Imprinted Polymers Prepared by Aqueous Suspension Polymerization*

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ABSTRACT: The feasibility of preparing cholesterol-imprinted polymers by aqueous suspension polymerization was investigated by the preparation of ethyleneglycol dimethacrylate and divinylbenzene-based beads imprinted using cholesteryl(4-vinyl)-phenyl carbonate as the template. A low volatility porogen to replace a 4:1 hexane/toluene mixture was selected on the basis of solubility parameters and consisted of dioctyl phthalate/*n*-decane 77:23 v/v. Beads were prepared using a range of porogen contents with the best results obtained at 5.5–6.5 mL /5 g of monomer. Uptake of cholesterol by suspension polymers was broadly similar to that of the corresponding "bulk" polymers, but suffered from higher nonspecific binding. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 1841–1850, 2000

Key words: suspension polymerization; molecular imprinting; cholesterol

INTRODUCTION

Polymers prepared by molecular imprinting; so-called plastic antibodies,[1–4] have potential in applications as diverse as chromatography,[5–7] capillary electrophoresis,[8–12] sensors,[13–15] analytical sample preparation[16–21] and assays,[22–25] catalysis,[26–31] and solid-state chemistry,[32–35] among others. Typically, monolithic slabs of imprinted polymer are made by polymerizing a mixture of monomers in the presence of a molecular template and a small volume of a porogenic solvent. A large excess of crosslinker ensures that the solid framework that forms around each molecule of template is relatively rigid, such that the presence of the template creates a "cavity" in the polymer structure. Monomer-bound functional groups that were interacting with those of the template before polymerization are trapped at the walls of this "cavity." The effect is to create a binding site specifically tailored to the steric and electrostatic requirements of the template. The interactions between functional monomers and templates can be either covalent[36] or noncovalent[37] in nature. Polymers produced in this way have to be broken into pieces and mechanically ground before the template can be removed, either by extensive washing or by a chemical treatment. After template removal, the resulting imprinted polymer can show a sufficiently high degree of selectivity in the binding of this molecule to enable, for example, chromatographic resolution of its enantiomers.

In principle, imprinted polymers could have major applications in the chemical, pharmaceutical, and food industries, provided they can be produced cheaply and on an appropriate scale. Industrial-scale production is clearly not feasible when materials have to be made as monoliths by "bulk" polymerization and ground before use. In fact, due to the exothermic nature of the polymerization reaction, bulk polymers cannot be prepared safely on more than ~100 g scale. The preferred industrial methods are therefore emul-

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sion and suspension polymerization, where dispersion in an aqueous phase helps dissipate the heat of reaction. Of these two techniques, suspension polymerization is particularly suited to the formation of crosslinked porous beads.[38–40]

The availability of affinity separation media and chromatographic materials in the form of spherical beads enables the production of packed or fluidized beds, and allows for a greater degree of process control than could be obtained with irregular particulates. It would be highly advantageous, therefore, to be able to produce imprinted polymers as spherical beads, ideally by a fully scaleable, aqueous-based suspension polymerization. However, a major obstacle to the preparation of imprinted polymers in these systems is the relative incompatibility of either conventional covalent or noncovalent methods with a bulk water phase, where readily hydrolyzable linkages and water soluble monomers are used respectively. Despite this, there have been some reports of the successful preparation of noncovalently imprinted polymer beads by a two-stage polymerization process[41–44] and microorganisms have been imprinted on the surface of beads formed by polymerization of the core of a microcapsule prepared by interfacial polycondensation.[45,46] Alternatively the use of water can be avoided altogether, by employing fluorinated solvent as the bulk phase[47,48] or performing polymerization in an aerosol.[49] However, these methods may not be suitable for all industrial applications on the grounds of either cost or convenience.

One way to overcome the limitations of conventional molecular imprinting is to use an approach where the underlying chemistry is not sensitive to the presence of water. Byström et al. prepared divinylbenzene (DVB)-based polymeric beads, imprinted with sterol acrylates by aqueous suspension polymerization.[34] These were converted to "templated polymeric reducing agents" by reductive cleavage of the acrylate ester bond and attachment of a metal hydride center to the resultant hydroxyl. While these polymers showed a high degree of regio- and stereochemical control in the reduction of steroid ketones (assumed to be due to shape complementarity at the site of reaction), the polymers themselves are unlikely to exhibit good binding characteristics. The reason is that hydrolysis (or reduction) of an ester bond would produce a binding site that is too "crowded" to fit the template's functional groups. These problems can be overcome if covalent template molecules incorporating sacrificial spacers are used,[50-52] for example in the imprinting of cholesterol *via* the carbonate ester.[50] One of the advantages of this approach is compatibility with a broad range of solvents, including water. In this article we describe the preparation and characterization of polymer beads imprinted with cholesterol using the carbonate ester methodology.

