Imprinted Polymers for Selective Adsorption of Cholesterol from Gastrointestinal Fluids

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A series of highly cross-linked terpolymers of methacryloylated cholesterol or bile acid methyl esters, methacrylic acid, and ethyleneglycol dimethacrylate were prepared in the presence or absence of cholesterol acting as a template molecule. The polymers were freed from cholesterol by washing and the resulting adsorbents tested for cholesterol rebinding in the chromatographic mode using methanol−water as mobile phase or in the batch mode using an intestinal mimicking fluid of concentrated bile acids in water. The polymers prepared in polar solvents, favoring the apolar association of the template and the apolar face of the amphiphilic monomers, exhibited selective rebinding of cholesterol as compared to a nonimprinted blank polymer prepared identically but without cholesterol. The strongest rebinding was seen for the polymer prepared using 3β-methacryloyl-cholesterol as the functional monomer. Using a physiologically relevant intestinal-mimicking solution of cholesterol (1 mM), these polymers adsorbed ca. 17 mg cholesterol per gram dry adsorbent, whereas a nonimprinted blank polymer adsorbed ca. 13 mg. The imprinted polymers showed the highest uptake of cholesterol as compared to other adsorbents (<13 mg/g) that were expected to show high affinity for cholesterol.

Introduction

There is overwhelming evidence that hypercholesterolemia is the major risk factor for the early development of atherosclerosis in man and thus the leading cause of coronary heart and peripheral atherosclerotic disease.[1] On the basis of results from various intervention studies it is well-established that drastic lowering of blood cholesterol concentration is followed by a reduction of clinical events, morbidity, and total mortality.

Cholesterol homeostasis is regulated by the amount of cholesterol absorbed from the diet, by hepatic cholesterol synthesis and metabolism, and by hepatic sterol excretion. With the introduction of a new family of substances (HMG-CoA reductase inhibitors) it became possible to increase hepatic LDL receptor activity and thereby cholesterol excretion. Specific inhibitors of cholesterol absorption from the diet are not available so far and attempts to achieve cholesterol reduction by such a strategy were less impressive.[2,3] Because in clinical practice it is often necessary to efficiently interfere with both absorption and excretion of cholesterol to achieve the recommended blood cholesterol concentrations, one approach would be to develop cholesterol-selective adsorbents that are biocompatible, easily accessible, and clinically efficient.

One way of imparting molecular recognition properties to a material is by way of molecular imprinting.[4−6] A few approaches to imprint cholesterol have been described to date.[7−9] Whitcombe et al. showed that cholesterolselective adsorbents can be prepared by a covalent molecular imprinting strategy. The system made use of an easily cleavable carbonate ester linkage between a phenol monomer and cholesterol during polymerization. After polymerization and removal of the template, rebinding was driven by hydrogen bonding between the hydroxyl group of cholesterol and the polymeric phenols.[7] The rebinding of cholesterol to these materials was evaluated in hexane and showed a fairly homogeneous population of binding sites with a dissociation constant of 0.59 mM and a binding capacity of 114 μmol/g. These materials were only evaluated in hexane and no data is given for the rebinding in water. It is also possible to obtain enhanced binding of cholesterol using systems based on noncovalent self-assembly of the monomer and cholesterol.[8,9] Recently, Asanuma et al. described the recognition properties exhibited by polymers prepared by cross-linking of a macrocyclic host, β-cyclodextrin, with diisocyanates in the presence

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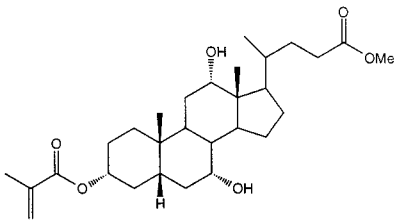
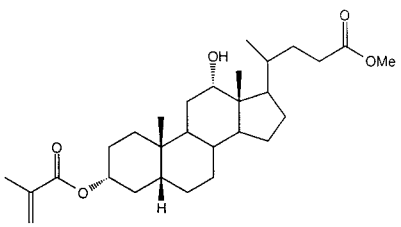
Scheme 1

CAMe1MAA:



DCAMe1MAA:



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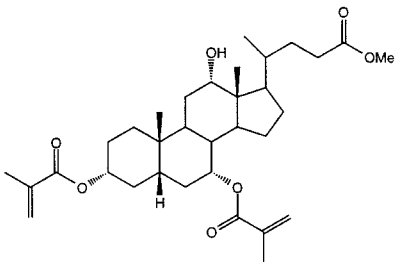

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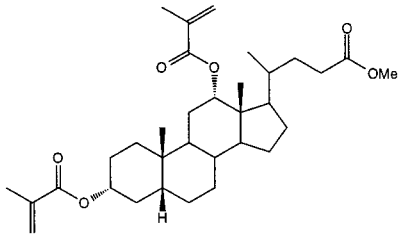
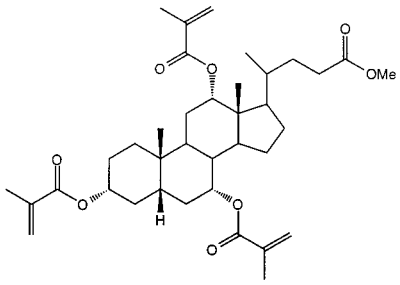
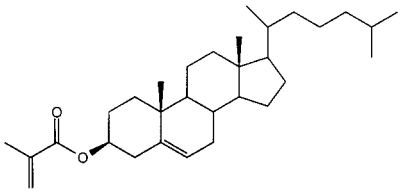


CAMe3MAA:



ChoMAA:



of cholesterol.[9] The materials were capable of rebinding cholesterol also in aqueous media.

Strong and selective rebinding in water depends on the hydrophobic effect and requires a large van der Waals contact area between cholesterol and the host.[10,11] This can be provided by cyclodextrins (vide supra) but, as we describe in this report, may also be obtained by imprinting of cholesterol using amphiphilic monomers under conditions favoring apolar association between the monomers and the template.

Molecular imprinting based on entropically driven association between the functional monomers and the template was previously described in the imprinting of 2,4-dichlorophenoxyacetic acid.[12] In this case, best recognition was seen for materials prepared at higher temperatures using polar protic solvents, all in agreement with expectations on entropically driven associations. Furthermore, a number of examples have shown that compounds can rebind to imprinted sites with a specific hydrophobic driving force.[13,14]

In this report we have synthesized polymerizable derivatives of cholesterol and bile acids (Scheme 1) to be used as amphiphilic monomers in the imprinting of highly cross-linked methacrylates with cholesterol. The polymers have been prepared under conditions favoring apolar intermolecular interactions (Scheme 2) and have been evaluated in the chromatographic mode or in intestinal mimicking fluids in the batch mode.

Experimental Section

Chemicals. The monomers ethylene glycol dimethacrylate (EDMA) (98%) (Aldrich) and methacrylic acid (MAA) (99%) (Aldrich) were purified as previously described.[18] EDMA was washed with 10% sodium hydroxide, dried with anhydrous sodium sulfate, and distilled, whereas MAA was purified by distillation. The initiator α,α′-azobis(isobutyronitril) (AIBN) was obtained from Janssen and purified by recrystallization from methylene chloride and stored dry and cold.

The HPLC-grade ethanol (Aldrich) used in the polymerizations contained 5% 2-propanol and 5% methanol and was stored over 4 Å molecular sieves. The other solvents used (Merck) were of pa grade and were stored over 4 Å molecular sieves, and the water used was collected from a Millipore water purification system.

Cholic acid (≥99%), desoxycholic acid (≥99%), cholesterol (Cho) (from Lanolin, ≥99%), testosterone (Tes) (≥99%), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (∼99%), 3-glycidyloxypropyltrimethoxysilane (≥97%), α-tomatine (lycopersicin) (∼99%), and sodium desoxycholate (≥99%) were purchased from Fluka and used without further purification. Stigmasterol (Sti) (95%), β-sitosterol (Sit) (50%), ergosterol (Erg) (95%), 4-(dimethylamino)pyridine (DMAP) (99%), sodium cholate (98%), acetic acid (99.8%), potassium dihydrogen phosphate (99+%), sodium hydrogen carbonate (99%), sodium hydroxide (97+%), and borotrifluoride−ethyl etherate (redistilled) came from Aldrich.

