Molecularly Imprinted Nanoparticles Prepared by Core-Shell Emulsion Polymerization

NATALIA PÉREZ, MICHAEL J. WHITCOMBE, EVGENY N. VULFSON

Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, United Kingdom

Received 12 July 1999; accepted 17 December 1999

ABSTRACT: Submicron core-shell polymer particles, with molecularly imprinted shells, were prepared by a two-stage polymerization process. Particles of this type, prepared with a cholesterol-imprinted ethyleneglycol dimethacrylate shell and in the absence of porogen, were found to be 76 nm in diameter with a surface area of 82 $m^2\ g^{-1}$. Cholesterol uptake from a 1 m*M* solution in isohexane was measured at both 10 and 30 mg mL^{-1}, with the imprinted polymer showing considerable binding (up to 57%). Imprinted but not hydrolyzed and hydrolyzed nonimprinted polymers showed very low uptakes (≤4.5%) and a phenol-imprinted polymer showed reduced binding (36%) under the same conditions. Imprinted shells were also prepared over superparamagnetic polymer cores and over magnetite ferrocolloid alone. The cholesterol binding to magnetic particles was very similar to that of equivalent nonmagnetic materials. Magnetic particles could be sedimented in as little as 30 s in a magnetic field. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 1851–1859, 2000

Key words: molecular imprinting; emulsion polymerization; core-shell particles; nanoparticles; magnetic particles

INTRODUCTION

Polymers prepared by molecular imprinting provide a means of creating specific recognition and catalytic sites similar to those found in biological systems such as enzymes or antibodies. At present most molecularly imprinted polymers are synthesized by bulk polymerization as porous materials that need to be ground before use. In the accompanying article, we have demonstrated the feasibility of preparing imprinted polymers in the form of spherical beads using an aqueous-based suspension polymerization method.[1] The resulting materials have been characterized in terms of their binding properties and morphology and compared with analogous polymers synthesized by conventional "bulk" polymerization. The beads were 2–10 μm in diameter and the preparations had a relatively broad size distribution. In this communication, we describe the preparation of much smaller imprinted "nanoparticles" using the technique of core-shell emulsion polymerization.[2] This approach enables one to exercise much better control over the particle size and to narrow significantly the size distribution. Imprinting of the outer shell layer ensures that the template sites are only situated close to the surface of the beads which should allow for the rapid diffusion of ligands to and from the imprint sites.

Core-shell polymerization is a two-stage process that starts with the preparation of a seed latex that can be prepared from a large variety of materials, for example styrene (St), divinylbenzene (DVB), alkyl acrylate, and methacrylate ester-based seeds have all been reported.[3–18] The seed latex particles are generally monodisperse

Correspondence to: M. J. Whitcombe. E-mail: michael.whitcombe@bbsrc.ac.uk

Contract grant sponsor: BBSRC.

Journal of Applied Polymer Science, Vol. 77, 1851–1859 (2000)

and can vary in size from approximately 30 nm to more than 1 μm in diameter. This seed is then used in the second stage when it is either mixed with another monomer/mixture of monomers before polymerization (batch process) or, in the case of a semi-continuous process, the monomer is fed into a reactor containing the seed under carefully controlled conditions. The size, morphology, and physico-chemical properties of the core-shell polymer particles can be controlled by the composition and structure of monomers and the reaction conditions used.[14–20]

Traditionally, core-shell particles are very lightly crosslinked, with the amounts of difunctional monomer rarely exceeding 8%. However, for the preparation of imprinted polymers, a shell of this composition would not be sufficiently rigid to maintain the precise spatial arrangement of functional groups in the binding site and to preserve the overall shape of the template, all prerequisites for efficient imprinting.[21–23] Several compositions of imprinted core-shell particles incorporating highly crosslinked shells were therefore prepared and characterized to assess their suitability as a protocol for the synthesis of submicron imprinted polymer particles. Once again, cholesterol was used as a model template to ensure that adequate comparisons with materials prepared by different polymerization methods could be made. Cholesterol was imprinted using the carbonate ester sacrificial spacer methodology.[24]

EXPERIMENTAL

Materials and Methods

DVB (80%), ethyleneglycol dimethacrylate (EGDMA), St, and methyl methacrylate (MMA) 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 lauryl sulphate, ammonium peroxodisulfate, both from Aldrich, and all the other solvents and reagents were used as received. High-pressure liquid chromatography (HPLC) solvents were purchased from Fisher Scientific (HPLC grade) or Riedel-de-Haën (Chromsolve). Deionized water was used throughout.

