbonyloxy)dodecanesulfate (**TS**), which acted both as a surfactant and as a template. Removal of the template left hydrophobic cavities on the surface of charged particles or particles bearing benzyl alcohol groups, dependent on the protocol adopted. Particles were shown to rebind cholesterol in 60% 2-propanol in water, and a poly(ethylene glycol) (PEG) modified with cholesterol at both chain ends was capable of flocculating the imprinted particles. No flocculation was seen either when a monocholesteryl PEG was used or when nonimprinted particles were used.

Introduction

Molecular imprinting is now an established technique for creating small molecule recognition sites in cross-linked polymeric matrixes[1] and the technique has recently been extended to larger entities such as proteins,[2] bacteria,[3,4] and even inorganic crystals.[5] The method of rebinding of ligands to the recognition sites is usually through the interaction of functionality on the template with complementary groups attached to the polymer, whose placement is a consequence of complexation with the template before and during polymerization. The most commonly utilized interactions are hydrogen bonds. However, the observation that some imprints are almost as good at binding the template in purely aqueous media as in nonpolar organic solvents suggests that the hydrophobic interaction[6,7] and stereochemical "fit" are also important contributors to recognition. Indeed such interactions have been exploited in the preparation of imprinted polymers in polar solvents, e.g., the imprinting of cholesterol with cyclodextrins cross-linked with isocyanates in DMSO,[8] and amino acids in water,[9] among others.[10]

We recently reported the preparation of nonporous, submicrometer particles, prepared by a two-stage core−shell emulsion polymerization, which are imprinted with cholesterol in the outer layer.[11] These polymers have a specific surface area approaching that of conventional "bulk" polymers prepared in the presence of a porogenic solvent, by virtue of their small particle size. We reasoned that a modification of this procedure can be developed to give particles with hydrophobic recognition cavities in the surface of a hydrophilic bead by using a specially designed template-surfactant. The distinguishing feature of this type of surface imprinting unlike, for example, polymers designed to bind certain metal ions[12] or other ligands,[13] is that the template molecule, in addition to its conventional function, is designed to position the template at a predefined distance from the polymer surface and at the same to stabilize the particles during the second polymerization step. In practical terms, the proposed methodology may form the basis for the preparation of specific "ligand-responsive" colloids, i.e., imprinted polymer particles which would undergo a controlled flocculation in the presence of bifunctional ligands in a manner analogous to the immunoprecipitation of a biological target by antibodies.

Results and Discussion

Synthesis of Surface-Imprinted Particles. To create hydrophobic recognition sites in the surface of submicrometer beads, we needed to achieve two goals: first to prepare a surfactant-like template that would sit at the polymer−water interface with the template region embedded in the monomer phase to create the recognition site; and second to design a method of differentiating the surface of the particle from the templated regions. This differentiated surface had previously been achieved in micrometer-sized beads by modification with a low surface energy polymer in the presence of the templates, in that case bacteria.[4] However a different approach was required for small molecule-imprinted beads. We therefore decided to design a polymerizable surfactant molecule which would allow us to anchor hydrophilic groups to the particle surface.

The structures of the template surfactant (**TS**) and polymerizable surfactant (**PS**) and the synthetic schemes for their preparation are given in Figure 1. Both have the same sulfate "head" group and (C_{12}) carbon spacer length; however, the template cholesterol is attached via a carbonate ester to a diol-derived spacer, and the carbon chain of **PS** is connected to a vinylbenzyl alcohol group via the corresponding hydroxy acid. The double bond of **PS** ensured that it would be covalently bound to the outer cross-linked shell of the polymer particles, and the structure allowed us to make beads with two different surface functionalities: In the first case, the core−shell latex particles were ultrafiltered to remove **TS** and residual SDS from the seed emulsion, the polymerizable nature of **PS** meaning that a considerable charge would remain on the surface of the emulsion

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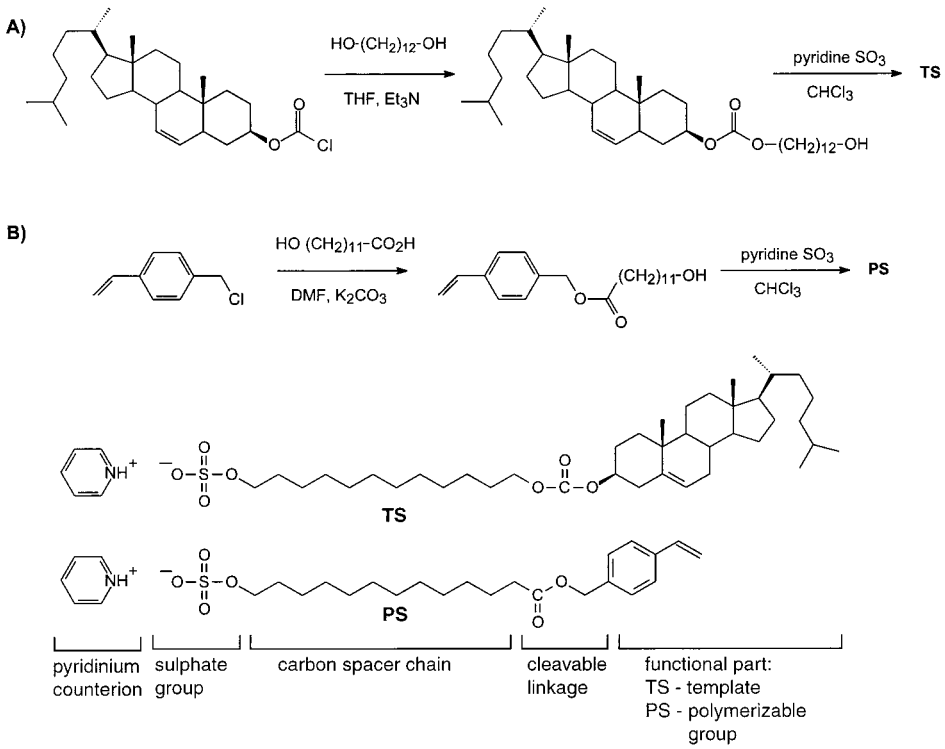

Figure 1. Synthesis of (A) the template surfactant (**TS**) and (B) the polymerizable surfactant (**PS**) used in the preparation of surface-imprinted core−shell particles.