The enzyme assay used for the determination of free cholesterol (Cholesterol 50) was from Sigma and was stored at 4 °C. The adsorbent Amberlite XAD2000 was purchased from Supelco, whereas LiChrosorb Si 100 (10 μm) was kindly

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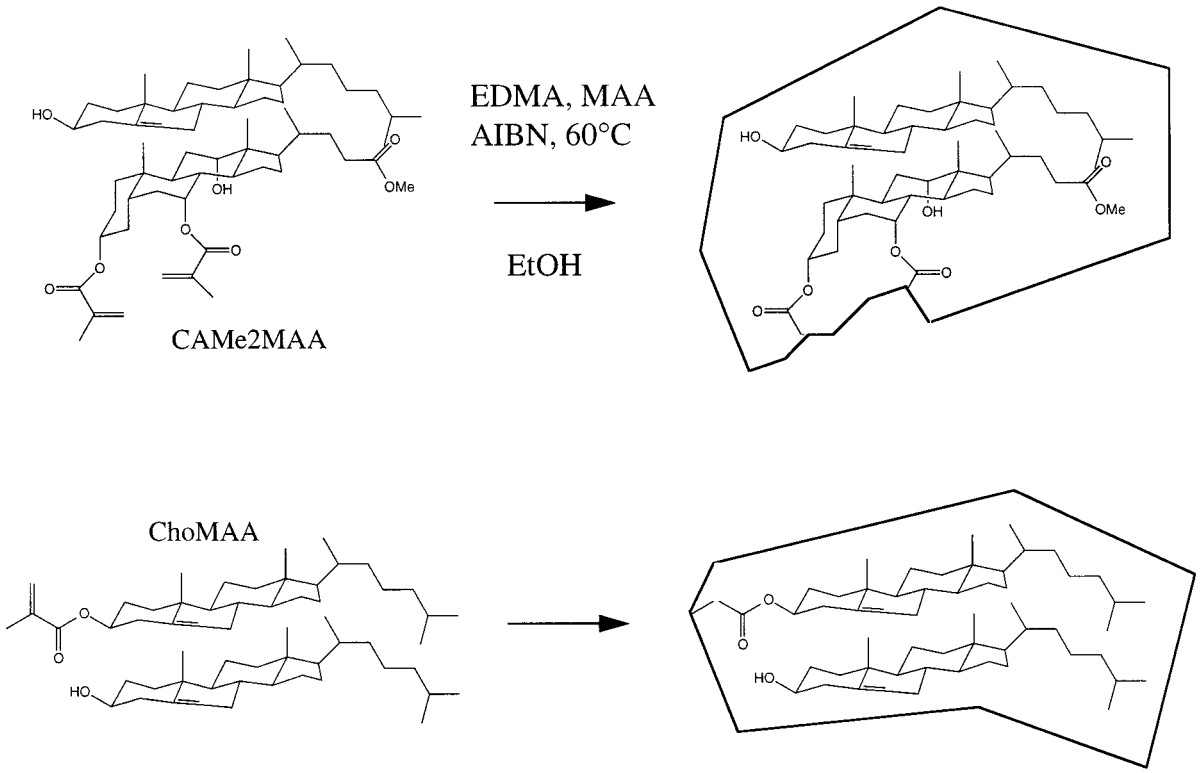
Scheme 2



provided by Dr. K.-F. Krebs (Merck KGaA, Darmstadt, Germany). The C18 modified silicas used in the batch experiments (M5.15, M5.20) were provided by Dipl.-Chem. Frank Grossmann (Universität Mainz).

Equipment and Characterization. The temperature during polymerization was regulated using an automated thermostat (Fryka-Therm FT 800). The UV lamp used in the photopolymerization was a high-pressure mercury vapor lamp (Philips, HPK 125 W). The polymers were ground in a ball mill (Pulverizette 05.202, Fritsch) and sieved using stainless steel sieves from Retsch (DIN 4188). The polymers were packed in chromatographic columns using an air-driven liquid pump (Haskel DSTV-122). The chromatographic evaluations of the imprinted polymers were done using a Hewlett-Packard instrument (HP1050) equipped with a quaternary pump, an autosampler, a variable wavelength detector, and an HP workstation. The pore- and surface area analysis was done by nitrogen sorption using a ASAP 2010 instrument from Micromeritics, and the scanning electron micrographs were obtained at the University of Mainz pathology department. The polymer density was estimated by weighing an amount of polymer (25−36 μm) corresponding to 1 mL into graduated NMR tubes. The swelling was then estimated by equilibrating the polymer in acetonitrile overnight followed by tapping until no further change in bed height was observed. The amount of extracted template was determined after two different extraction procedures. In the first, the polymers were stirred in methanol for 2 × 24 h followed by evaporation of the extracts and enzymatic assay for cholesterol. In the second, the polymers were subjected to consecutive Soxhlet extractions for 5 h in methanol and methylene chloride, respectively, and then drying of the polymer at 60 °C overnight. The extracts were evaporated and redissolved in $CDCl_3$ containing benzene as internal standard. The cholesterol content was then estimated by comparing the ^{1}H NMR integrals.

The log *P* values of the steroids were estimated by the incremental method using the software ACD/log P 1.13 (Toronto, Canada), available on the Internet.

Synthesis of Imprinted Polymers. The polymers were synthesized following the general imprinting protocol shown in Scheme 2 and the monomer compositions given in Table 1. A typical procedure would be as follows. EDMA (5.88 g, 30 mmol), MAA (0.52 g, 6 mmol), and the steroid monomer (3 mmol) were dissolved in ethanol or dichloromethane (9 mL). For the imprinted polymers, cholesterol (0.58 g, 1.5 mmol) was added and the solution gently heated. Thereafter, the initiator AIBN (50 mg) was added. The clear solution was transferred to a thick-walled glass polymerization tube, cooled on ice, degassed by sparging with nitrogen gas for 10 min, and then sealed. In the thermochemically initiated polymerizations, the tube was immersed into a water bath maintained at 60 °C (ethanol) or 38 °C (dichloromethane). In the photochemically initiated polymerizations, the tubes were allowed to equilibrate at 10 °C for 10 min. Then the tubes were irradiated using a high-pressure Hg lamp and rotating the tube once 180° within the first 15 min. The polymerization time was in all cases 16 h. Following polymerization, the polymer monolith was

Table 1. Preparation of Adsorbents for the Cholesterol Binding Experiments[a]

polymer	steroid monomer	template	porogen	procedure
P1	DCAMe1MAA	cholesterol	EtOH	60 °C
P2	DCAMe1MAA		EtOH	60 °C
P3	DCAMe1MAA	cholesterol	CH_2Cl_2	38 °C
P4	DCAMe1MAA		CH_2Cl_2	38 °C
P5	DCAMe2MAA	cholesterol	EtOH	60 °C
P6	DCAMe2MAA		EtOH	60 °C
P7		cholesterol	CH_2Cl_2	photo. 10 °C
P8			CH_2Cl_2	photo. 10 °C
P9	DCAMe2MAA	cholesterol	CH_2Cl_2	photo. 10 °C
P10	DCAMe2MAA		CH_2Cl_2	photo. 10 °C
P11	CAMe2MAA	cholesterol	EtOH	60 °C
P12	CAMe2MAA		EtOH	60 °C
P13	ChoMAA	cholesterol	EtOH	60 °C
P14	ChoMAA		EtOH	60 °C

[a] The polymers were prepared as described in the Experimental Section using EDMA (5.88 g, 30 mmol) as cross-linking monomer, the steroid monomer (3 mmol), and MAA (0.52 g, 6 mmol). The templated polymers were prepared in the presence of cholesterol (0.58 g, 1.5 mmol). The porogen was either ethanol or dichloromethane (9 mL) and the polymers were polymerized either by thermochemical initiation at 60 °C or 38 °C or photochemically at 10 °C.

crushed in a mortar and then ground in wetted state by means of a mechanical ball mill followed by sieving. The procedure was optimized to obtain the maximum yield of the required size fraction, 25−35 μm.

Synthesis of Tomatin−Silica Adsorbents. *Synthesis of Epoxy−Silica*. To a suspension of LiChrosorb Si 100 (10 μm, 5 g) in toluene (200 mL) was added 3-glycidyloxypropyltrimethoxysilane (18 g, 76 mmol) dropwise. After heating of the suspension to reflux for 5 h, the gel was filtered off and washed with acetone, methanol, acetone, and diethyl ether followed by drying under vacuum. Yield: 5.91 g of dry solid. Anal. Found: C, 7.74; H, 1.64.

Synthesis of Tomatin-Silica.[15] To a solution of α-tomatin (lycopersicin) (200 mg, 0.19 mmol) in 20 mL of 1,4-dioxane was added dry epoxy−silica (1 g). After addition of borotrifluoride−ethyl etherate (1 mL), the reaction was left for 48 h at room temperature and the flask was shaken three times. The modified silica was filtered and washed with 1,4-dioxane, methanol, methanol/water, methanol, 1,4-dioxane, and diethyl ether. Thereafter it was dried at 80 °C under vacuum. Yield: 1.05 g of dry solid. Anal. Found: C, 10.98; H, 2.13; N, 0.07.