The purity of the synthesized products was checked by Fourier transform infrared (FTIR) spectroscopy, thin layer chromatography (TLC), and nuclear magnetic resonance (NMR). FT-NMR spectra were obtained on a Jeol EX-270. FTIR spectra of samples dispersed in KBr were recorded on a Perkin-Elmer Series 1600 FTIR spectrophotometer by diffuse reflectance IR spectroscopy. The submicron polymeric particles obtained were characterized using a Philips transmission electron microscope (TEM), or by dynamic light scattering using a Malvern Instruments Zetasizer 3000. 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 light-scattering mass detector and a Shimadzu SIL-9A autosampler. Samples were analyzed 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 over 6 min. Magnetic separations were performed using a 12.5 × 6.0 cm BioMag Separator 8-4101S containing 24 magnetic disks each of 27 megagauss Oersteads strength (Advanced Magnetics Inc., Cambridge, MA).

Synthesis of Cholesteryl (4-vinyl)phenyl carbonate (CVPC) and phenyl (4-vinyl)phenyl carbonate (PVPC)

CVPC and PVPC compounds were prepared by reaction of 4-vinylphenol and either cholesteryl or phenyl chloroformate in tetrahydrofuran/triethylamine as described previously.[24] Melting point, IR, and NMR spectra were identical to the published data.

Preparation of Seed Latex

The preparation of submicron core-shell particles was performed by a two-step polymerization method. In a typical polymerization, the seed was first prepared by batch emulsion polymerization in a 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. Monomer (6.7%) and a solution of $NaHCO_3$ (0.16%) in distilled water (92.8%) were pre-emulsified in the presence of sodium lauryl sulphate (0.16%) by stirring at 80°C for at least 15 min, before addition of ammonium peroxodisulfate (0.088%) to start the polymerization reaction. The temperature was maintained at 80°C for at least 20 h. The stirring rate was 260 rpm.

Emulsion Polymerization in the Presence of a Seed

A conventional three-necked glass reactor was used. Before charging, 50 mL of seed latex was stirring with a solution of the crosslinker monomer (14.9%) and the template CVPC (0.8%) for 2 h. The reactor was then charged in the following order: First, a solution of sodium lauryl sulphate (0.58%) in deionized water (79%), followed by the swollen seed containing monomer and template. This mixture was kept under a nitrogen atmosphere and stirred at 260 rpm for 30 min before the addition of ammonium peroxodisulfate (0.046%). The reaction was maintained under a nitrogen atmosphere at 60°C for 24 h with stirring before cooling to room temperature. The final latex was filtered and no coagulum was obtained.

Preparation of Magnetic Seed Latex

Oleic acid/sodium dodecylbenzene sulfonate stabilized ferrofluid was prepared from iron (II) and iron (III) chlorides based on the method of Wooding et al.[25] The ferrofluid solution was ultrafiltered to eliminate excess oleic acid and surfactant. The magnetic seed particles were prepared by mixing the ferrrofluid with the aqueous part of the polymerization mixture.

Template Removal by Hydrolysis

Thirty milliliters of the 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 removal of the template was followed by TLC and IR.

Ultrafiltration

This was performed 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 the 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.

Binding Experiments

Polymers (10 mg or 30 mg) were weighed into 2-mL–capacity screw cap vials (Wheaton) fitted with PTFE-lined caps. A 1 m*M* solution of cholesterol in 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, UK) fitted to 5-mL disposable syringes (gravimetric experiments with polymer suspensions in pure solvent showed that all of the polymer was retained by the filters and hence was in an agglomerated state in isohexane). The concentration of cholesterol remaining in the supernatant was determined by HPLC, calibrated against dilutions of the stock solution.