EXPERIMENTAL

Materials and Methods

Ethyleneglycol dimethacrylate (EGDMA), divinylbenzene, 55% tech. (DVB), styrene, dioctyl phthalate, *n*-decane, and poly(vinyl alcohol) (87–89% hydrolyzed, M_w = 85,000–146,000) were obtained from Aldrich. Inhibitor was removed from monomers by distillation or washing with aqueous NaOH, as appropriate. Azo-*bis*-isobutyronitrile (AIBN) was obtained from Fluka. Cholesteryl (4-vinyl)phenyl carbonate (CVPC) was prepared as previously described.[50] HPLC analyses were performed using Gilson 303 pumps equipped with an ACS light-scattering mass detector and a Shimadzu SIL-9A autosampler. Samples were analysed on a 25 cm, 5 μm Spherisorb column (Hichrom), at room temperature, using a flow rate of 1.5 mL min^{-1}. Elution was with a linear gradient from 10% ethyl acetate:*n*-hexane to 100% ethyl acetate for 6 min. Scanning electron micrographs were taken on a Hitachi S570 electron microscope. 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. Water was from a Millipore Q water system. Sodium hydroxide (AR) and methanol (Chromsolve) was purchased from Riedel-de-Haën. To remove dissolved carbon dioxide from the solutions, the water was boiled and allowed to cool while purging with nitrogen before use, and the polymer suspensions were purged with nitrogen during the titrations.

Polymer Synthesis

Poly(vinyl alcohol) (7 g), was dissolved in 100 mL of water by stirring at 90–95°C under a nitrogen atmosphere in a 250 mL flanged reactor flask fitted with a mechanical stirrer, reflux condenser, nitrogen inlet, and dropping funnel. After cooling to room temperature, a solution of monomers (see Table III, total mass 5 g) and AIBN (0.1 g) in the dioctyl phthalate:*n*-decane mixture (77:23 v/v, see

Table III for volumes) was admitted to the flask. The mixture was stirred at 650 rpm under a gentle stream of nitrogen while the temperature was raised to 65°C. The polymerization was allowed to proceed for 24 h. After cooling to room temperature, the polymer was washed by repeated sedimentation from water in a centrifuge at 13,000 rpm at a temperature of 10°C for 30 min. Polymers were dried in a vacuum oven at 80°C overnight.

Template Removal

The template was removed from imprinted polymers by hydrolysis in 1M methanolic NaOH, as previously described.[50] Hydrolyzed polymers were washed with methanol in a soxhlet apparatus for 12 h before vacuum drying, as above. Template cleavage was assessed by the mass of cholesterol recovered by extraction from the hydrolysate.[50]

Binding Experiments

Polymers (10, 20, and 40 mg) were weighed into 2 mL capacity screw cap vials (Wheaton) fitted with poly(tetrafluoroethylene) (PTFE)-lined caps. A 2 m*M* solution of cholesterol in isohexane (2 mL) was added to each vial and the solutions were incubated in a shaker at room temperature overnight. Solutions were filtered into HPLC vials using 5 mL disposable syringes fitted with 13 mm, 2 μm porosity PTFE-membrane syringe filters (Whatman). The concentration of cholesterol remaining in the supernatant was determined by HPLC, calibrated against dilutions of the stock solution.

Conductometric Titrations

Polymer (0.1–0.15 g) was suspended in 5:1 water/methanol and titrated with a solution of sodium hydroxide in the same solvent mixture. The conductance of the suspension was measured for each incremental addition of sodium hydroxide solution. Conductance was then plotted against volume of sodium hydroxide solution added. The end point was determined from the intersection of the descending and ascending portions of the titration curve by linear extrapolation.

RESULTS AND DISCUSSION

The Role of Solvent

The carbonate spacer methodology was previously used in our laboratory to prepare "bulk" polymers imprinted with cholesterol in hexane and hexane-toluene solvent mixtures.[50] As the nature of porogenic solvent is expected to have a significant effect on the polymer microstructure, and consequently, may influence the polymer's binding properties, a series of preliminary experiments were conducted to evaluate the importance of this parameter. To this end, several bulk polymers were prepared in hexane, toluene, and propan-2-ol containing mixtures, the resulting materials exhibiting a range of surface areas varying from about 2 to 440 $m^2\ g^{-1}$ (Table I). In accordance with the data obtained in simple model systems,[53,54] our results confirm that vastly different morphologies can be obtained for chemically identical materials, merely by changing the nature of the porogen. Thus, a small increase in the proportion of toluene in hexane from 1:9 to 1:4 led to a 5-fold increase in surface area for EDGMA-based imprinted materials (**BI2** and **BI3**). However, the dependence of surface area on solvent followed a different pattern for EGDMA homopolymers (nonimprinted polymers), showing that copolymerization with the template also has a profound effect on the polymer morphology. The question therefore arises as to how these morphological differences affect the binding properties of otherwise chemically identical polymers.