Synthesis of Steroid Monomers. *Cholic Acid Methyl Ester*. Cholic acid (40 g, 98 mmol) was dissolved in methanol (200 mL) followed by addition of 1.5 mL of concentrated HCl and heating of the solution to reflux for 30 min. This resulted in a dark yellow solution. After leaving the solution at room temperature, crystallization started. The solution was left for 48 h at 4−8 °C and thereafter filtered cold. This gave 35.8 g of colorless crystals. Yield: 86.1%. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.64 (s, 3H, 18-H_3), 0.85 (s, 3H, 19-H_3), 0.94 (d, 3H, 21-H_3, $J_{21-H,20-H}$ = 6 Hz), 1.33−1.86 (steroid), 2.16 (m, 2H, 22-H_2), 2.34 (m, 2H, 23-H_2), 3.43 (m, 1H, 3β-H), 3.63 (s, 3H, COOCH_3), 3.80 (s, 1H, 7β-H), 3.92 (s, 12β-H). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.25 (18-C), 17.09 (21-C), 22.23 (19-C), 23.02, 26.09, 27.31, 27.92, 30.09, 30.72, 30.91, 34.45, 34.57, 35.11, 39.27, 41.28, 41.37, 46.20, 46.77, 50.33, 51.29 (COOCH_3), 68.25 (7-C) 71.68 (3-C), 72.89 (12-C), 174.66 (COOCH$_3$). EI-MS: *m*/*z* (%): 422 (1; M^+), 404 (7; $M^+ - H_2O$), 386 (100; $M^+ - 2\ H_2O$), 371 (10; 386 − CH_3), 368 (37; $M^+ - 3\ H_2O$), 355 (10; 386 − OCH_3), 353 (14; 368 − CH_3), 271 (33), 253 (16). IR (KBr): ν/cm^{-1} = 3401, 2934, 2870, 1738, 1447, 1379, 1171, 1080, 1032, 982. Anal. Calcd for $C_{25}H_{42}O_5$ (422.61): C, 71.05, H, 10.02. Found: C, 70.89; H, 10.14

Desoxycholic Acid Methyl Ester. Desoxycholic acid (50 g, 127 mmol) was dissolved in 200 mL of methanol under gentle heating followed by addition of 1.5 mL of concentrated HCl and heating of the solution to reflux for 30 min. This resulted in a red-brown solution. After addition of a small volume of water, the solution was left for 48 h at 4−8 °C, and thereafter crystals were separated by filtration. This gave 37.9 g of colorless crystals. Yield: 73.3%. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.64 (s, 3H, 18-H_3), 0.87 (s, 3H 19-H_3), 0.92 (d, 3H, 21-H_3, $J_{21-H,20-H}$ = 5.7 Hz), 1.21−1.79 (steroid), 2.19 (m, 2H, 22-H_2), 2.33 (m, 2H, 23-H_2), 3.56 (m, 1H, 3β-H), 3.62 (s, 3H, COOCH_3), 3.94 (s, 1H, 12β-H). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.50 (18-C), 17.04 (21-C), 22.92 (19-C), 23.48, 25.93, 26.94, 27.29, 28.42, 30.18, 30.69, 30.92, 33.38, 33.90, 35.02, 35.06, 35.81, 36.18, 41.87, 46.27, 47.02, 47.98, 51.29 (COOCH_3), 71.46 (3-C), 72.89 (12-C), 174.55 (COOCH$_3$). EI-MS: *m*/*z* (%): 406 (4; M^+), 388 (59; $M^+ - H_2O$), 370 (100; $M^+ - 2\ H_2O$), 357 (19; 388 − OCH_3), 355 (27; 370 − CH_3), 273 (72), 255 (60). IR (KBr): ν/cm^{-1} = 3432, 2938, 2864, 1742, 1449, 1377, 1169, 1044. Anal. Calcd for $C_{25}H_{42}O_4$ (406.61): C, 73.85; H, 10.41. Found: C, 73.61; H, 10.52.

3α-Methacryloyldesoxycholic Acid Methyl Ester (DCAMe1MAA). Desoxycholic acid methyl ester, (6 g, 14.8 mmol), DCC, (3.36 g, 16.3 mmol), and DMAP (0.21 g, 1.7 mmol) were dissolved in dichloromethane (100 mL). Thereafter, MAA (1.41 g, 16.4 mmol) was added dropwise and the reaction allowed to proceed overnight with stirring. The dicyclohexylurea was filtered off and the solution washed with water, 5% acetic acid, 0.5 N sodium bicarbonate, and brine (each 60 mL). After drying of the organic phase with anhydrous sodium sulfate the solution was taken down, resulting in a white solid. The solid was treated with water (80 mL) and heated to 50 °C for 15 min and the remaining solid was filtered and dried. Yield: 5.93 g (84.4%) of a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.65 (s, 3H, 18-H_3), 0.89 (s, 3H 19-H_3), 0.94 (d, 3H, 21-H_3, $J_{21-H,20-H}$ = 6.2 Hz), 1.02−1.95 (steroid), 1.89 (s, 3H, 3-O(CO)C(CH_3)=CH_2), 2.22 (m, 2H, 22-H_2), 2.33 (m, 2H, 23-H_2), 3.63 (s, 3H, COOCH_3), 3.96 (s, 1H, 12β-H), 4.74 (m, 1H, 3β-H), 5.47, 6.03 (s, 1H, 3-O(CO)C(CH$_3$)=CH_2). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.52 (18-C), 17.12 (21-C), 18.14 (3-O(CO)C(CH_3)=CH_2), 22.93 (19-C), 23.39, 25.80, 26.34, 26.77, 27.22, 28.57, 30.69, 30.82, 31.98, 33.44, 33.50, 33.94, 35.78, 41.69, 46.28, 47.13, 48.10, 51.28 (COOCH_3), 57.93, 72.93 (3-C), 74.32 (12-C), 124.75 (3-O(CO)C(CH$_3$)=CH_2), 136.68 (3-O(CO)C(CH$_3$)=CH_2), 166.85 (3-O(CO)C(CH$_3$)=CH_2), 174.49 (COOCH$_3$). EI-MS: *m*/*z* (%): 474 (1; M^+), 456 (2; $M^+ - H_2O$), 388 (18; M^+ − MAA), 370 (49; 388 − H_2O), 355 (15; 370 − CH_3), 341 (17), 273 (16), 255 (100). IR (KBr): ν/cm^{-1} = 3551, 2934, 2864, 1739, 1699, 1630, 1452, 1298, 1188, 1044. Anal. Calcd for $C_{29}H_{46}O_5$ (474.68): C, 73.38; H, 9.77. Found: C, 73.15; H, 9.81.

3α,12α-Dimethacryloyldesoxycholic Acid Methyl Ester (DCAMe2MAA). Starting with desoxycholic acid methyl ester (6.1 g, 15 mmol), DCC (6.82 g, 33 mmol), DMAP, (0.4 g, 3.3 mmol), and MAA (2.92 g, 34 mmol), the synthesis took place as described for DCAMe1MAA. The white residue obtained after evaporation was recrystallized from 25 mL of acetone. Yield: 7.13 g (87.6%) of colorless crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.69 (s, 3H, 18-H_3), 0.94 (s, 3H 19-H_3), 0.97 (d, 3H, 21-H_3, $J_{21-H,20-H}$ = 6 Hz), 1.18−2.01 (steroid), 1.93 (s, 3H, 3-O(CO)C(CH_3)=CH_2), 1.99 (s, 3H, 12-O(CO)C-(CH_3)=CH_2), 2.24 (m, 2H, 22-H_2), 2.41 (m, 2H, 23-H_2), 3.67 (s, 3H, COOCH_3), 4.78 (m, 1H, 3β-H), 4.93 (s, 1H, 12β-H), 5.53, 6.08 (s, 1H, 3-O(CO)C(CH$_3$)=CH_2). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.54 (18-C), 17.15 (21-C), 18.15 (3-O(CO)C(CH$_3$)=CH_2), 19.81 (12-O(CO)C(CH$_3$)=CH_2), 22.94 (19-C), 24.47, 25.33, 25.81, 26.25, 26.36, 28.53, 30.70, 30.83, 32.43, 33.52, 33.96, 35.80, 41.71, 46.30, 47.17, 48.13, 51.30 (COOCH_3), 58.12, 72.98 (12-C), 74.30 (3-C), 124.74 (3-O(CO)C(CH$_3$)=CH_2), 136.70 (3-O(CO)C(CH$_3$)=CH_2), 166.85 (3-O(CO)C(CH$_3$)=CH_2), 174.48 (COOCH$_3$). EI-MS: *m*/*z* (%): 456 (15; M^+ − MAA), 438 (1; 456 − H_2O), 370 (96; M^+ − 2 MAA), 355 (17; 370 − CH_3), 341 (12), 255 (100). IR (KBr): ν/cm^{-1} = 3061, 2936, 2859, 1709, 1649, 1452, 1350, 1175, 1017. Anal. Calcd for $C_{33}H_{50}O_6$ (542.75): C, 73.03; H, 9.29. Found: C, 72.91; H, 9.43.