Characterization of Particles by TEM

To prepare samples for TEM, one drop of polymerized latex was added to 2 mL of water (in the case of the acrylic particles they were previously negatively stained with one drop of 1% phosphotungstic acid solution) and one drop of this mixture was put on a Formvar-coated copper grid of 400 mesh. The micrographs were taken using a Philips TEM.

RESULTS AND DISCUSSION

Initially, seed latices were prepared using conventional (meth)acrylates and styrene monomers according to protocols available in the literature,[9,26,27] with some modifications (see Experimental section), as illustrated schematically in Figure 1. The choice of seed was dictated by particle size requirements because we wanted the seed, and hence the final particles, to be as small as possible. Because only the shell is imprinted, the core would be nonfunctional in terms of binding interactions and in any case is unlikely to be accessible to ligands. Smaller core-shell particles will exhibit a higher surface area per unit mass than larger beads and our intention was to see whether sufficiently high surface areas could be achieved in imprinted nanoparticles without the need for incorporating porogens in the polymerization mixture. For a polymer of density ρ (in

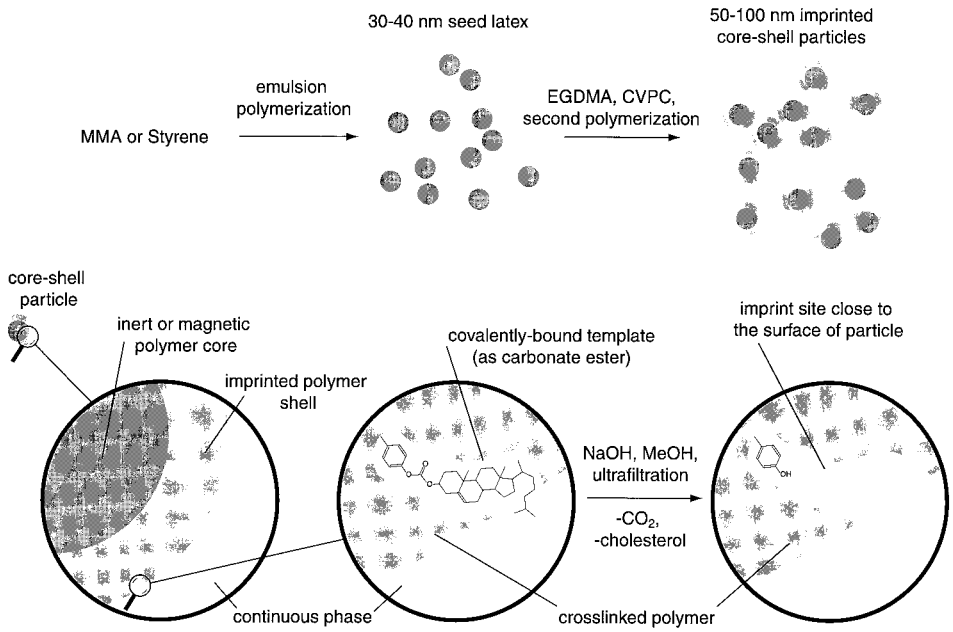

Figure 1 Schematic diagram of the synthesis of submicron core-shell particles with cholesterol-imprinted shells.

g cm^{-3}) consisting of spherical particles of radius r (in m), the surface area is given by Eq. (1) (in m^2 g^{-1}). From this formula it is evident that to achieve a surface area of approximately 100 m^2 g^{-1} requires the formation of particles of approximately 60 nm in diameter (assuming a density of 1 g cm^{-3}).

Area per unit mass for

$$\text{spherical particles} = \frac{3 \times 10^{-6}}{\rho \times r} \quad (1)$$

In addition to the seed latices prepared according to literature methods, those based on St and MMA were also made with the inclusion of a small amount of a compatible crosslinker, i.e., DVB in the case of St and EGDMA in the case of MMA. This would enable us to assess the compatibility of different imprinted shell-forming mixtures with each of the seed compositions and to compare the binding properties of materials made with the same shell over different seeds. The particle and latex characteristics for the five seed preparations are summarized in Table I.