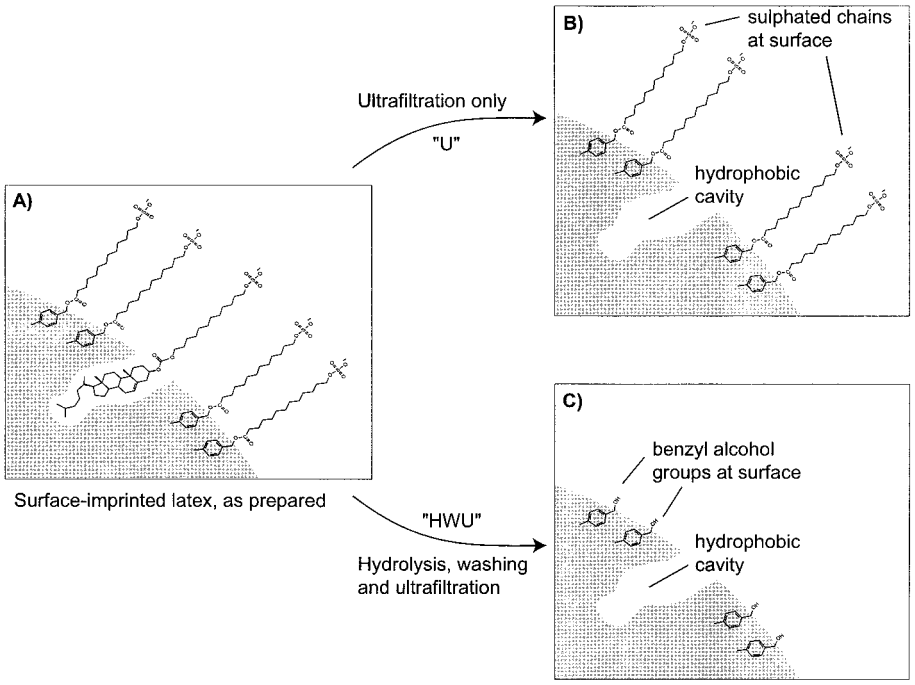

Figure 2. Schematic diagram of the creation of hydrophobic surface imprints in particles with a hydrophilic surface layer: (A) particles as prepared using a mixture of template surfactant (**TS**) and polymerizable surfactant (**PS**); (B) ultrafiltration, removing excess adsorbed surfactant, including **TS**, to leave template cavities in among sulfated surfactant chains, covalently bound to the surface; (C) hydrolysis, followed by washing and ultrafiltration cleaving the ester bonds of **PS**, in addition to removing **TS**, to leave template cavities in a surface covered with benzyl alcohol groups.

particles by virtue of the sulfate groups. If, however, the polymer was subjected to a basic hydrolysis before ultrafiltration, it was expected that both **TS** and **PS** would be cleaved at the carbonate and ester sites respectively and the subsequent fragments removed in the ultrafiltration to leave a surface covered with benzyl alcohol groups. These schemes are illustrated in Figure 2. The polymer particles were then tested for their ability to bind cholesterol from aqueous solutions. Cyclodextrin-based imprinted polyurethanes were shown to bind cholesterol from 70% THF in water. We therefore tested the binding in this solvent mixture and also in an 2-propanol−water mixture.

An aqueous dispersion of a polystyrene/divinylbenzene (DVB) seed latex, prepared as previously described,[11] was treated with DVB and a mixture of **PS**

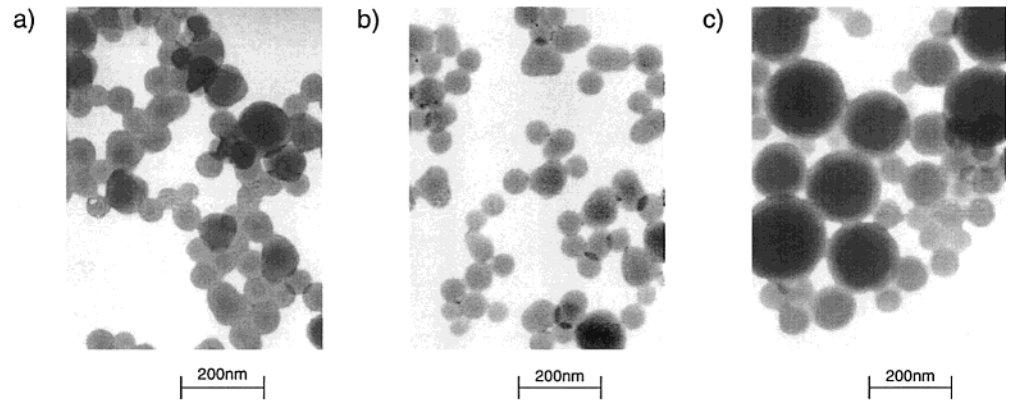

Figure 3. Transmission electron microscope (TEM) photographs: (A) polymer **P0** (B) polymer **P2** showing relatively monodisperse small, slightly irregular beads, and C) polymer **P5** showing two populations of particle sizes.