3α-Methacryloylcholic Acid Methyl Ester (CAMe1MAA).[16b] Starting with cholic acid methyl ester (7.2 g, 17 mmol), DCC (3.87 g, 18.7 mmol), MAA (1.61 g, 18.7 mmol), and DMAP (0.24 g, 1.9 mmol), the synthesis took place as described for DCAMe1MAA. The solid obtained after evaporation was recrystallized from 40 mL of ethyl acetate. Yield: 7.70 g (92%) of white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.65 (s, 3H, 18-H_3), 0.86 (s, 3H 19-H_3), 0.96 (d, 3H, 21-H_3, $J_{21-H,20-H}$ = 5.5 Hz), 1.02−2.08 (steroid), 2.02 (s, 3H, 3-O(CO)C-(CH_3)=CH_2), 2.19 (m, 2H, 22-H_2), 2.35 (m, 2H, 23-H_2), 3.63 (s, 3H, COOCH_3), 3.96 (s, 1H, 12β-H), 4.74 (m, 1H, 3β-H), 5.51 (d, 1H, 3-O(CO)C(CH$_3$)=CH_2, $J_{vinyl-H}$ = 16 Hz). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.25 (18-C), 17.09 (21-C), 18.07 (3-O(CO)C-(CH$_3$)=CH_2), 22.24 (19-C), 23.01, 24.46, 26.13, 27.30, 27.93, 30.14, 30.88, 32.39, 34.46, 34.55, 35.08, 39.28, 41.28, 41.41, 46.21, 46.77, 51.28 (COOCH_3), 57.94, 68.24 (7-C), 71.66 (3-C), 72.86 (12-C), 124.69 (3-O(CO)C(CH$_3$)=CH_2), 136.71 (3-O(CO)-C(CH$_3$)=CH_2), 166.84 (3-O(CO)C(CH$_3$)=CH_2), 174.56 (COOCH$_3$). EI-MS: *m*/*z* (%): 454 (1; $M^+ - 2\ H_2O$), 404 (1; M^+ − MAA), 386 (7; 404 − H_2O), 368 (6; 368 − H_2O), 271 (6), 253 (7). IR (KBr): ν/cm^{-1} = 3509, 3067, 2934, 2857, 1739, 1697, 1628, 1452, 1348, 1215, 1080, 910. Anal. Calcd for $C_{29}H_{46}O_6$ (490.68): C, 70.99; H, 9.45. Found: C, 70.64; H, 9.42.

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3α,7α-Dimethacryloylcholic Acid Methyl Ester (CAMe2MAA). Cholic acid methyl ester (6.4 g, 15 mmol), DCC (6.86 g, 33 mmol), MAA (2.92 g, 34 mmol) and DMAP (0.4 g, 3.3 mmol) were dissolved in dichloromethane (100 mL). The synthesis was carried out as described for DCAMe1MAA. The white amorphous solid obtained after evaporation of dichloromethane was recrystallized from 50 mL of acetone/water 85/15 (v/v). Yield: 5.87 g (69.4%) of white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.65 (s, 3H, 18-H$_3$), 0.86 (s, 3H 19-H$_3$), 0.92 (d, 3H, 21-H$_3$, $J_{21-H,20-H}$ = 3.5 Hz), 1.12−2.12 (steroid), 1.89 (s, 3H, 3-O(CO)C(C*H*$_3$)=CH$_2$), 1.94 (s, 3H, 7-O(CO)C(C*H*$_3$)=CH$_2$), 2.24 (m, 2H, 22-H$_2$), 2.31 (m, 2H, 23-H$_2$), 3.61 (s, 3H, COOC*H*$_3$), 3.95 (s, 1H, 12β-H), 4.66 (m, 1H, 3β-H), 5.00 (s, 1H, 7β-H), 5.48 (d, 2H, 3-O(CO)C(CH$_3$)=C*H*$_2$, 7-O(CO)C(CH$_3$)=C*H*$_2$, $J_{vinyl-H}$ = 16 Hz), 6.02 (d, 2H, 3-O(CO)C(CH$_3$)=C*H*$_2$, 7-O(CO)C(CH$_3$)=C*H*$_2$, $J_{vinyl-H}$ = 16 Hz). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.57 (18-C), 17.08 (21-C), 18.07 (3-O(CO)C(*C*H$_3$)=CH$_2$), 19.72 (7-O(CO)C(*C*H$_3$)=CH$_2$), 22.24 (19-C), 23.01, 24.49, 25.02, 26.13, 26.37, 27.27, 30.49, 30.64, 30.80, 32.29, 34.49, 35.04, 39.19, 41.05, 46.28, 46.92, 51.25 (COO*C*H$_3$), 57.46, 67.98 (7-C), 72.75 (3-C), 74.47 (12-C), 124.61 (3-O(CO)C(CH$_3$)=*C*H$_2$), 125.14 (7-O(CO)C(CH$_3$)=*C*H$_2$), 136.48 (7-O(CO)*C*(CH$_3$)=CH$_2$), 136.68 (3-O(CO)*C*(CH$_3$)=CH$_2$), 166.80 (3-O(*C*O)C(CH$_3$)=CH$_2$),166.67 (7-O(*C*O)C(CH$_3$)=CH$_2$), 174.56 (*C*OOCH$_3$). EI-MS: *m*/*z* (%): 292 (6), 211 (15), 167 (9), 98 (22), 86 (24), 69 (100). IR (KBr): ν/cm^{-1} = 3542, 2936, 2863, 1739, 1628, 1448, 1331, 1175, 1074, 1016, 912. Anal. Calcd for $C_{33}H_{50}O_7$ (558.75): C, 70.94; H, 9.02. Found: C, 70.76; H, 9.04.

3α,7α,12α-Trimethacryloylcholic Acid Methyl Ester (CAMe3MAA). Cholic acid methyl ester (6.8 g, 16 mmol), DCC (10.95 g, 53 mmol), MAA (4.56 g, 53 mmol) and DMAP (0.7 g, 5.7 mmol) were dissolved in dichloromethane (100 mL) and stirred overnight. The synthesis was carried out as described for DCAMe1MAA. The organic phase was washed with water, 5% acetic acid, saturated sodium bicarbonate solution, water, and brine and then dried over anhydrous sodium sulfate. The white amorphous solid obtained after evaporation was recrystallized from 30 mL of acetone. Yield: 6.42 g (76.6%) of a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.64 (s, 3H, 18-H$_3$), 0.90 (s, 3H 19-H$_3$), 0.92 (d, 3H, 21-H$_3$, $J_{21-H,20-H}$ = 8.0 Hz), 1.12−2.27 (steroid), 1.85 (s, 3H, 3-O(CO)C(C*H*$_3$)=CH$_2$), 1.91 (s, 3H, 12-O(CO)C(C*H*$_3$)=CH$_2$), 1.94 (s, 3H, 7-O(CO)C(C*H*$_3$)=CH$_2$), 2.27 (m, 2H, 22-H$_2$), 2.31 (m, 2H, 23-H$_2$), 3.61 (s, 3H, COOC*H*$_3$), 4.61 (m, 1H, 3β-H), 4.95 (s, 1H, 7α-H), 5.09 (s, 1H, 12β-H), 5.48 (d, 3H, 3-O(CO)C(CH$_3$)=C*H*$_2$, 7-O(CO)C(CH$_3$)=C*H*$_2$, 12-O(CO)C(CH$_3$)=C*H*$_2$, $J_{vinyl-H}$ = 15 Hz), 6.04 (t, 3H, 3-O(CO)C(CH$_3$)=C*H*$_2$, 7-O(CO)C(CH$_3$)=C*H*$_2$, 12-O(CO)C(CH$_3$)=C*H*$_2$, $J_{vinyl-H}$ = 16 Hz, 16 Hz). ^{13}C NMR ($CDCl_3$): δ/ppm = 12.30 (18-C), 17.16 (21-C), 18.05 (3-O(CO)C(*C*H$_3$)=CH$_2$), 18.27 (12-O(CO)C(*C*H$_3$)=CH$_2$), 19.79 (7-O(CO)C(*C*H$_3$)=CH$_2$), 22.34 (19-C), 22.77, 24.46, 25.04, 25.32, 26.23, 26.45, 27.04, 30.55, 30.81, 32.40, 34.50, 34.74, 38.21, 41.94, 47.02, 49.31, 51.29 (COO*C*H$_3$), 58.01, 70.90 (7-C), 72.40 (3-C), 73.88 (12-C), 125.16 (3-O(CO)C(CH$_3$)=*C*H$_2$, 7-O(CO)C(CH$_3$)=*C*H$_2$, 12-O(CO)C(CH$_3$)=*C*H$_2$), 136.68 (3-O(CO)*C*(CH$_3$)=CH$_2$, 7-O(CO)*C*(CH$_3$)=CH$_2$, 12-O(CO)*C*(CH$_3$)=CH$_2$), 166.71 (3-O(*C*O)C(CH$_3$)=CH$_2$, 7-O(*C*O)C(CH$_3$)=CH$_2$, 12-O(*C*O)C(CH$_3$)=CH$_2$), 174.56 (*C*OOCH$_3$). EI-MS: *m*/*z* (%): 292 (6), 211 (15), 167 (9), 98 (22), 86 (24), 69 (100). IR (KBr): ν/cm^{-1} = 2934, 2858, 1739, 1699, 1628, 1452, 1333, 1194, 1015, 910. Anal. Calcd for $C_{33}H_{50}O_7$ (626.83): C, 70.90; H, 8.68. Found: C, 70.72; H, 8.59.