In general, all the seed particles were of similar size, typically between 30 and 45 nm as determined by TEM [Table I and Fig. 2(a,c)]. These seeds were then used for the second stage polymerization to introduce the crosslinked shell. In standard experiments, the seeds were swollen with EGDMA or DVB for 2 h before initiation (at 60°C) by the addition of ammonium peroxodisulfate. In the case of imprinted polymers, 1.5–2.0 mol % template was added with the crosslinking monomer. Polymerization was allowed to proceed for 24 h after which time the polymer was filtered to remove any coagulum that may have formed and the particles recovered by precipitation with methanol. The carbonate ester method requires a

Table I Composition of Seed Particles

Code	Seed Composition[a]	SDS/M (%)	d, nm[c]	Solid Content (%)[d]
C1	MMA, 6.7%[b]	2.47	32	7.2
C2	MMA/EGDMA (9 : 1), 7.3%[b]	2.26	30	7.7
C3	St, 14.3%[b]	3.98	45	14.7
C4	St/DVB (9 : 1), 16.7%[b]	3.87	35	9.7
C5	St/Na acrylate (9 : 1), 9.2%[b]	5.18	41	9.8

[a] Prepared at 330 rpm using ammonium peroxodisulfate as initiator.
[b] Percentage by weight of monomer mixture in the dispersion.
[c] Determined by TEM.
[d] Determined by gravimetric analysis.