Table 1. Molar Percentages of Template Surfactant (TS) and Polymerizable Surfactant (PS) and the TS:PS Ratios for Nonimprinted (P0) Polymer and Surface-Imprinted Polymer Beads (P1−P8)

	mol % wrt monomers (mass, g)			
polym	TS	PS	ratio TS:PS	particle diam (nm)[a]
P0	0 (0)	2.75 (0.2214)	0:100	62.1
P1	0.068 (0.0089)	2.68 (0.2156)	2.5:97.5	65.6
P2	0.14 (0.0178)	2.61 (0.2101	5:95	57.8
P3	0.21 (0.0266)	2.54 (0.2045)	7.5:92.5	86−209 (2 pop.)[b]
P4	0.28 (0.0355)	2.47 (0.1990)	10:90	115−180 (2 pop.)[b]
P5	0.41 (0.0532)	2.34 (0.1878)	15:85	67−179 (2 pop.)[b]
P6	0.55 (0.0710)	2.20 (0.1769)	20:80	288.9
P7	0.83 (0.1065)	1.92 (0.1548)	30:70	30−63 (2 pop.)[b]
P8	1.71 (0.2211)	1 (0.0831)	62.4:37.6	56

[a] Measured from TEM photographs. [b] (2 pop.) = two populations of particles.

and **TS** to assemble the imprinted shell layer. The shell was subsequently polymerized at 65 °C using ammonium persulfate as initiator. The **TS**/**PS** ratio was adjusted from 0/100 to 10/90 in steps of 2.5% and subsequently in larger steps (Table 1). It was expected that recognition would be greatest when there was a reasonable degree of site isolation at the polymer surface, and therefore, a low **TS**/**PS** ratio would prove to be optimal. DVB was chosen as the matrix material for the outer shell layer since this is resistant to the hydrolysis conditions necessary for cleavage of **PS**. It was also felt that hydrophobic recognition would be enhanced with the hydrocarbon matrix over methacrylate-based cross-linkers such as ethylene glycol dimethacrylate and trimethylolpropane trimethacrylate.

The morphology of core−shell particles was monitored by transmission electron microscopy (TEM) (Figure 3), and the measured particle sizes are given in Table 1. The particles imprinted with a low percentage of template, between 0 and 5%, showed a good morphology with a small particle diameter. When the amount of template was increased with a consequent reduction in the concentration of **PS**, the quality of the latex obtained deteriorated in terms of monodispersity and secondary nucleation (see for example Figure 3c).

The samples of imprinted core−shell latex were subjected to two treatments to remove template, either ultrafiltration alone (U) or hydrolysis, washing and ultrafiltration (HWU). The removal of **PS** and **TS** in "HWU" samples was shown by the loss of the carbonyl bands (~1732 cm^{-1}) in the FT−IR spectra of treated polymer in addition to the disappearance of bands at

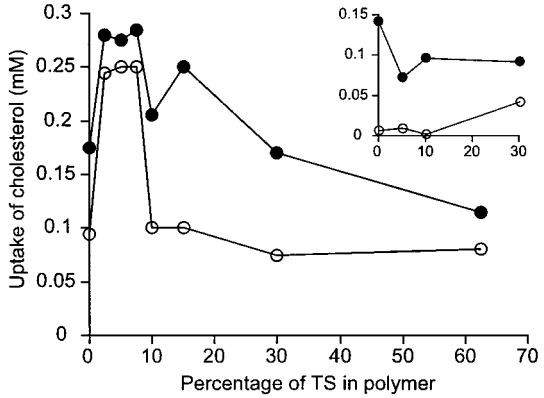

Figure 4. Binding characteristics of polymers: Uptake of cholesterol from a 0.5 mM solution in 60:40 2-propanol:water by polymers **P0**−**P8** at 20 mg mL^{-1}, for "**U**" (ultrafiltered only) materials (open circles) and "**HWU**" (hydrolyzed, washed and ultrafiltered) particles (closed circles). Inset: As above but with 0.5 mM cholesterol in isohexane.

1136 and 1266 cm^{-1} (attributed to sulfate) and the appearance of a broad, shallow OH band around 3200−3500 cm^{-1}. Unfortunately, it was not possible to conclusively show the removal of **TS** in the "U" samples since the carbonate and ester carbonyl bands of **TS** and **PS** overlap; however, ultrafiltration was carried out exhaustively until the conductivity of the effluent was the same as that of pure water, implying that all soluble surfactants, including the template, had been removed. Both types of sample were obtained as dry solids which were resuspended in the analyte solution for determining the polymer's binding characteristics.

Binding Properties. The binding of cholesterol (0.5 mM in 60:40 2-propanol:water) was determined for each of the polymers present at a concentration of 10 mg/mL. The results are shown graphically in Figure 4, which shows the pattern of cholesterol uptake across the range of **TS**/**PS** ratios. Both "U" and "HWU" samples show a fairly flat maximum in the range between 2.5 and 7.5% (**P1**−**P3**), with uptake dropping off at higher template ratios. This probably reflects the loss of site isolation which will be the consequence of too high a template concentration. While the "HWU" treatment produced the highest uptake in any polymers, the background (nonspecific binding) level, as shown by the figure for the nonimprinted particles, was lowest in the case of polymers only receiving the "U" treatment. This probably reflects the more hydrophobic nature of the polymer once the surface of the particles no longer carries the charged sulfate residues.