Batch binding experiments were carried out on the polymers, using a 2 m*M* solution of cholesterol in hexane and 10 mg mL^{-1} polymer concentration, for imprinted polymers before (data not shown) and after hydrolysis to remove the template and hydrolyzed nonimprinted polymers, the results being shown in Table I. The nonspecific contribution to binding can be estimated from the binding to hydrolyzed, nonimprinted, and nonhydrolyzed imprinted polymers. In all cases this was low, $\leq$10% and typically 0–8%, except for **B4**, where the figures were 31 and 32%, respectively. This probably was to be expected for such high surface area (>400 $m^2\ g^{-1}$) polymers. The specific uptake, however (defined as the difference between the total and nonspecific binding), was found to be very similar (22–32%) for most of the polymers studied. This result is probably not too surprising because the sacrificial spacer (as well as the covalent) methodology allow the functional groups to be positioned exclusively in the binding sites of imprinted polymers. Hence, as our results suggest, it is the nonspecific rather than the specific ("in the site") binding that is most affected by the polymer morphology— i.e., the higher the surface area, the higher the nonspecific adsorption.

Table I Composition, Surface Area, and Binding Properties for Cholesterol-Imprinted and Nonimprinted Polymers Prepared by Bulk Polymerization

Polymer	Porogenic Solvent[a]	Polymer Composition[b] (Mole %)		Surface Area ($m^2\ g^{-1}$)	Template Removal (%)[c]	Uptake (%)[d]
		Template	Crosslinker			
BI1	*n*-Hexane	5% CVPC	95% EGDMA	26	104	32
BN1	*n*-Hexane	—	100% EGDMA	44	—	10
BI2	*n*-Hexane : toluene 9 : 1 (v/v)	5% CVPC	95% EGDMA	52.5	73	33
BN2	*n*-Hexane : toluene 9 : 1 (v/v)	—	100% EGDMA	2	—	7
BI3	*n*-Hexane : toluene 4 : 1 (v/v)	5% CVPC	95% EGDMA	255.5	93	34
BN3	*n*-Hexane : toluene 4 : 1 (v/v)	—	100% EGDMA	2.5	—	<2
BI4	Propan-2-ol : toluene 3 : 1 (v/v)	5% CVPC	95% EGDMA	440	48	54
BN4	Propan-2-ol : toluene 3 : 1 (v/v)	—	100% EGDMA	409	—	31
BI5	Propan-2-ol	5% CVPC	95% DVB	9	32[e]	<2
BI6	Toluene	5% CVPC	95% DVB	39	nd[f]	nd
BI7	Toluene : acetonitrile 1 : 1 (v/v)	5% CVPC	95% DVB	382	78	17
BI8	THF	5% CVPC	95% DVB	45	nd	nd

[a] All polymers were prepared with 2 mL of porogen per gram of monomer mixture.
[b] CVPC: cholesteryl (4-vinyl)phenyl carbonate; EGDMA: ethyleneglycol dimethacrylate; DVB: divinylbenzene.
[c] Estimated gravimetrically as the percentage of the theoretical amount of cholesterol produced on hydrolysis.
[d] Uptake of cholesterol from a 2 m*M* solution in *n*-hexane by hydrolyzed polymer (10 mg mL^{-1}).
[e] Estimated by HPLC.
[f] nd: Not determined.

It was also of interest to investigate the effect of surface area on the kinetics of cholesterol uptake. The uptake of cholesterol by **BI4** (a high surface area polymer) over an initial period of 30 min is shown in Figure 1. The hydrolyzed polymer bound around 20% in the first few minutes followed by a slower increase in binding to 35% after 30 min and 53% overnight. Binding to nonhydrolyzed polymer was established at around 5–10% after 5 min and was practically unchanged overnight. Similar results were obtained for **BI2**, a medium surface area polymer (not shown) but with slower overall kinetics. Reliable curves could not be established for low surface area polymers, nor for those based on DVB, on account of larger errors in the determination of cholesterol uptake from polymers showing low overall binding, which hampered a more detailed investigation of their kinetics.

In general, the observations made above have implications for the optimization of imprinted polymer preparations designed for different uses. For example, the rapid diffusion of ligand in and out of a polymeric matrix is crucial for chromatographic separations where a somewhat higher nonspecific binding may be tolerated as the final resolution depends on multiple adsorption/desorption events. On the other hand, for batch separations nonspecific binding may well be more of a problem but slower kinetics may not matter too much. In both cases, however, better control of particle morphology would be advantageous.