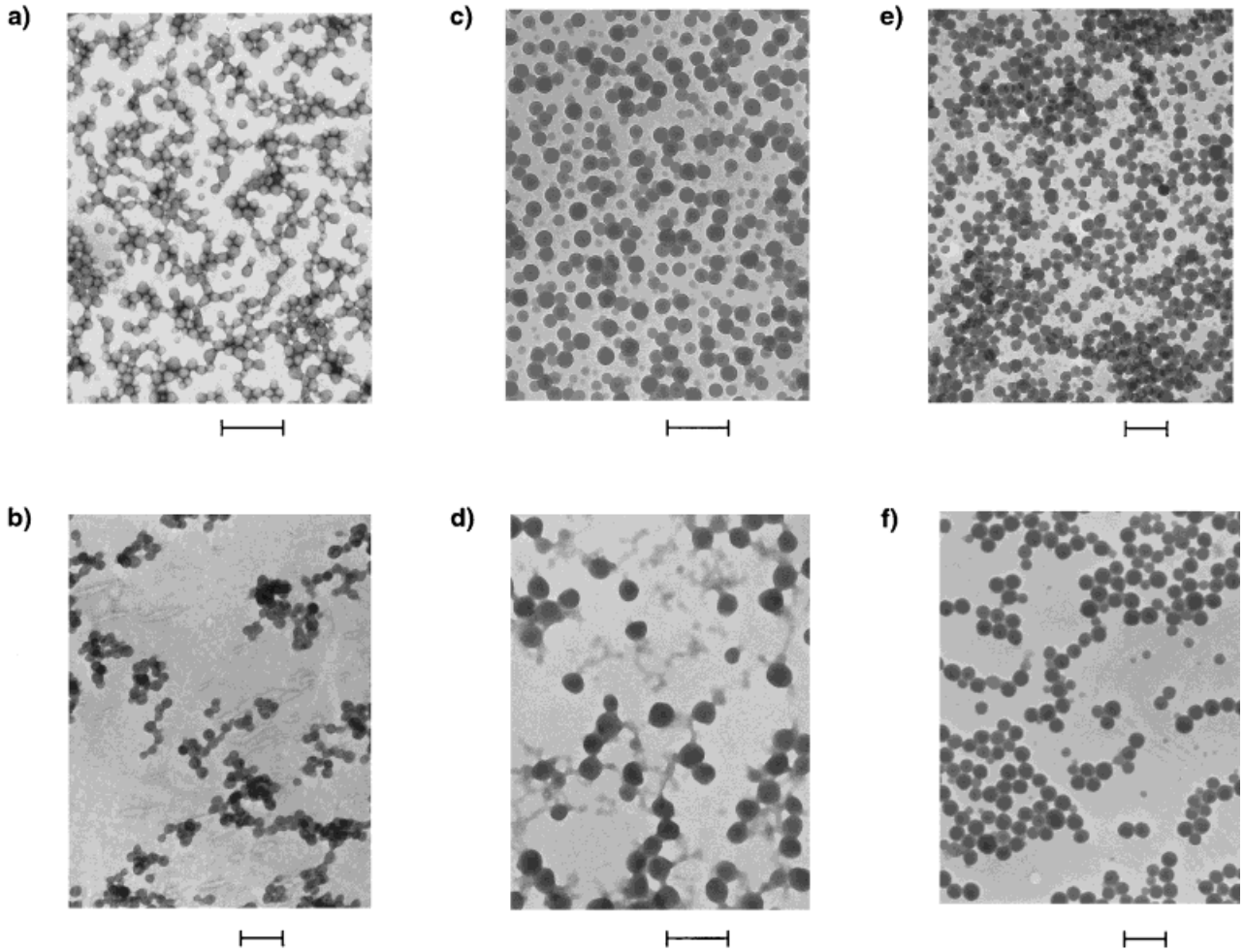

Figure 2 TEM photographs of polymeric nanoparticles (scale bars represent 200 nm). (a) Seed particles C1, MMA, d = 32 nm. (b) Core-shell particles CS1-EIH, EGDMA-based imprinted shell over MMA core, d = 58 nm, after hydrolysis, washing and ultrafiltration. (c) Seed particles C3, St, d = 45 nm. (d) Core-shell particles CS3-EI, EGDMA-based imprinted shell over St core, d = 76 nm. (e) Core-shell particles CS5-DI, DVB-based imprinted shell over St/Na acrylate core, d = 52 nm. (f) Core-shell particles CS3-DI, DVB-based imprinted shell over St core, d = 71 nm. (d), (e), and (f) are the latex particles as prepared (before removal of template and surfactant).

hydrolysis step to free the template and hence polymers were hydrolyzed under standard conditions[24] followed by ultrafiltration to remove the template and the surfactant present (see Experimental section). The polymer compositions and the results of surface area measurements are presented in Table II and typical photomicrographs of imprinted core-shell polymers are shown in Figure 2(b,d–f). As can be seen from Table II, the surface areas of imprinted core-shell particles from TEM observations were in the range 80–120 $m^2\ g^{-1}$. In selected examples, this was compared with the values obtained by nitrogen porisimetry (BET method) which agreed fairly well with the results calculated on the basis of microscopic observations.

Once imprinted core-shell polymers were obtained they were tested for their ability to bind cholesterol from isohexane solution at 1 mM concentration. Imprinted polymers that had been hydrolyzed and ultrafiltered to remove template and surfactant were compared with the same polymer that had been ultrafiltered only (template still in place) and a blank preparation, incorporating the shell material but no template, which had also been hydrolyzed and ultrafiltered. The morphol-

Table II Composition of Imprinted Core-Shell Particles

				Surface Area (m^2 g^{-1})	
Code	Core	Shell[a]	d, nm[c]	Calculated	Measured[d]
CS1-EI	C1	EGDMA	49	122.4	79
CS1-DI	C1	DVB	54	110.6	n.d.
CS2-EI	C2	EGDMA	43	138.5	n.d.
CS2-DI	C2	DVB	21–63	2 populations of particles	
CS3-EI	C3	EGDMA[b]	76	78.7	82
CS3-DI	C3	DVB	71	85.2	n.d.
CS4-EI	C4	EGDMA	n.d.	Low yields of polymer obtained	
CS5-EI	C5	EGDMA	21–95	2 populations of particles	
CS5-DI	C5	DVB	52	114.8	n.d.

n.d., not determined.
[a] Core-shell polymers were prepared at 3.5 to 1 ratio of DVB or EGDMA in the shell to core by weight except b.
[b] Prepared at 6 to 1 ratio.
[c] If the shell is formed as intended, the particle size should increase in a predictable manner, as given by the equation: $(dp)^3/(dp_{seed})^3 = (V_M + V_{seed})/V_{seed}$ (dp, particle diameter, V, volume, assuming a density of 1 g/mL). The calculated figures for CS1-E1 (52 nm) and CS3-EI (74.5 nm) and are in close agreement with the measured values (above), indicating that for these particles the shell polymerization has proceeded as expected, without significant secondary nucleation.
[d] BET surface area determined by nitrogen adsorption porosimetry.

ogy of imprinted and blank preparations were found to be very similar by TEM (not shown) with the expectation that nonspecific adsorption should likewise be similar for both preparations. The results are presented in Table III.