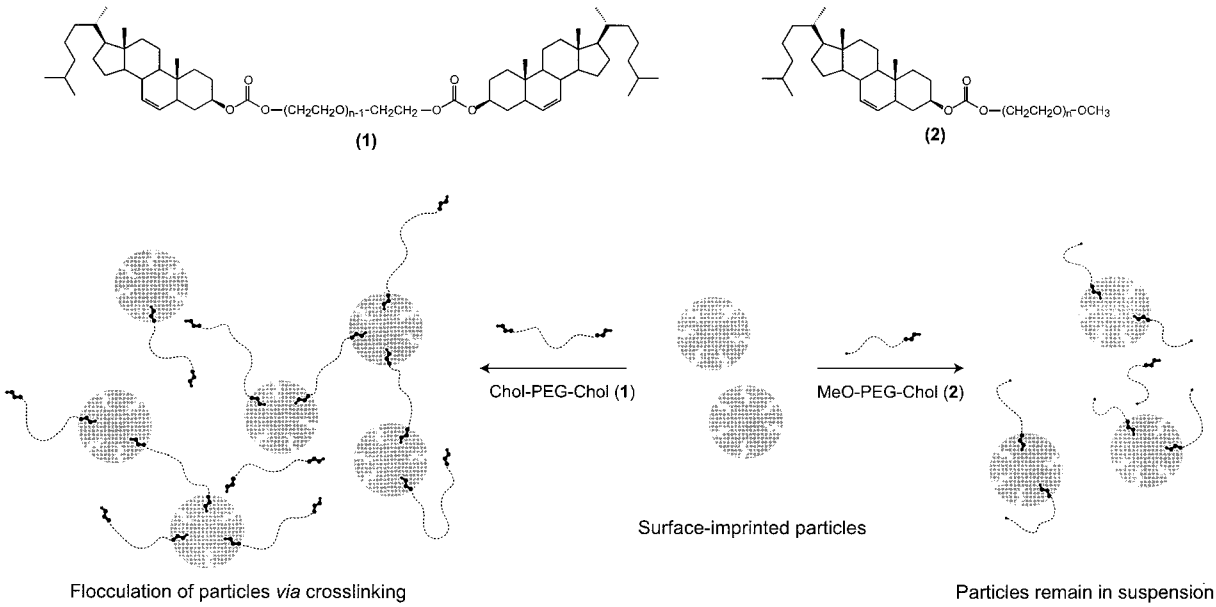

Figure 5. Structures of: α,ω-PEG-di(cholesteryl carbonate), (Chol−PEG−Chol, **1**); monomethoxy−PEG−cholesteryl carbonate (MeO−PEG−Chol, **2**) and a schematic diagram of the "immunoprecipitation" of surface-imprinted particles with **1** but not in the presence of **2**.

To provide further evidence for the hydrophobic nature of the interactions at the polymer's binding site, uptake experiments were also performed in a nonpolar solvent, in this case isohexane. Cholesterol is reasonably soluble in this solvent and therefore will tend to remain in solution rather than partition on the polymer surface, and therefore, there should be no energetic advantage in inhabiting the hydrophobic imprinted sites. Thus, one would expect to see relatively little binding of cholesterol in isohexane unlike the situation in 2-propanol/water, which is exactly the opposite phenomenon to that which was observed with cholesterol-imprinted polymers which rely on hydrogen bonding interactions in the recognition site.[11,14] This prediction was indeed confirmed experimentally (Figure 4, insert). In contrast to the situation in 2-propanol/water, the "U"-treated samples bound virtually no cholesterol while the "HWU"-treated particles showed a slight uptake. The latter can be explained by a moderate degree of nonspecific binding to the particle surface via hydrogen bonds between surface-bound alcohols and the cholesterol hydroxyl.

Flocculation by "Immunoprecipitation". Having shown appreciable binding of cholesterol to polymers **P1**−**P3** in 2-propanol−water, we proceeded to investigate the ability of these materials to undergo ligand-induced flocculation. This "immunoprecipitation" type reaction should result from noncovalent cross-linking of polymer particles, carrying multiple recognition sites on their surface, on exposure to multidentate ligands. We would therefore expect flocculation to occur in the presence of a poly(ethylene glycol) (PEG) modified with two molecules of cholesterol (**1**), but not in the presence of a monocholesteryl PEG (**2**), as illustrated schematically in Figure 5. Particles, both imprinted and nonimprinted, were incubated with a range of concentrations of **1** and **2** followed by determination of their average particle diameter by dynamic light scattering measurements. The results are in agreement with the predicted behavior and are shown graphically in Figure 6.

It is evident from the data presented in Figure 6 that the apparent particles sizes of **P1** and **P2** increased dramatically to about 3500 nm in the presence of

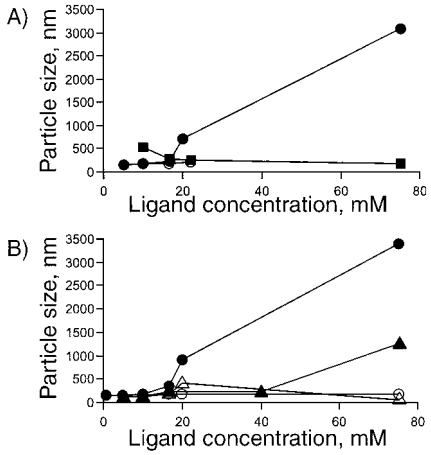

Figure 6. "Immunoprecipitation" of surface-imprinted polymers−change in apparent particle size on addition of cholesterol-modified PEGs to (A) polymer **P2** "**U**" with **1** (closed circles) and with **2** (open circles) and polymer **P0** "**U**" with **1** (squares) in 60:40 2-propanol:water; (B) polymer **P1** "**U**" with dicholesteryl PEG (**1**) (closed circles) and with monocholesteryl PEG (**2**) (open circles) in 60:40 2-propanol:water; polymer **P1** "**U**" with **1** (closed circles) and with **2** (open circles) in 70:30 THF:water.

increasing concentrations of the bifunctional ligand (**1**). This effect was not observed with the control polymer **P0** nor when the imprinted polymers were incubated with the monocholesteryl−PEG (**2**). The flocculation was also seen in solvents other than 2-propanol−water as illustrated in Figure 6b, where 70% THF also was employed as the solvent in a similar experiment. However, in the latter case the average particle size increased to a lesser extent (1500 nm vs 3500 nm in 2-propanol water) which was consistent with the lower uptake of cholesterol by the particles from THF−water mixtures.