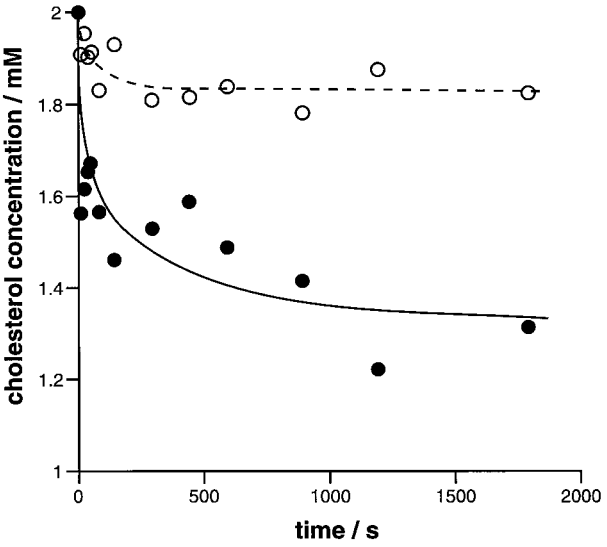

Figure 1 Kinetics of binding to **BI4**: for nonhydrolyzed (open circles) and hydrolyzed (closed circles) polymers, shown as cholesterol concentration in the supernatant against time. Initial concentrations were 2 m*M* cholesterol and 10 mg mL^{-1} polymer.

Table II Solubility Parameters for Porogenic Solvents and Solvent Mixtures

Solvent	Solubility Parameter, δ, $(MPa)^{0.5}$
Toluene	18.2
n-Hexane	14.9
Dioctyl phthalate	16.2
n-Decane	13.5
Toluene : *n*-hexane, 1 : 4 (v/v)	15.6
Dioctyl phthalate : *n*-decane, 77 : 23 (v/v)	15.6

Unfortunately, as these results (and those below) demonstrate, it is rather difficult at the moment to predict the surface area of imprinted polymers prepared in different solvents because the presence of template, at 5 or even 2.5 mol % loading, has a significant effect on the polymer microstructure.

For rapid kinetics, combined with a relatively low nonspecific component, mixtures of *n*-hexane and toluene appeared to be satisfactory. While these solvent mixtures could be used in "bulk" polymerizations, they were clearly too volatile to use in suspension polymerization at 65°C, and maintain adequate control over the composition and volume of solvent in the dispersed organic droplets. We therefore needed to find a porogen for suspension polymerization that would give us beads with a similar porosity to "bulk" polymers prepared using a 1:4 toluene: hexane mixture. As these two solvents were considered inappropriate on the grounds of their volatility, we chose dioctyl phthalate (DOP) and *n*-decane as more suitable alternatives and compared the solubility parameters δ[55] (Table II) obtained from the literature[56] for all four solvents. From the relationship of Lloyd et al.,[57] eq. (1), which relates the solubility parameter for a mixture of solvents to that of the individual solvents and their volume fractions ϕ,

Table III Composition of Polymers Prepared by Suspension Polymerization

Polymer	Polymer Composition[a] (mole %)			Porogen Volume (mL)[b]	Surface Area ($m^2\ g^{-1}$)	Template Removal (%)[c]
	Template	Styrene	Crosslinker			
EN0	—	5%	95% EGDMA	0	<5	
EI3.5	2.5% CVPC	2.5%	95% EGDMA	3.5	242	74
EN3.5	—	5%	95% EGDMA	3.5	202	
EI4.5	2.5% CVPC	2.5%	95% EGDMA	4.5	202	79
EN4.5	—	5%	95% EGDMA	4.5	98	
EI5.5	2.5% CVPC	2.5%	95% EGDMA	5.5	316	78
EN5.5	—	5%	95% EGDMA	5.5	62	
BEI5.5[d]	2.5% CVPC	2.5%	95% EGDMA	5.5	328	39
BEN5.5[d]	—	5%	95% EGDMA	5.5	110	
EI6.5	2.5% CVPC	2.5%	95% EGDMA	6.5	121	38
EN6.5	—	5%	95% EGDMA	6.5	<5	
EI8.0	2.5% CVPC	2.5%	95% EGDMA	8.0	236	78
EN8.0	—	5%	95% EGDMA	8.0	15	
EI9.0	2.5% CVPC	2.5%	95% EGDMA	9.0	191	78
EN9.0	—	5%	95% EGDMA	9.0	19	
DN0	—	5%	95% DVB	0	<5	
DI4.5	2.5% CVPC	2.5%	95% DVB	4.5	375	52
DN4.5	—	5%	95% DVB	4.5	305	
DI6.0	2.5% CVPC	2.5%	95% DVB	6.0	464	50
DN6.0	—	5%	95% DVB	6.0	441	
DI7.5	2.5% CVPC	2.5%	95% DVB	7.5	437	49
DN7.5	—	5%	95% DVB	7.5	475	

[a] CVPC: cholesteryl (4-vinyl)phenyl carbonate; EGDMA: ethyleneglycol dimethacrylate; DVB: divinylbenzene.
[b] Per 5 g of monomer mixture.
[c] Estimated gravimetrically as the percentage of the theoretical amount of cholesterol produced on hydrolysis.
[d] Bulk polymers BEI5.5 and BEN5.5 were prepared using the same porogen and porogen volume ratio as the equivalent suspension polymers, EI5.5 and EN5.5.