In general, the best results were obtained with polymers prepared with the MMA core and EGDMA shell (CS1-E series) but the performance of these polymers deteriorated significantly when either a small proportion of EGDMA was added to the core (polymers based on CS2) or when the EGDMA shell was replaced by DVB (compare lines 1–7 with lines 8–13, Table III). It is not clear why the addition of only 10% EGDMA to the MMA seed preparation should have such a dramatic effect on the uptake of cholesterol. This result is surprising because template removal was confirmed (although not quantified) by TLC, implying that imprint sites were also present in this material. When EDGMA-based shells were prepared over a styrene core (CS3-E series) satisfactory uptakes were also seen after template removal (compare lines 6 and 7, Table III). However, very low yields of core-shell particles were obtained when the same shell was applied to the St-DVB core (CS4-E polymers) and insufficient material was recovered to perform uptake measurements. In all instances, imprinted shells based on DVB bound very little cholesterol. This phenomenon has been observed previously for other DVB-based imprinted polymers[1,24] and was found to be true regardless of seed composition. These observations were consistent with the general view that DVB-based imprinted polymers tend to be inferior to their methacrylate-based counterparts.[28]

In previous experiments with bulk[24] and suspension polymers,[1] imprinted using the same chemistry, high nonspecific binding was occasionally seen with high surface area polymers, irrespective of hydrolysis. This is clearly not the case with imprinted core-shell nanoparticles because negligible binding to nonhydrolyzed imprinted polymers was observed despite identical particle morphologies. In addition, whereas in our earlier work some binding may have been due to partial hydrolysis of the EGDMA matrix, in the case of core-shell particles, binding to hydrolyzed nonimprinted particles remained very low. These results suggest that binding to CS1-EIH and CS3-EIH is almost entirely due to hydrogen bonding to the vinylphenol residue in the imprint sites revealed by template removal. The imprinting of a smaller template, PVPC, in place of CVPC had previously been observed to give polymers, which, although functionally identical to cholesterol imprints, showed substantially reduced cholesterol binding. The same effect was evident in CS1-EPH

Table III Uptake of Cholesterol by Imprinted, Nonhydrolyzed and Nonimprinted Core-Shell Particles from 1 mL of a 1 m*M* Solution of Cholesterol in Isohexane

				Cholesterol Uptake Percentage Bound (μmol/g)			
No.	Polymer[a]	Shell	Template	10 mg mL^{-1}		30 mg mL^{-1}	
1	CS1-EIH	EGDMA	CVPC	19%	(19)	57%	(19)
2	CS1-EI	EGDMA	CVPC-nonhydrolyzed	2.7%	(2.7)	4.5%	(1.5)
3	CS1-ENH	EGDMA	None	<2.0%	(<2)	<2.0%	(<1)
4	CS2-EIH	EGDMA	CVPC	<2.0%	(<2)	<2.0%	(<1)
5	CS2-EI	EGDMA	CVPC-nonhydrolyzed	<2.0%	(<2)	3.0%	(1)
6	CS3-EIH	EGDMA	CVPC	18%	(18)	41%	(14)
7	CS3-EI	EGDMA	CVPC-nonhydrolyzed	<2.0%	(<2)	4%	(1.4)
8	CS1-DIH	DVB	CVPC	<2.0%	(<2)	<2.0%	(<1)
9	CS1-DNH	DVB	None	<2.0%	(<2)	<2.0%	(<1)
10	CS2-DIH	DVB	CVPC	<2.0%	(<2)	7%	(2)
11	CS2-DI	DVB	CVPC-nonhydrolyzed	<2.0%	(<2)	5.7%	(2)
12	CS3-DIH	DVB	CVPC	<2.0%	(<2)	8%	(2.7)
13	CS3-DI	DVB	CVPC-nonhydrolyzed	<2.0%	(<2)	3.6%	(1)
14	CS1-EPH	EGDMA	PVPC	10.5%	(10.5)	35.6%	(12)

[a] Polymers prepared with seed C4 (Table I) are not included because these materials were obtained in low yields.