Conclusions

It is possible that the specificity of the materials could be greatly improved by the use of an alternative polymerizable surfactant or the use of a different cross-

linker or mixture of monomers in the "shell" layer. It may also be possible to improve the imprinting efficiency by the use of alternative surfactant structures. These experiments remain to be done; nevertheless, the particles described in this report represent a unique kind of imprinted material: submicrometer, relatively monodisperse particles with a moderately high surface area, imprinted with hydrophobic cavities in a hydrophilic surface. These particles are designed to bind nonpolar templates dissolved in aqueous media and their applications therefore should be compatible with procedures where biological receptors (antibodies) might otherwise be used, e.g., ELISA-type assays. Note that the incorporation of a fluorescent dye into the polymer matrix would provide a simple detection method to visualize, and possibly quantify, immobilized ligands. Similarly, standard biological methods such as cell cytometery can be used in place of light scattering to analyze the degree of aggregation of fluorescently labeled particles. Other possible applications of the materials described would include the preparation of imprinted membranes either by attaching the particles to prefabricated sheets through the available functionality or simply by covalently cross-linking.

Experimental Section

Materials and Methods. Divinylbenzene 80% (DVB) and styrene (St), from Aldrich, were washed with 1 M aqueous sodium hydroxide to remove the inhibitor, dried over $MgSO_4$, and stored over calcium chloride at 4 °C until required. Sodium dodecyl sulfate (SDS), ammonium peroxodisulfate (APS), and all other solvents and reagents were used as received. HPLC solvents were purchased from Fisher Scientific (HPLC grade) or Riedel-de-Haën (Chromsolve). Deionized water was used throughout.

FT−NMR spectra were obtained on a JEOL EX-270. FT−IR spectra of samples dispersed in KBr were recorded on a Perkin-Elmer Series 1600 FTIR spectrophotometer by diffuse reflectance IR spectroscopy. The submicrometer polymeric particles obtained were characterized using a Philips transmission electron microscope (TEM) and by dynamic light scattering using a Malvern Instruments Zetasizer 3000 or an ALV 5000 series laser light scattering system. Conductivity was measured with a WPA CM 35 conductivity meter with a CM 25B dip cell (from WPA Linton Cambridge UK) with a cell constant $K = 0.96$. HPLC analyses were performed using Gilson 303 pumps equipped with an ACS or a Sedex 55 light-scattering mass detector, and a Shimadzu SIL-9A or a Gilson 234 autosampler. Samples were analyzed on a 25 cm, 5 μm Spherisorb normal phase column (Hichrom), at room temperature, using a flow rate of 1.5 mL min^{-1} or 0.75 mL min^{-1}. Elution was with a linear gradient from 10% ethyl acetate:*n*-hexane to 100% ethyl acetate over 6 min or isocratic ethyl acetate. The critical micelle concentration (cmc) of **PS** was determined by addition of aliquots of a concentrated solution of **PS** to water at 26 °C, measuring the conductivity after each addition. The cmc was estimated to be at the discontinuity of the graph of conductivity against concentration, determined by extrapolation of the two linear portions of the graph.

Synthesis of Surfactants. Cholesteryl, 12-Hydroxydodecanyl Carbonate. 1,12-Dodecanediol (9 g., 44,4 mmol) was dissolved in 150 mL of dried THF and 3.5 mL of triethylamine in a round-bottomed flask equipped with a drying tube and a dropping funnel. A solution of cholesteryl chloroformate (10 g, 22.2 mmol) dissolved in 40 mL of THF was added dropwise, and the mixture was stirred for 22 h. After solvent removal the residue was taken up with CH_2Cl_2 and washed with water. After extraction of the aqueous phase with CH_2Cl_2, the organic phase was dried over $MgSO_4$ and filtered. The solvent was evaporated under vacuum and the purity of the product checked by TLC (silica gel, 50:50 v/v chloroform:THF, $R_f = 0.84$) and NMR. The final yield was 91.2%, and the compound was characterized by NMR. FT−IR (KBr), cm^{-1}: 3543(ν_{-OH}); 3000−2850 (ν_{-CH}); 1731 ($\nu_{C=O\ ester}$); 1076 ($\nu_{C-O\ ester}$); 1176 ($\nu_{C-O\ alcohol}$). ^{13}C NMR, δ, ppm ($CDCl_3$): 154.00(−CO−); 67.82 (−CH_2−O−CO−); 63.07(−CH_2−OH)); 122.39 (−CH−, C6); 140.07, 42.28, 36.46 (3 × −C−, C5, C13, C10); 79.01, 56.65, 56.10, 49.99, 35.76, 31.80, 27.98 (8 × −CH−, C6, C3, C14, C17, C9, C20, C8, C25); 30.71, 39.48, 39.19, 37.10, 36.13, 31.89, 28.84, 28.19, 24.26, 23.81, 21.00 (11 × −CH_2−, C4, C12, C24, C1, C22, C7, C2, C16, C15, C23, C11); 38.0, 29.54, 29.49, 29.43, 29.40, 29.18, 28.64, 28.21, 27.67, 25.69 (10 × −CH_2− chain); 22.80, 22.53, 19.28, 18.67, 11.82 (5 × −CH_3, C27, C26, C21, C19, C18). ^1H NMR, δ, ppm ($CDCl_3$): 5.3 (d, 1H, −C*H*═C−, $J = 5.3$ Hz); 4.3 (m, 1H, −O−C*H*−); 4.03 (t, 2H, CH_2−O−CO); 3.5 (t, 2H, CH_2−OH); 2.05−1.0 (m's, 27H, −CH_2−); 1.05−0.69 (m, 15H, −CH_3-). Mp = 83−85 °C.