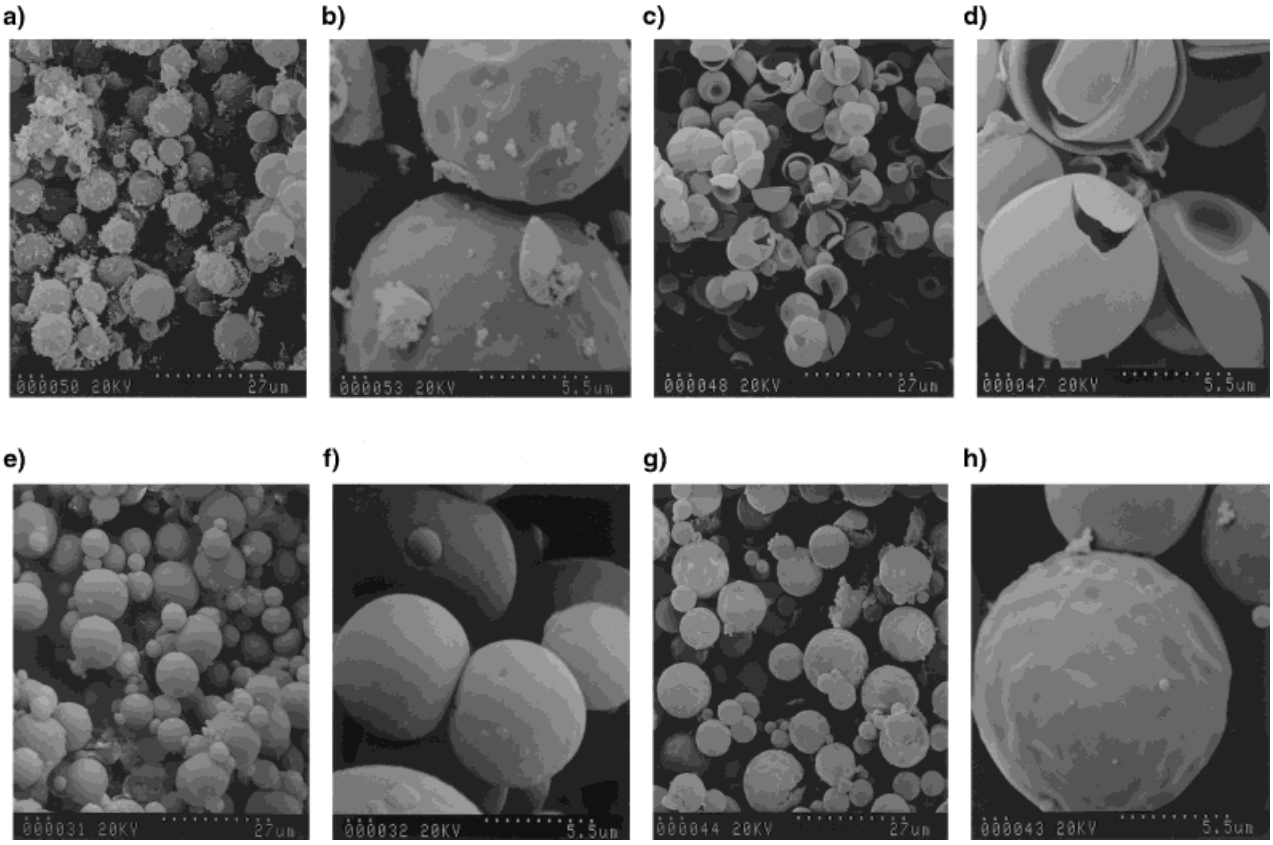

Figure 2 SEMs of EGDMA-based polymer beads: (a) and (b) polymer **EI8.0** (cholesterol-imprinted, porogen volume 8.0 mL); (c) and (d) polymer **EN8.0** (nonimprinted, 8.0 mL); (e) and (f) polymer **EI4.5** (cholesterol-imprinted, 4.5 mL); (g) and(h) polymer **EN4.5** (nonimprinted, 4.5 mL).

we calculated the value of δ for a 1:4 v/v mixture of toluene: *n*-hexane (Table II):

$$\delta^2 = \phi_1\delta_1^2 + \phi_2\delta_2^2 \quad (1)$$

Using this formula, we then calculated that a 77:23 v/v DOP:*n*-decane mixture would have the same solubility parameter as a 1:4 v/v toluene and *n*-hexane mixture. Consequently, this low volatility mixture was used in the preparation of imprinted polymers in aqueous suspensions.