3β-Methacryloylcholesterol (ChoMAA). Cholesterol (5 g, 13 mmol), DCC (3.5 g, 17 mmol), MAA (1.45 g, 17 mmol), and DMAP (0.2 g, 1.7 mmol) were dissolved in dichloromethane (100 mL) and stirred for 48 h at room temperature. After filtereing off of the dicyclohexylurea, the organic phase was washed with water, 5% acetic acid, saturated sodium bicarbonate solution, water, and brine and then dried over anhydrous sodium sulfate. The colorless oil obtained after evaporation was recrystallized from 20 mL of ethyl acetate. Yield: 5.56 g (94%) of white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm = 0.63 (s, 3H, 18-H$_3$), 0.83 (d, 3H, 27-H$_3$, $J_{27-H,25-H}$ = 1.7 Hz), 0.88 (d, 3H, 21-H$_3$, $J_{26-H,25-H}$ = 6.5 Hz), 0.99 (s, 3H, 19-H$_3$), 1.04−2.32 (steroid), 1.89 (s, 3H, 3-O(CO)C(C*H*$_3$)=CH$_2$),4.61 (m, 1H, 3α-H), 5.33 (m, 1H,6-H), 5.48 (s, 1H, 3-O(CO)C(CH$_3$)=C*H*$_2$), 6.03 (s, 1H, 3-O(CO)C(CH$_3$)=C*H*$_2$). ^{13}C NMR ($CDCl_3$): δ/ppm = 11.62 (18-C), 18.13 (21-C), 18.51 (3-O(CO)C(*C*H$_3$)=CH$_2$), 19.14 (21-C), 20.84 (19-C), 22.36, 22.67, 23.64, 24.08, 27.57, 28.03, 27.04, 31.67, 31.74, 35.59, 35.98, 36.80, 37.91, 39.38, 39.54, 42.11, 49.82, 55.93, 56.49, 56.57, 74.01, 122.44 (6-C), 124.74 (3-O(CO)C(CH$_3$)=*C*H$_2$), 136.66 (3-O(CO)*C*(CH$_3$)=CH$_2$), 139.49 (5-C), 166.67 (−O(*C*O)C(CH$_3$)=CH$_2$). EI-MS: *m*/*z* (%): 375 (18), 369 (24; M$^+$ − MAA), 354 (66; 369 − CH$_3$), 129 (89), 117 (100), 91 (81). IR (KBr): ν/cm^{-1} = 2936, 2854, 1721, 1649, 1466, 1375, 1294, 1169, 1013, 935. Anal. Calcd for $C_{31}H_{52}O_2$ (454.73): C, 81.52; H, 11.48. Found: C, 81.51; H, 11.41.

Chromatographic Evaluation of the Imprinted Polymers. The polymer particles (size 25−36 μm) were sedimented in 100 mL of methanol followed by sedimentation twice in methanol/water 80/20 (v/v), the second time accompanied by sonication. The particles were then slurry-packed into HPLC columns (125 × 4 mm, Merck) in 80% aqueous methanol at pressures of 200−300 bar.

The columns were equilibrated in methanol until a stable baseline was achieved, usually within 30−45 min. The flow rate was 1 mL/min, the UV detector wavelength was 220 nm (cholesterol, stigmasterol, and β-sitosterol), 271 nm (ergosterol), 241 nm (testosteron), or 254 nm (acetone), and the chromatography was run at room temperature with duplicate injections unless otherwise stated. The retention, k', was calculated as $k' = (t - t_o)/t_o$ where t_o is the elution time of the void marker acetone, which normally eluted as a sharp peak with a maximum plate number, N, of approximately 10 000/m. A volume of 10 μL of stock solutions of the steroids (2 mg/10 mL) in the mobile phase was injected separately.

Batch Rebinding Experiments in Intestinal-Mimicking Medium. *Preparation of Intestinal-Mimicking Medium (A).* To 125 mL of a 0.2 M potassium dihydrogen phosphate solution and 95 mL of a 0.2 M sodium hydroxide solution was added 200 mL of water. Then 24.5 g of sodium desoxycholate (NaDC) and 16.5 g sodium cholate (NaC) were added and dissolved by stirring. This gave the solution a light yellow color. The pH was adjusted to 7.5 ± 0.1 with 0.2 M sodium hydroxide and water added to a final volume of 500 mL. After sparging with nitrogen for 30 min, the solution was stored in darkness at room temperature.

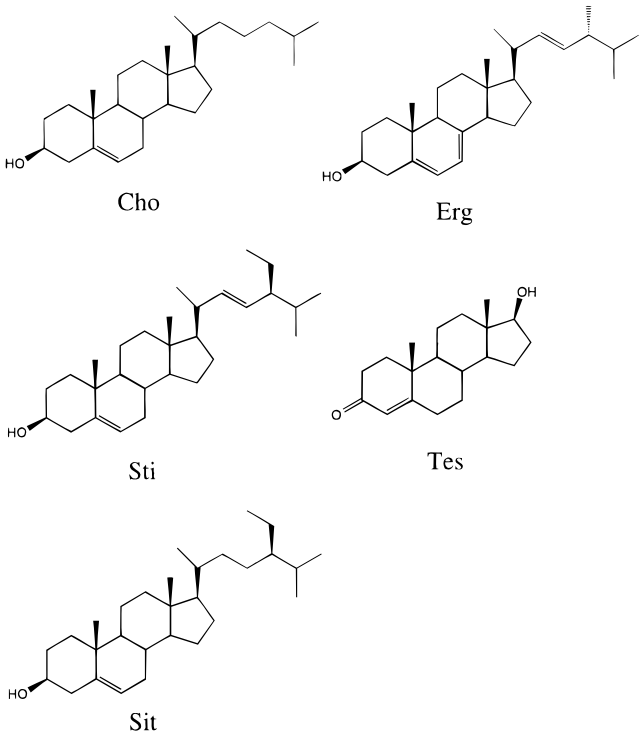
Preparation of Cholesterol Standard Solution (B). To 500 mL of A was added cholesterol (901.7 mg) and the solution treated for 3 h at 50 °C under sonication. The solution was then sparged with nitrogen for 30 min and stored in darkness at room temperature.

Rebinding Experiment. The dry adsorbents (30 mg) were weighed into 20 mL glass vials, and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, or 5.0 mL of the cholesterol standard solution (B) was added followed by addition of solution A to a final volume of 5 mL. The samples were then stirred in a circularly shaking bath at 37 °C for 24 h. After sedimentation of the adsorbents, 30 μL of the supernatant was transferred to an enzyme reagent solution (3 mL) (Sigma kit no. 352-50) and left for 5 min at 37 °C for completion of the reaction. The absorbance at 500 nm was then measured. The amount of cholesterol adsorbed was calculated by subtraction using a calibration curve obtained from the same experiment leaving out the adsorbent. The experiment was repeated at least twice for each adsorbent.