(line 14, Table III) although not as marked as in the bulk polymer case.

Encouraged by these results, we investigated the possibility of preparing magnetic imprinted core-shell polymers using essentially the same approach. To this end, aqueous-dispersed ferrofluid was prepared as described in detail elsewhere[29] for incorporation into the core. It was thought that a degree of crosslinking was desirable in a magnetic core to stabilize the magnetite particles and reduce the possibility of interference in the binding process by surfactants present in the ferrofluid. The seed latex compositions were therefore modified until a suitable magnetic core preparation was found. This consisted of a 9:1 MMA to EGDMA mixture prepared with 14.5% aqueous ferrofluid (based on solids) without additional surfactant, polymerized at 70°C using 0.46% initiator. Both this polymeric seed and the ferrocolloid itself were used as cores in the preparation of EGDMA-based imprinted core-shell latices CSM-EI and CSF-EI, respectively. The composition and particle size data are presented in Table IV together with the uptake of cholesterol by hydrolyzed and nonhydrolyzed magnetic polymers.

It is evident from the results presented in Table IV that the magnetite-loaded core-shell polymers, CSM-EI(H), both in terms of their morphology and binding characteristics, were very similar to materials prepared with nonferrofluid-containing cores. This is despite the fact that a crosslinked MMA core was used (compare with CS2-EIH, Table III). More interestingly it also proved possible to use the ferrofluid itself as a seed with very similar results being obtained, with the added bonus that the resultant particles could be very easily dispersed and could be rapidly sedimented with a magnetic field.

In conclusion, we have shown that imprinted polymer particles in the 50–100-nm size range can be readily prepared by a modified core-shell polymerization procedure. The resulting materials displayed binding properties similar to that of analogous "bulk" polymers prepared by conventional imprinting techniques. Surface areas of approximately 80 m^2 g^{-1} can therefore be achieved without incorporating a porogenic solvent in the polymerization mixture. It is possible, therefore, to predetermine the surface area of imprinted polymers by controlling particle morphology alone, thus eliminating the unpredictable effects of porogen, monomer composition, template loading, and crosslink density on the accessible polymer surface.

Table IV Composition, Characterization, and Cholesterol Uptake by Magnetic Core-Shell Polymer Particles

Code	Core	Shell	Template	d, nm[b]	Cholesterol Uptake[c] Percentage Bound (μmol/g) 10 mg mL^{-1}		30 mg mL^{-1}		Time to Sediment[d] Magnet[e]	Gravity
CSM-EIH	MMA/EGDMA (9 : 1)[a]	EGDMA	CVPC		28%	(28)	60%	(20)		
CSM-EI	MMA/EGDMA (9 : 1)[a]	EGDMA	CVPC-nonhydrolyzed	210	6%	(6)	11%	(3.7)	4 min	4–5 h
CSF-EIH	Ferrofluid	EGDMA	CVPC		15%	(15)	40%	(13.3)		
CSF-EI	Ferrofluid	EGDMA	CVPC-nonhydrolyzed	74	4%	(4)	11%	(3.7)	30 s–1 min	>75 h

[a] Contains 14.5% magnetite as aqueous ferrofluid.
[b] Determined by dynamic light scattering.
[c] From a 1 m*M* solution in isohexane.
[d] From a dispersion in water.
[e] See Materials and Methods for magnet details.

Both MMA and St-based cores were successfully overlaid with imprinted EGDMA shells. Furthermore, the core can be a magnetite-loaded polymer, or consist entirely of magnetite, to produce superparamagnetic imprinted nanoparticles. The magnetite loading of the particles was shown to be sufficient for practical usage in the laboratory with separations as rapid as 30 s in a magnetic field whereas the same particles took in excess of 75 h to sediment under the influence of gravity alone. The aqueous-based polymer chemistry presented herein may prove to be more advantageous than systems based on fluorocarbon solvents[30] (for the production of magnetic imprinted beads) or extensive grinding of bulk polymers[31] (to produce irregularly shaped micron-sized or submicron particles), combining the features of both in a simple procedure. The imprinted core-shell polymers are expected to find applications in biomedical and food analyses and possibly in relatively small-scale separations of high added value products. The feasibility of the latter is currently being investigated in our laboratory.