Suspension Polymerization

Having selected a suitable solvent on the basis of solubility parameters, we used a standard procedure[39] to prepare imprinted polymer beads, varying both the volume of porogen and the crosslinker. Due to the relatively low solubility of CVPC in the polymerization mixture, a template loading of 2.5 mol % was used. However, in order for the polymers to resemble those from bulk polymerization, it was decided to fix the level of crosslinker at 95 mole %, the remaining 2.5% consisting of styrene. Polymerization was initiated with AIBN, and poly(vinyl alcohol) was used as the stabilizer. A summary of the polymer preparations is presented in Table III.

Polymer Morphology and Surface Area of Suspension Polymers

Scanning electron micrograph (SEM) photographs of representative preparations are given in Figures 2 and 3, and surface area data are presented in Table III. As expected, EGDMA-based polymers differ from their DVB-based counterparts, both in their overall morphology and in their internal structures. As our results[50] and those of other authors[58] suggest that (meth)acrylate-based crosslinkers are superior in molecular imprinting studies to DVB, we will consider the EGDMA-based materials first.

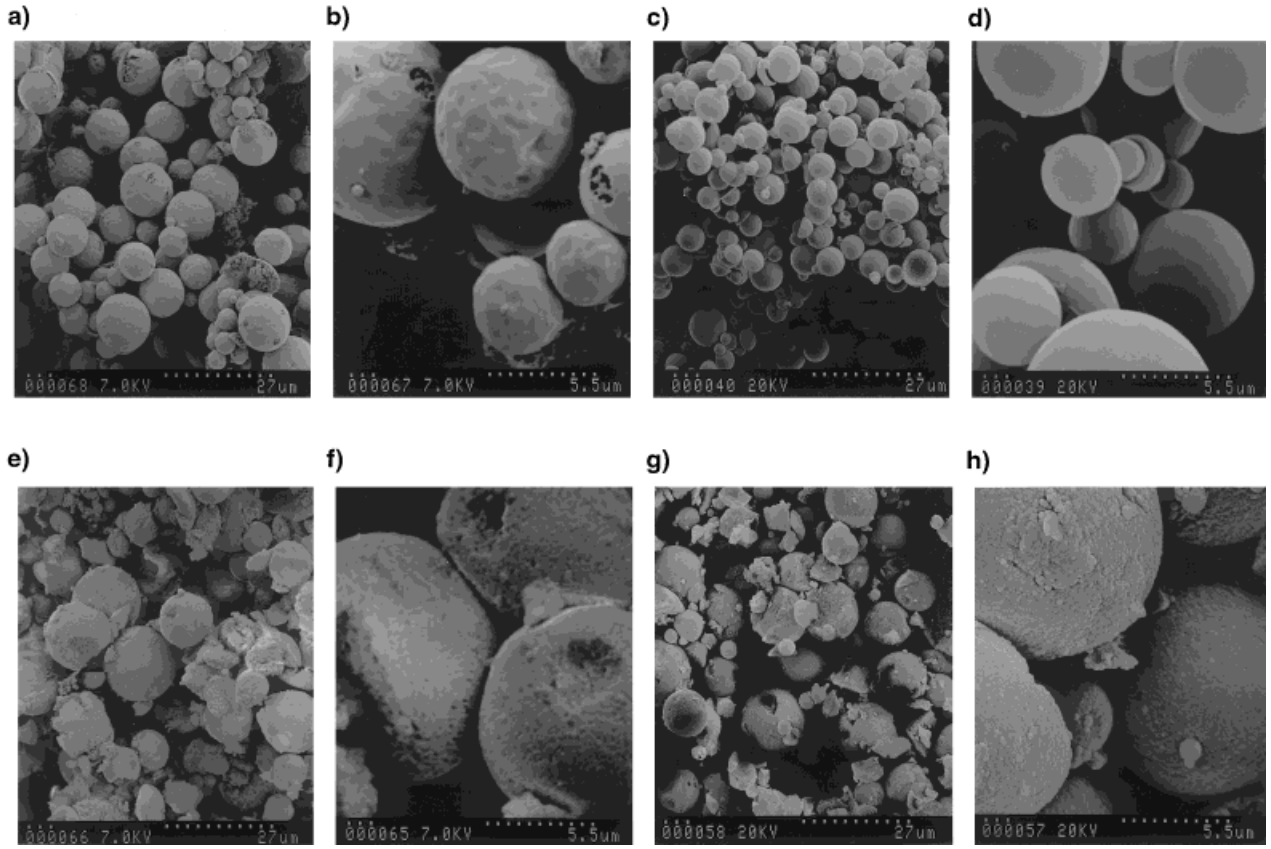

Figure 3 SEMs of EGDMA-based polymer beads: (a) and (b) polymer **EI6.5** (cholesterol-imprinted, porogen volume 6.5 mL); (c) and (d) polymer **EN6.5** (nonimprinted, 6.5 mL) and of DVB-based polymer beads: (e) and (f) polymer **DI7.5** (cholesterol-imprinted, porogen volume 7.5 mL); (g) and(h) polymer **DI4.5** (cholesterol-imprinted, 4.5 mL).