Results and Discussion

Functional Monomer Design. Cholesterol is a hydrophobic steroid (estimated log P_{ow} = 9.8) with a relatively planar structure and with one polar hydroxyl substituent in the A ring (Schemes 2 and 3). As in the biological recognition elements, efficient recognition of hydrophobic molecules is achieved using hosts with lipophilic binding sites offering a large apolar contact area toward the guest.[10,11] As shown in a previous work, molecular imprinting can produce sites capable of

Scheme 3



discriminating cholesterol from epicholesterol, its 3-OH epimer, in hexane.[7] Addition of only small amounts of more polar solvents completely supressed the selective binding. In water, no synthetic recognition element exhibits, to our knowledge, this kind of selectivity. In this work our objective was to explore the steroid backbone as a building block in the templating of recognition sites for cholesterol. Bile acids belong to one class of easily accessible steroids that possess amphiphilic properties with a polar face that can be further derivatized.[2,10] They have therefore been used in the construction of macrocycles for molecular recognition.[10] Furthermore they have, as their monomethacrylate derivatives, been copolymerized with polar methacrylate monomers to form random bile acid containing copolymers.[16] In the light of these facts, we considered them a suitable first choice in the cholesterol-templating work. We thus synthesized a number of mono-, di-, and trimethacrylate-substituted bile acid methyl esters (Scheme 1). Due to the low reactivity of the 7α and 12α OH groups, acylation using methacryloyl anhydride or methcryloyl chloride failed. In this case only DMAP-catalyzed esterification gave the desired products in good yield. Parallel to the synthesis of the bile acid derivatives, 3*β*-methacryoloylcholesterol was also synthesized. In view of the crystal structure of cholesterol,[17] it may itself provide the most complementary surface for binding cholesterol.

Polymer Synthesis and Physical Characterization. Polymers imprinted with cholesterol were synthesized following a previously described procedure with some modifications.[18] The polymers were all prepared by free radical terpolymerization of a mixture of methacrylic acid (MAA), the cross-linking monomer ethylene glycol dimethacrylate (EDMA), and the steroid monomer as described in the Experimental Section and summarized in Table 1. MAA was used in order to obtain hydrogen bonding to the cholesterol hydroxyl group and to provide the polymer with negative charges for repelling of the bile acids in the intestine. By the use of polar protic solvents it was anticipated that apolar association of the steroid monomers and cholesterol would be favored. Using an excess of functional monomer intermolecular assemblies of the type shown in Scheme 2 would provide the hydrophobic binding sites necessary for a strong and selective rebinding of cholesterol. Obviously this relies on a preference for intermolecular association of the type A−B at the expense of self-association, i.e., A−A and B−B, respectively. After polymerization, the polymers were freed from cholesterol by washing with methanol at room temperature. This was compared with a Soxhlet extraction in methanol and dichloromethane. The yield of cholesterol after a wash of the polymers in methanol at room temperature was 57% for P11, 53% for P13, and 39% for P9, whereas after the Soxhlet extraction cholesterol was quantitatively extracted from P13. P11−P14 were characterized by nitrogen sorption analysis, scanning electron microscopy (SEM), and swelling measurements (Table 2**)**. This revealed small differences between the imprinted and nonimprinted polymers and larger differencies between the polymers prepared using the different porogens. First of all, previous characterization of linear copolymers of MAA and 3α-methacryloylcholic acid showed a high yield of polymerization and a random incorporation of the monomers. In the present work, the NMR spectra of the Soxhlet extracts showed no peaks that could be assigned to unreacted monomer. The swelling and porosity of the polymers were in agreement with previous observations.[18] Thus, polymers prepared using ethanol as porogen can be characterized as macroporous with relatively low swelling, whereas polymers prepared using dichloromethane as porogen are gel-like with low porosity in the dry state and a high swellability. Typically the nitrogen adsorption isotherms as well as the SEMs of the ethanol polymers (Figure 1) indicated a significant amount of pores with a diameter larger than 1000 Å. Furthermore, these pores appeared more frequently in the imprinted compared to the nonimprinted polymers. The former polymers showed furthermore a larger swelling in acetonitrile and a higher dry density than the latter polymers. Previously small differences between imprinted and blank polymers have been observed.[18] These have been explained by considering the cross-linking function of the template. After its removal, the imprinted polymer will be less densely cross-linked than the blank nonimprinted polymer and will thus swell more in good solvating solvents.[19]

Chromatographic Characterization. In Figure 2 the calculated capacity factors for the different steroids injected on the different columns packed with cholesterol-imprinted and nonimprinted polymers are plotted. The first evaluation was done using polymers that had not been subjected to extractions using the Soxhlet extractor. Thus the template was extracted on line in the

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Table 2. Characterization of Adsorbents Prepared for the Cholesterol Binding Experiments[a]

polymer	surface area[b] (m^2/g)	pore volume[c] (cm^3/g)	pore diameter[d] (nm)	micropore volume[e]	swelling[f] (mL/mL)	density[g] (g/mL)
P11	184	0.79	172	0.080	1.32	0.42
P12	198	0.92	186	0.088	1.20	0.34
P13	273	1.05	154	0.12	1.24	0.32
P14	291	1.03	142	0.13	1.16	0.32

[a] Physical characterization of the 25−36 μm particle size fraction. Prior to characterization the polymers were extracted in a Soxhlet apparatus in methanol and dichloromethane and dried at 60 °C as described in the Experimental Section. In the nitrogen adsorption measurements, the polymers were outgassed at 40 °C for 12 h. [b]BET surface area using a 40 point pressure table. [c]Total pore volume of pores less than 2600 Å. [d]Average pore diameter (BJH). [e]DR method micropore volume. [f]Swelling in acetonitrile. [g]Weight of 1 mL of dry polymer (25−36 μm).

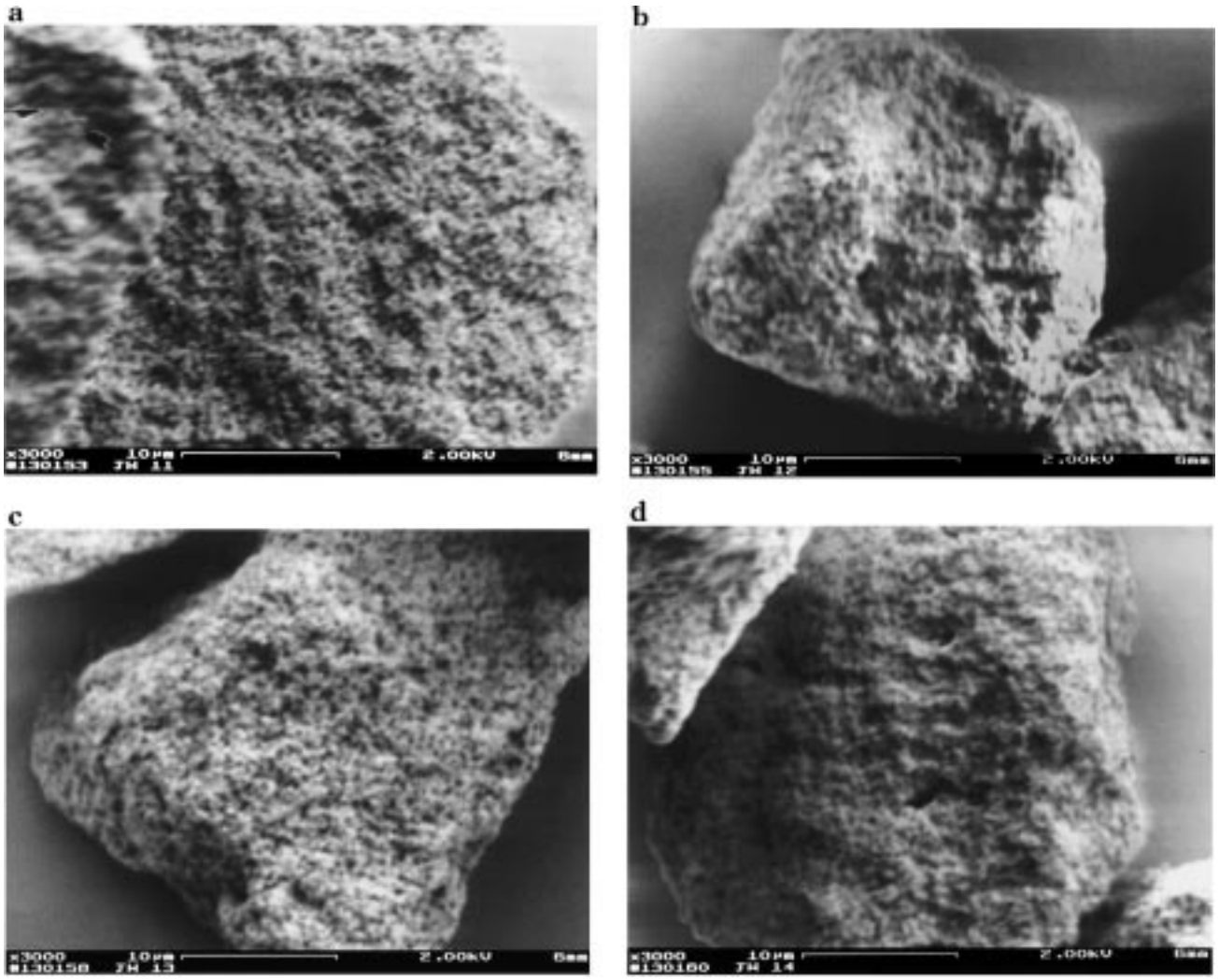

Figure 1. Scanning electron micrographs of polymers P11 (a), P12 (b), P13 (c), and P14 (d) photographed at 3000× magnification.