Pyridinium 12-(Cholesteryloxycarbonyloxy)dodecanesulfate (TS). Cholesteryl, 12-hydroxydodecanyl carbonate (2.6 mmol), was dissolved in 85 mL of $CHCl_3$ in a flask equipped with a reflux condenser. Then 5.2 mmol of solid pyridine sulfur trioxide complex ($PySO_3$) was added and the mixture heated at 59 °C for 30−40 min with stirring. The reaction mixture was then cooled to 4 °C, filtered, and washed with hexane. After evaporation of the solvent, the solid obtained was washed with ether and its purity checked by TLC (silica gel, chloroform, $R_f = 0.04$) and NMR. The final yield was 48%. FT−IR (KBr), cm^{-1}: 3000−2850 (ν_{-CH}); 1738 ($\nu_{C=O\ ester}$); 1270−1250 (2 bands: $\nu_{-SO2;}$ $\nu_{C-O\ carbonate}$). ^{13}C NMR, δ, ppm ($CDCl_3$): 154.64(−CO−); 145.8, 142.19, 127.19 (3 × −CH−, Py); 68.69 (−CH_2−O−SO_3−); 67.89(−CH_2−O−CO−); 140.07, 42.28, 36.46 (3 × −C−, C5, C13, C10); 145.84, 142.21, 127.20 (3 × −CH−, Py); 122.39 (−CH−, C6), 79.01, 56.65, 56.10, 49.99, 35.76, 31.80, 27.98 (8 × −CH−, C6, C3, C14, C17, C9, C20, C8, C25); 30.71, 39.48, 39.19, 37.10, 36.13, 31.89, 28.84, 28.19, 24.26, 23.81, 21.00 (11 × −CH_2−, C4, C12, C24, C1, C22, C7, C2, C16, C15, C23, C11); 38.02, 29.50, 29.45, 29.31, 29.27, 29.22, 28.64, 27.67, 25.75, 25.71 (10 × −CH_2− chain); 22.80, 22.53, 19.28, 18.67, 11.82 (5 × −CH_3, C27, C26, C21, C19, C18. ^1H NMR, δ, ppm ($CDCl_3$): 9 (Py, d, 2H, −CH_a−); 8.42, (Py, dd, 2H, −CH_b−); 7.8 (Py, dd, 1H, −CH_c−); 5.3 (d, 1H, −C*H*═C−, $J = 5.3$ Hz); 4.4 (m, 1H, −O−C*H*−); 4.05 (m, 4H, CH_2−O−CO and −CH_2−O−SO_3); 2.05−1.0 (m's, 27H, −CH_2−); 2.05−1.0 (m's, 27H, −CH_2−); 1.05−0.69 (m, 15H, −CH_3-). Mp = 112−117 °C.

4-Vinylbenzyl 12-Hydroxydodecanoate. A 100 mL flask equipped with a condenser was charged with 9.2 mmol (2 g) of 12-hydroxydodecanoic acid in DMF (50 mL) with 9.2 mmol (1.4 mL) of 4-vinylbenzyl chloride, 9.2 mmol of K_2CO_3 (1.2 g), 50 mg of BHT to inhibit polymerization, and a trace of KI. The flask was placed in an oil bath and heated under stirring at 80 °C for 40 min until the reaction was complete (followed by TLC: silica gel, ethyl acetate). The cooled mixture was diluted with water and extracted into ether. The ethereal extracts were washed with water (at least three times) and dried over $MgSO_4$, and the residue was recrystallized from methanol/water. The final yield was 63%, and the purity of the product was analyzed by NMR. FT−IR (KBr), cm^{-1}: 3307 (ν_{-OH}); 2920 (ν_{-CH}); 1731 ($\nu_{C=O}$); 1172 ($\nu_{C-O\ alcohol}$). ^{13}C NMR, δ, ppm ($CDCl_3$): 173.69 (−CO−); 136.29 (CH_2═CH−); 135.54 (−C−); 128.41, 126.30, 137.46 (−CH−arom); 114.25 (−CH_2); 63.02 (−CH_2OH); 65.76 (−CH_2−O−CO−); 24.89, 25.68, 29.05, 29.16, 29.36, 29.43, 29.50, 32.74, 34.28 (9 × −CH_2−). ^1H NMR, δ, ppm ($CDCl_3$): 7.33, 7.23 (aromatic AB system, 4H, $J_{A,B}$ = 8.2 Hz, 20 Hz, −C*H*−); 6.67, 6.61 (dd, 1H, J_{trans} = 17.5 Hz, J_{cis}= 11 Hz-CH_{2cis}−); 5.68 (dd, 1H, J_{trans} = 17.48 Hz, −CH_{2trans}−); 5.12 (d, 1H, $J = 10.9$ Hz, −CH_{2cis}−); 3.56 (*t*, 2H, $J = 7$ Hz, −CH_2−OH); 2.27 (*t*, 2H, $J = 8$ Hz, −CH_2−CO−). Mp = 44−47 °C.