Figure 2 shows beads prepared with 8.0 mL of porogen (both imprinted, **EI8.0** and nonimprinted **EN8.0**), and 4.5 mL (imprinted, **EI4.5**, and nonimprinted, **EN4.5**). At the highest volume of porogen [Fig. 2(a,b)] the internal structure of the particles is very open. Although the outer surface of beads is relatively smooth [Fig. 2(b)], there are many broken fragments [Fig. 2(a)]. The situation is even worse in the case of nonimprinted beads at high porogen content which consist of a smooth shell of polymer with a solvent-filled void in the centre. On drying the shell splits, giving the appearance of torn and dimpled spheres [Fig. 2(c,d)] or a sample resembling broken eggshells. At 4.5 mL, good beads are produced in both the imprinted and nonimprinted materials; however, while the imprinted beads [Fig. 2(c,d)] are relatively smooth, the nonimprinted beads [Fig. 2(e,f)] have a dimpled surface, similar to **EI8.0** [Fig. 2(a,b)]. These differences are also reflected in the surface area measurements. In all cases, the surface area of nonimprinted polymers are significantly lower than their imprinted counterparts, the mismatch being much greater when the porogen volume exceeds 4.5–5.5 mL. Evidently, the quality of beads prepared at lower porogen volumes was better in terms of their overall shape and structural integrity [compare Fig. 2(e–h) with 2(a–d)]. The imprinted beads prepared with 6.5 mL of porogen, **EI6.5** [Fig. 3(a,b)], represent an intermediate state, with fewer broken beads than at 8.0 mL but with the same open internal structure. The nonimprinted sample, **EN6.5** [Fig. 3(c,d)], consisted of very smooth and regular particles that have no measurable porosity (Table III), very similar to polymers prepared without porogen (not shown).

In the case of the DVB-based materials the surface areas of imprinted and nonimprinted materials were high, and all fell within the range 305–475 $m^2\ g^{-1}$ (with the exception of **DN0**, made without porogen). The polymer particles

Table IV Percentage Uptake of Cholesterol from a 2 m*M* Solution in Hexane for Imprinted and Nonimprinted Polymers

Imprinted Polymer	Polymer Concentration (mg mL^{-1})	Cholesterol Uptake (%)		Nonimprinted Polymer	Cholesterol Uptake (% Hydrolyzed)
		Nonhydrolyzed	Hydrolyzed		
EI4.5	5	2	30	**EN4.5**	21
EI5.5	5	4	27	**EN5.5**	23
BEI5.5	5	4	19	**BEN5.5**	7
EI6.5	5	1	20	**EN6.5**	14
EI8.0	5	2	27	**EN8.0**	25
EI4.5	10	4	47	**EN4.5**	33
EI5.5	10	5	46	**EN5.5**	34
BEI5.5	10	8	32	**BEN5.5**	13
EI6.5	10	2	37	**EN6.5**	24
EI8.0	10	3	44	**EN8.0**	39
DI4.5	20	2	7	**DN4.5**	6
DI6.0	20	3	12	**DN6.0**	6
DI7.5	20	2	7	**DN7.5**	6

were also similar in appearance, having a rough surface {compare **DI7.5** [(Fig. (3e,f)] and **DI4.5** [Fig. 3(g,h)]. However, the DVB-based polymers were somewhat friable and broken fragments were seen in all preparations, again with the exception of **DN0**, which gave smooth and featureless spheres. The size distribution of both DVB and EGDMA particles were broadly similar, (Figs. 2 and 3) whilst the differences in surface features can clearly be seen.

Binding Characteristics of Suspension Polymers

The binding of cholesterol from a 2 m*M* solution in hexane, determined in batch experiments for a number of suspension polymers, is presented in Table IV. In the case of EGDMA-based polymers, data are presented for cholesterol uptakes at polymer concentration of 5 mg mL^{-1}. Due to lower overall uptake, higher amounts of polymer (20 mg mL^{-1}) were used in binding experiments with DVB-based polymers. In all cases, the binding to nonhydrolyzed imprinted polymer, the same polymer after hydrolysis, and the hydrolyzed nonimprinted polymer are compared. We felt this was necessary because the nonimprinted polymers, having significantly different morphology and surface area, could not serve as adequate controls.