chromatographic mode. Considering the particle size of the packings, the number of theoretical plates, 200−1000, was acceptable, indicating a fair column efficiency. For lower plate numbers it was verified that no void had been created at the column inlet. On all columns, the retention of the hydrophobic steroids cholesterol (Cho) and ergosterol (Erg) increased strongly with the water content in the mobile phase, whereas the less hydrophobic steroid testosterone (Tes) was relatively weakly retained and responded much less to the addition of water. The linear dependence of log k' on the water content (Figure 3B) further supports that the retention is mainly controlled by the hydrophobic effect. Relatively strong retention was seen on the polymers prepared using dichloromethane as porogen (P3, P4, P9, P10). This may be related to the more efficient solvation of the hydrophobic monomers in this porogen, leaving them more exposed after removal of the porogen. Comparing polymers P7 and P8 with P9 and P10 reveals that the cholic acid monomer promotes an almost doubling of the capacity factors for the hydrophobic steroids. For these polymers (P3, P4, P7−10), however, the difference between the retention on the imprinted and the blank polymers was small. When dichloromethane was used as mobile phase, the retention was weak on both the imprinted and the blank polymer. A different picture emerged when comparing the polymers prepared using ethanol as porogen. The capacity factors for cholesterol were two times larger on the imprinted (P1, P5, P11) compared to on the blank polymers (P2,

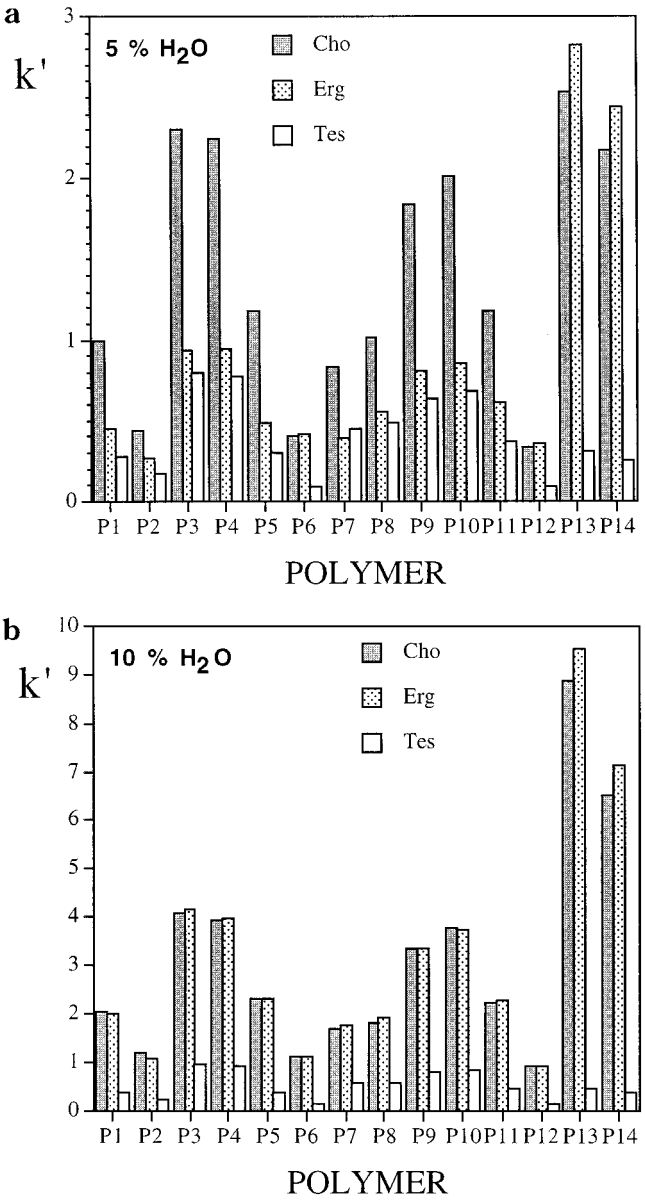

Figure 2. Capacity factors for cholesterol (Cho), ergosterol (Erg), and testosterone (Tes) on columns packed with the adsorbents shown in Table 1 in a mobile phase consisting of (a) methanol/water 95/5 (v/v) and (b) methanol/water 90/10 (v/v). Conditions otherwise as described in the Experimental Section.

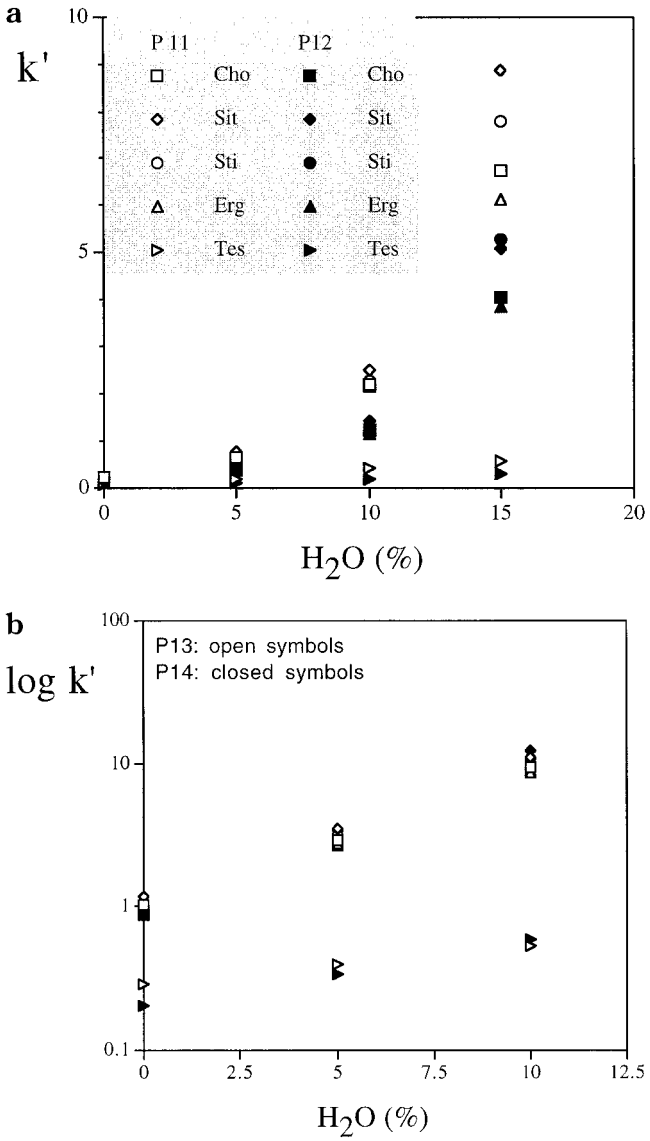

Figure 3. Capacity factors for the various steroids versus the amount of water in the mobile phase using columns packed with polymers subjected to extraction in Soxhlet apparatus and drying: (a) P11 and P12 and (b) P13 and P14 (log k′). The estimated hydrophobicity values of the steroids are as follows: Cho, 9.8; Sti, 10.2; Sit, 10.7; Erg, 9.3; Tes, 3.5.

P6, P12) prepared using the cholic acid monomers. Furthermore, using the mobile phase consisting of 5% water in methanol, the former polymers preferentially retained cholesterol over the similar steroid ergosterol, an effect that dissappeared at higher aqueous contents (Figure 2). These observations support a templating mechanism driven by apolar association of monomers and template prior to polymerization. Using dichloromethane as porogen, such association is unlikely due to efficient solvation of the apolar parts of the monomers and the template. In this case, however, hydrogen bonding between MAA and the hydroxyl group of the template can be expected to occur. As has been observed by other workers, this stabilization is not strong enough to cause an observable templating effect of cholesterol.[7] The type of cholic acid monomer only seems to have a small influence on the templating effect. However, the polymers prepared using 3β-methacryloylcholesterol (P13, P14) instead of the cholic acid monomers behaved quite differently. These gave the strongest retention of the hydrophobic steroids among the tested polymers. Although the imprinted polymer retained cholesterol more strongly than the blank polymer, the difference was smaller compared to the difference seen using the cholic acid polymers. Nevertheless, these polymers clearly exhibited the strongest retention of cholesterol among the materials synthesized. However, even stronger retentions were observed on a commercial reversed phase C18 column under the same conditions. On a column with similar dimensions using methanol/water 95/5 (v/v) as mobile phase, the capacity factor for cholesterol was 18. However, as will be discussed in the next section, the affinity of the phases for cholesterol in the presence of high concentrations of bile salts is a better indicator for the materials usefullness under physiological conditions. Chromatographic evaluation of P11−P14 was also done after the Soxhlet extraction treatment. As seen in Figure 3, the

retention of the steroids was similar as before extraction, whereas the selectivity of the imprinted polymers was lower. Porous MIPs are known to be less thermally stable than nonporous ones.[18] Thus it is possible that the extraction procedure and the subsequent drying of the polymers at 60 °C has to some extent denatured the binding sites.