Pyridinium 12-(4-Vinylbenzyloxycarbonyl)dodecanesulfate (PS). 1 mmol of 4-vinylbenzyl 12-hydroxydodecanoate and 100 mg of BHT were dissolved in $CHCl_3$ in a flask equipped with a reflux condenser before the addition of 2 mmol of $PySO_3$. The mixture was heated under stirring to 80 °C for

30−40 min, before cooling to room temperature, and finally filtered and washed with hexane, $CHCl_3$ and ether. The purity of the final product was checked by TLC (silica gel, ethyl acetate, $R_f = 0.53$) and NMR. The yield was 90%. FT−IR (KBr), cm^{-1}: 2916 (ν_{-CH}); 1732 ($\nu_{C=O}$); 1249−1219 (2 bands, ν_{-SO_2}). ^{13}C NMR, δ, ppm ($CDCl_3$): 173.6 (−CO−); 145.80, 142.24, 128.39 (−CH− from Py); 135.5, 137 (2 × −C−); 136.28 (CH_2═CH−); 127.17, 126.3 (2 × −CH− arom); 114.26 (−CH_2−); 68.55 (−CH_2OSO_3); 65.76 (−CH_2−O−CO); 34.28, 29.45, 29.36, 29.31, 29.23, 29.18, 29.05, 25.73, 24.89 (−CH_2−). ^1H NMR, δ, ppm ($CDCl_3$): 9.1 (y, d, 2H, −CH_a−); 8.6, (Py, dd, 2H, −CH_b−); 8 (Py, dd, 1H, −CH_c−); 7.5, 7.4 (aromatic AB system, 4H, $J_{A,B}$ = 8.25 Hz, −CH−); 6.8, 6.7 (dd, 1H, J_{trans} = 17.6 Hz, J_{cis} = 11 Hz, CH_{2cis}−), 5.85 (d, 1H, J_{trans} = 18 Hz, CH═CH_{2b}−); 5.35 (d, 1H, J_{cis} = 11 Hz, CH═CH_{2c}); 4.2 (*t*, 2H, J = 7 Hz, −CH_2−OSO_3); 2.4 (*t*, 2H, J = 7.5 Hz, −CH_2−CO−). Mp = 70−80 °C. Cmc = 2.15 mmol/L (measured by conductivity).

Synthesis of PEG Derivatives. Bis(cholesteryloxycarbonyloxy)−PEG (dicholesteryl PEG, 1). Cholesteryl chloroformate (2 g, 4.4 mmol) was dissolved in dry THF (10 mL) containing 0.6 mL of Et_3N. Then a solution containing 2.2 g of poly(ethylene glycol) (PEG; 2.2 mmol; M_w = 1000) in anhydrous THF (5 mL) was added dropwise and the mixture stirred overnight. After filtration and evaporation of the solvent, the product was dissolved in ethyl acetate and precipitated in hexane. TLC conditions for both PEG derivatives were silica gel, eluted with 2-propanol:water, 60:40 v/v. ^1H NMR, δ, ppm ($CDCl_3$): 3.6 (m, PEG−CH_2, 494H); 1.7−2.4 (ms, 8H), 0.8−1.6 (ms, 42H); 0.64 (s, methyl, 3H).

Cholesteryloxycarbonyloxy−PEG Methyl Ether (Monocholesteryl PEG, 2). A similar procedure to that for the synthesis of dicholesteryl PEG was followed, with the exception that equimolar amounts (2.2 mmol) of cholesteryl chloroformate and poly(ethylene glycol) methyl ester (CH_3−O−PEG; M_w = 750) were used. ^1H NMR, δ, ppm ($CDCl_3$): 5.31 (m, CH, 1H); 4.46 (m, CH, 1H); 3.6 (m, PEG-CH_2, 32H); 3.21 (m, −OCH_3, 2H); 2.2−2.4 (m, 2H); 1.7−2.0 (m, 5H); 1.2−1.6 (ms, 12H); 0.8−1.2 (ms, 26H); 0.63 (s, methyl, 3H).

Synthesis of Microparticles. Preparation of the Seed Latex. The seed was prepared by batch emulsion polymerization in a 500 mL three-necked glass reactor equipped with a condenser, a mechanical stirrer, and a gas inlet to maintain a nitrogen atmosphere. The reactor was immersed in an oil bath with thermostatic control to maintain the desired temperature to ±1 °C. The monomer mixture, 9:1 styrene: divinylbenzene (16.7%), (all percentages are by mass) and a solution of $NaHCO_3$ (0.13%) in distilled water (82.3%) were pre-emulsified in the presence of SDS (0.64%) by stirring at 80 °C for at least 10 min, before addition of ammonium peroxodisulfate (APS, 0.13%, dissolved in 2 mL of water) to initiate polymerization. The temperature was maintained at 80 °C for at least 24 h and the stirring rate was 260 rpm. The final latex was filtered and used as prepared in the next step.

Preparation of Surface-Imprinted Core−Shell Particles. A conventional three-necked glass reactor was used. Before charging, 3.43 mL of seed latex (solids content 17.6%) was stirring with divinylbenzene (4.13%), (all percentages are by mass) for 2 h. The reactor was then charged in the following order: first a solution of a mixture of **PS** and **TS** (see Table 1 for quantities) in deionized water (90.9%), followed by the swollen seed latex. This mixture was kept under a nitrogen atmosphere at 65 °C and stirred at 260 rpm for 15 min before the addition of APS (0.058%, dissolved in 2 mL of water). The reaction was maintained under a nitrogen atmosphere at 65 °C for 24 h with stirring before cooling to room temperature. The final latex was filtered and no coagulum was obtained.

Template Removal. By Ultrafiltration ("U"). This was carried out using a 76 mm Amicon YM 10 membrane. The membrane was rinsed with deionized water 4−5 times before use. The latex was placed into a stainless steel ultrafiltration unit and diluted with deionized water under gentle stirring. Pressure was applied (40 psi N_2) to allow passage of water and water-soluble compounds while retaining the polymer particles. Water passing from the unit was collected and the conductivity measured. This procedure was repeated several times by adding pure water to the system until the conductivity of the effluent was equal to that of the pure water. Polymer was recovered from the base of the filtration unit and dried to obtain a colorless powder.