It is evident from the results presented in Table IV that all nonhydrolyzed imprinted polymers showed remarkably little binding of cholesterol. On hydrolysis the uptake increased by about an order of magnitude from 1–4 to 20–36%. The binding to all the polymers was broadly similar, except for **EI6.5**, which showed somewhat lower cholesterol uptake, presumably due to a correspondingly lower degree of template removal (Table III). Perhaps the lower degree of template removal from **EI6.5** compared with the rest of the materials can be explained by the lower surface area of this particular polymer. Apart from **EI6.5**, there was remarkably little variation in cholesterol binding, despite the variation in surface area from 191 to 316 $m^2\ g^{-1}$. The binding to hydrolyzed nonimprinted polymers was also very similar and surprisingly independent of polymer surface area, which varied greatly across the range of materials studied (from <5 to 98 $m^2\ g^{-1}$). DVB-based polymers showed very modest binding of cholesterol despite high surface areas and moderately good degrees of hydrolysis (around 50% in all cases, see Table III). While the greatest effect was seen for **DI6.0**, the small overall binding makes drawing definite conclusions difficult.

A comparison of EGDMA-based suspension polymers with the corresponding bulk polymers prepared using the same volume of dioctyl phthalate:*n*-decane mixture showed that surface areas were roughly similar, (316 and 62 $m^2\ g^{-1}$ for **EI5.5** and **EN5.5** compared to 328 and 110 $m^2\ g^{-1}$ for **BEI5.5** and **BEN5.5**, respectively, Table III). The uptakes were generally higher for the suspension polymers, reflecting the higher level of template removal (78 against 39%, Table III).

Table V Carboxyl Content of Hydrolyzed and Nonhydrolyzed Polymers

	Carboxyl Content[a] (μmol g^{-1})	
Polymer	Nonhydrolyzed	Hydrolyzed
EN5.5	4.0	11.1
EI5.5	5.0	9.4
BI5.5	3.1	2.7
EI6.5	8.3	14.8
BN6.5	4.0	5.1

[a] Determined by conductometric titration with NaOH.

However, the level of binding to hydrolyzed nonimprinted polymers was much higher for the beads than the corresponding bulk polymers. In fact, all hydrolyzed, nonimprinted bulk polymers gave low uptakes with the exception of **BN4** (Table I). In order to determine whether hydrolysis of the methacrylate matrix was responsible for the level of nonspecific binding, a representative set of polymers were titrated with sodium hydroxide, the results being presented in Table V. The suspension polymers, whether imprinted or nonimprinted, all show a greater increase in carboxyl content on hydrolysis than the bulk polymers analyzed. While this clearly shows some subtle differences exist between the bulk and suspension polymers, the presence of around 10 μmol g^{-1} carboxyl groups can account for no more that 5% uptake at 10 mg mL^{-1} polymer, which is insufficient to explain the high binding observed.

CONCLUSIONS

Standard suspension polymerization techniques using aqueous dispersions of monomers can be employed in the preparation of imprinted polymer beads, suitable for chromatographic separations in packed and fluidized beds, provided the "sacrificial spacer" covalent imprinting methodology[50] is used. This imprinting methodology is tolerant of a wide range of polymerization solvents, including the presence of a bulk water phase, and therefore lends itself to conventional, aqueous-based emulsion[59] and suspension polymerization, which can be readily performed on an industrial scale. The latter can easily be performed in multikilogram batches, and dispenses with the requirement for expensive fluorocarbon-based surfactants and continuous phases demanded by other methods of imprinted bead formation.[47,48] Relatively low volatility porogen mixtures, selected on the basis of their solubility parameters, can be used to produce similar polymer characteristics to the corresponding bulk polymers. In this case, a 77:23 v/v mixture of dioctyl phthalate:*n*-decane was used in the formation of imprinted polymer beads as a substitute for 4:1 *n*-hexane:toluene, used in the formation of bulk polymers. For a given polymer composition, the porogen volume is an important parameter. High porogen volumes gave poor quality beads in the case of EGDMA, acceptable materials being formed when between 4.5 and 6.5 mL of solvent mixture/5 g of monomer were used. While binding to nonimprinted polymers was high, the effect of imprinting was seen in all cases for samples prepared within the range of porogen volumes mentioned above. In the accompanying paper[59] we demonstrate that nonporous, imprinted polymer particles prepared by emulsion polymerization techniques show specific binding of cholesterol, while the control particles (and unhydrolyzed imprinted polymers) exhibit much lower nonspecific binding while still possessing moderately high (~80 m^2 g^{-1}) surface areas.

DVB-based polymers were generally rougher in appearance, were more easily damaged, and possessed consistently higher internal surface areas than those prepared with EGDMA. Similar results were obtained with porogen volumes between 4.5 and 7.5 mL/5 g batch. Moderate binding of cholesterol, even at 20 mg mL^{-1}, indicated a small imprinting effect that appeared to be largest for a porogen volume of 6.0 mL, although the effect is probably too small to be certain.

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