Cholesterol Adsorption in Intestine-Mimicking Medium. To get an idea of the materials performance in vivo, the adsorption of cholesterol by the different adsorbents in a medium that closely would mimic the intestinal fluid was tested. The development of a suitable medium was the first task. The solubility of cholesterol in the medium needed to be sufficient to cover a concentration interval that would lead to saturation of the adsorbent binding sites. The medium needed further to be easily and reproducibly prepared. A mixture of the sodium salts of cholic and desoxycholic acid was found to best satisfy these criteria. An ionic strength and pH corresponding to that found in intestine (pH 7.5, Ciba Geigy) was adjusted by addition of sodium hydroxide and potassium dihydrogen phosphate. This led to a medium that dissolved 1.8 mg/mL of cholesterol.

The batch experiments were carried out at 37 °C under circular stirring, i.e., under conditions that would mimic the in vivo conditions. After 24 h, the adsorbents were allowed to settle, and the supernatant was analyzed enzymatically for cholesterol. The assay used is based on coupled reactions catalyzed by cholesterol oxidase and peroxidase resulting in the formation of a dye in amounts proportional to the amount of cholesterol. The dynamic range for the assay is 15.5 mM with expected values between 3.4 and 8.2 mM, according to the manufacturers specifications. Care was taken to control the temperature and the time of reaction and calibration was regularly repeated. The amount of cholesterol adsorbed was calculated by subtracting the amount found in the supernatant after adsorption from the amount of cholesterol present before addition of adsorbent. The resulting values were then plotted against the equilibrium concentration of free cholesterol in the supernatant (Figure 4). The amounts adsorbed by the C18 modified silica adsorbents were similar and increased almost linearly throughout the concentration range studied; i.e., no visible saturation occurred. Despite the higher specific surface area of Amberlite XAD2000, this adsorbent adsorbed significantly less cholesterol than the C18 silicas. This may be due to the more hydrophobic character of this adsorbent, leading to agglomeration and thus a smaller exposed surface area. Silica gel modified with tomatine, a complex glycosylated steroid known to complex cholesterol in aqueous media,[20] clearly adsorbed more cholesterol than the precursor epoxy−silica gel or naked silica gel. This was particularly apparant in the low concentration range below 2 mM.

The cholesterol-imprinted polymers exhibited the highest affinity for cholesterol among the tested adsorbents. Particularly striking is the strong adsorption exhibited by P13, the imprinted adsorbent prepared using 3β-methacryloylcholesterol as functional monomer. This polymer also retained cholesterol the most among the imprinted adsorbents in the chromatographic evaluation. Also worth noting are the differences in the binding exhibited by the imprinted and the nonimprinted blank polymers. Physiologically relevant concentrations of cholesterol are expected to lie below 1 mM. In this concentration range, P13 together with the

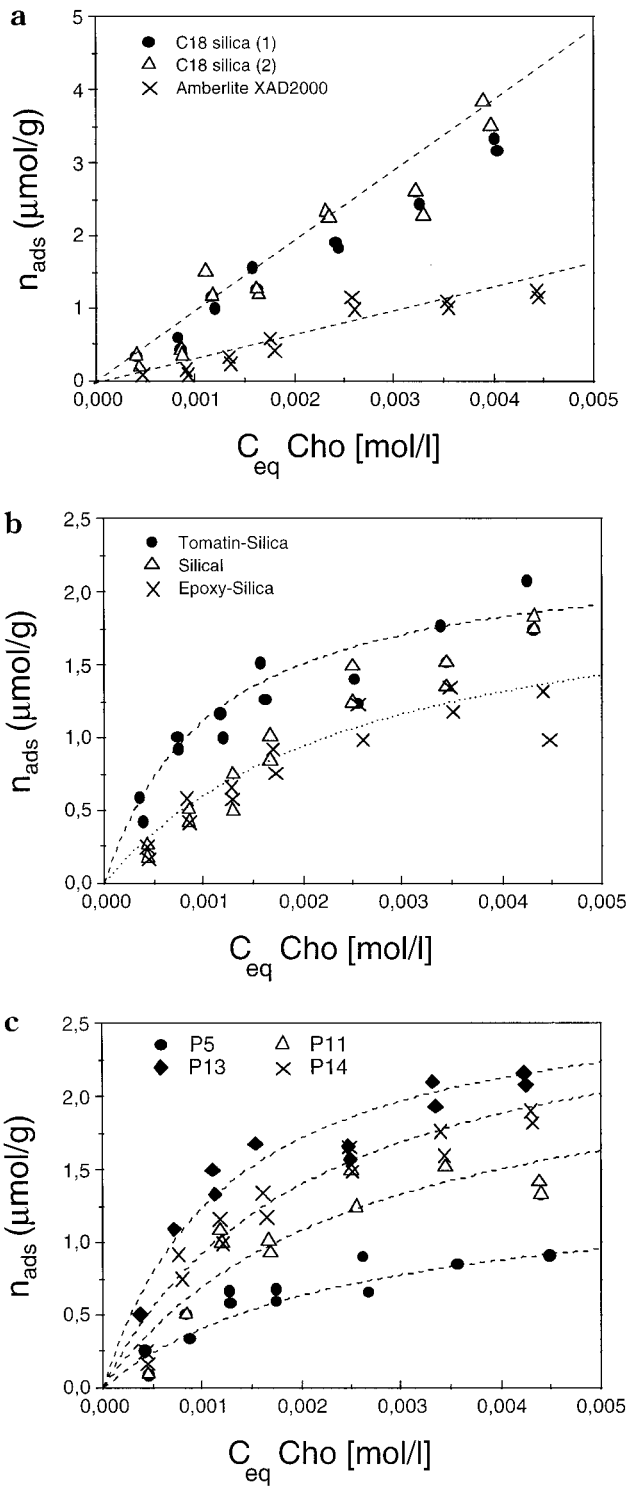

Figure 4. Adsorption isotherms of cholesterol in intestine-mimicking medium (5 mL) at pH 7.5 using the adsorbents (30 mg) described in the Experimental Section and in Table 1. The samples were stirred in a circularly shaking bath at 37 °C for 24 h and the amount of cholesterol in the supernatant was thereafter determined enzymatically. The experiment was repeated twice for each adsorbent.

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tomatin−adsorbent adsorbed cholesterol most strongly of all tested adsorbents. At a cholesterol concentration of 1 mM, P13 adsorbed about 45 μmol/g (ca. 17 mg/g) adsorbent, whereas the nonimprinted polymer P14 adsorbed ca. 33 μmol/g (ca. 13 mg/g)

Conclusions

Steroid functionalized polymers imprinted with cholesterol exhibit enhanced affinity and capacity for cholesterol in intestinal-mimicking media. The adsorptive capacity seems to be due to binding sites induced by the presence of steroid units in the polymer backbone and the presence of cholesterol during formation of the adsorbent. The templating effect of cholesterol probably involves apolar interactions with the apolar parts of the monomers during polymerization. This may result in hydrophobic binding pockets capable of accommodating cholesterol in the subsequent rebinding experiment. Cholesterol itself apparantly offers the best binding sites for cholesterol. The crystal structure of cholesterol monohydrate features layers of close-packed cholesterol molecules with a large apolar contact area and a polar sheet of hydrogen-bonded hydroxyl groups and water molecules.[17] The cholesterol monomer may interact with cholesterol in a similar fashion. It is possible that accessory monomers prepared from other steroids will be better suited for cholesterol binding materials. Methacryloyl derivatives of ergosterol, stigmasterol, testosterone, or β-sitosterol will thus be evaluated. Alternatively, another site of coupling of the functional monomer to cholesterol may lead to stronger interactions.

An alternative recognition mechanism involves binding sites stabilizing clusters of cholesterol. In this context it should be mentioned that the porogen ethanol is used as a recrystallization solvent for cholesterol. Moreover, the cholesterol monomer in P13 may stabilize such clusters. More detailed information regarding the imprinting mechanism may be obtained from a spectroscopic characterization of the solution complexes present prior to polymerization.[21] The adsorptive capacity exhibited by the cholesterol imprinted polymers, as well as their low cost and ease of preparation, appears promising for their future therapeutic use in the prevention of diet-cholesterol related diseases. The adsorbents may also be useful in other applications relying on strong and selective binding of steroids in aqueous media.

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