By Hydrolysis, Washing, and Ultrafiltration ("HWU"). The surface-imprinted core−shell latex was suspended in a 1 M solution of NaOH in methanol and heated to reflux in a round-bottomed flask for at least 2 h. The suspension was then filtered (Whatman No. 1 filter paper) to collect the nanoparticles which were washed repeatedly with methanol and isohexane to desorb the surfactant and the cholesterol from the surface of the particles. The particles were finally resuspended in pure water and ultrafiltered as above.

Binding Studies. Batch Binding. Polymers (10 mg) were weighed into 2 mL capacity screw cap vials (Wheaton) fitted with PTFE-lined caps. A 0.5 mM solution of cholesterol in 60: 40 2-propanol:water, (1 mL), 70:30 THF:water (1 mL) or isohexane (1 mL) was added to each vial and the solutions were incubated in a shaker at 20 °C overnight. Solutions were filtered into HPLC vials using 13 mm, 2 μm porosity PTFE-membrane syringe filters (HPLC Technology Ltd., Macclesfield, U.K.) fitted to 5 mL disposable syringes. The concentration of cholesterol remaining in the supernatant was determined by HPLC, calibrated against dilutions of the stock solution.

Flocculation Experiments. The dried polymeric particles were resuspended in the solvent mixture by immersion of the vial containing the mixture in an ultrasonic bath for at least 1 h until no sedimentation of particles was observed. A fixed amount of this resuspension was mixed with a solution containing **1**, **2**, or an equivalent concentration of cholesterol and PEG and left to interact for at least 14 h. After this time, a sample of the mixture was diluted with the appropriate solvent and the diameter measured by dynamic light scattering.

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References and Notes

(1) (a) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812−1832. (b) Sellergren, B. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 1031. (c) Mayes, A. G.; Mosbach, K. *Trends Anal. Chem.* **1997**, *16*, 321−332. (d) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166−173. (e) Vulfson, E. N.; Alexander, C.; Whitcombe, M. J. *Chem. Br.* **1997**, *33*, 23−26. (f) Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468−475. (g) Bartsch, R. A.; Maeda, M. *Molecular and Ionic Recognition with Imprinted Polymers*; ACS Symposium Series 703; American Chemical Society: Washington, DC, 1998.

(2) (a) Shi, H. Q.; Tsai, W. B.; Garrison, M. D.; Ferrari, S.; Ratner, B. D. *Nature* **1999**, *398*, 593−597. (b) Shi, H. Q.; Ratner, B. D. *J. Biomed. Mater. Res.* **2000**, *49*, 1−11.

(3) Aherne, A.; Alexander, C.; Payne, M. J.; Pérez, N.; Vulfson, E. N. *J. Am. Chem. Soc.* **1996**, *118*, 8771−8772.

(4) Alexander, C.; Vulfson, E. N. *Adv. Mater.* **1997**, *9*, 751−755.

(5) D'Souza, S. M.; Alexander, C.; Carr, S. W.; Waller, A. M.; Whitcombe, M. J.; Vulfson, E. N. *Nature* **1999**, *398*, 312−316.

(6) Yu, C.; Ramström, O.; Mosbach, K. *Anal. Lett.* **1997**, *30*, 2123-2140.

(7) Andersson, L. I. *Anal. Chem.* **1996**, *68*, 111−117.

(8) (a) Asanuma, H.; Kakazu, M.; Shibata, M.; Hishiya, T.; Komiyama, M. *Chem. Commun.* **1997**, 1971−1972. (b) Asanuma, H.; Kakazu, R.; Shibata, M.; Hishiya, T.; Komiyama, M. *Supramol. Sci.* **1998**, *5*, 417−421.

(9) Piletsky, S. A.; Andersson, H. S.; Nicholls, I. A. *Macromolecules* **1999**, *32*, 633−636.

(10) Ramström, O.; Ye, L.; Gustavsson, P. E. *Chromatographia* **1998**, *48*, 197−202.

(11) Pérez, N.; Whitcombe, M. J.; Vulfson, E. N. *J. Appl. Polym. Sci.* **2000**, *77*, 1851−1859.

(12) (a) Uezu, K.; Nakamura, H.; Goto, M.; Murata, M.; Maeda, M.; Takagi, M.; Nakashio, F. *J. Chem. Eng. Jpn.* **1994**, *27*, 436−438. (b) Uezu, K.; Nakamura, H.; Kanno, J.; Sugo, T.; Goto, M.; Nakashio, F. *Macromolecules* **1997**, *30*, 3888−3891. (c) Yoshida, M.; Uezu, K.; Goto, M.; Furusaki, S. *J. Appl. Polym. Sci.* **1999**, *73*, 1223−1230. (d) Yoshida, M.; Hatate, Y.; Uezu, K.; Goto, M.; Furusaki, S. *J. Polym. Sci. A, Polym. Chem.* **2000**, *38*, 689−696.

(13) (a) Yoshida, M.; Uezu, K.; Goto, M.; Furusaki, S.; Takagi, M. *Chem. Lett.* **1998**, 925−926. (b) Toorisaka, E.; Yoshida, M.; Uezu, K.; Goto, M.; Furusaki, S. *Chem. Lett.* **1999**, 387−388.

(14) Whitcombe, M. J.; Rodriguez, M. E.; Villar, P.; Vulfson, E. N. *J. Am. Chem. Soc.* **1995**, *117*, 7105−7111.

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