The authors thank the BBSRC for financial support and also express their gratitude to Prof. R. Burch and Miss. V. Caps of the Department of Chemistry, University of Reading for the surface area measurements, Mrs. Patricia Bland for assistance with TEM and to Dr. Andreas Stein of the Advanced Centre for Biochemical Engineering, University College London, for assistance with the dynamic light scattering measurements.

REFERENCES

1. Flores, A.; Cunliffe, D.; Whitcombe, M. J.; Vulfson, E. N. J Appl Polym Sci 2000, 77, 1841.
2. Lee, S.; Rudin, A. ACS Symp Ser 1992, 492, 234.
3. Tamai, H.; Nishida, Y.; Suzawa, T. J Colloid Interface Sci 1991, 146, 288.
4. Eshuis, A.; Leendertse, H. J.; Thoenes, D. Colloid Polym Sci 1991, 269, 1086.
5. Zou, D.; Ma, S.; Guan, R.; Park, M.; Sun, L.; Aklonis, J. J.; Salovey, R. J Polym Sci A 1992, 30, 137.
6. Okubo, M.; Kondo, Y.; Takahashi, M. Colloid Polym Sci 1993, 271, 109.
7. Kim, H. B.; Wang, Y. C.; Winnik, M. A. Polymer 1994, 35, 1779.
8. Lee, J.; Senna, M. Colloid Polym Sci 1995, 273, 76.
9. Kawaguchi, S.; Yekta, A.; Winnik, M. A. J Colloid Interface Sci 1995, 176, 362.
10. Bon, S. A. F.; Van Beek, H.; Piet, P.; German, A. L. J Appl Polym Sci 1995, 58, 19.
11. Nelliappan, V.; El-Asser, M. S.; Klein, A.; Daniels, E. S.; Roberts, J. E. J Appl Polym Sci 1995, 58, 323.
12. Okubo, M.; Hosotani, T.; Yamashita, T. Colloid Polym Sci 1996, 274, 279.
13. Okubo, M.; Ahmad, H. J Polym Sci A 1996, 34, 3147.
14. Lee, C. F.; Lin, K. R.; Chiu, W. Y. J Appl Polym Sci 1994, 51, 1621.
15. Segall, I.; Dimonie, V. L.; El-Asser, M. S.; Soskey, P. R.; Mylonakis, S. G. J Appl Polym Sci 1995, 58, 385.
16. Okubo, M.; Lu, Y. Colloid Polym Sci 1996, 274, 1020.
17. Okubo, M.; Lu, Y. Colloids Surf A 1996, 109, 49.
18. Cheong, I. W.; Kim, J. H. Colloid Polym Sci 1997, 275, 736.

19. He, W. D.; Cao, C. T.; Cai Yuan, P. J Appl Polym Sci 1996, 61, 383.
20. Wang, Q.; Fu, S. K.; Yu, T. Y. Prog Polym Sci 1994, 19, 703.
21. Wulff, G. Angew Chem Int Ed Engl 1995, 34, 1812.
22. Steinke, J.; Sherrington, D. C.; Dunkin, I. R. Adv Polym Sci 1995, 123, 81.
23. Vulfson, E. N.; Alexander, C.; Whitcombe, M. J. Chem Br 1997, 33, 23.
24. Whitcombe, M. J.; Rodriguez, M. E.; Villar, P.; Vulfson, E. N. J Am Chem Soc 1995, 117, 7105.
25. Wooding, A.; Kilner, M.; Lambrick, D. B. J Colloid Interface Sci 1992, 149, 98.
26. Nuno–DonLucas S.; Rhoton, A. I.; Corona–Galvan, S.; Puig, J. E.; Kaler, E. W. Polym Bull 1993, 30, 207.
27. Sarobe, J.; Forcada, J. Colloid Polym Sci 1996, 274, 8.
28. Wulff, G.; Vietmeier, J.; Poll, H. G. Makromol Chem 1987, 188, 731.
29. Khng, H. P.; Cunliffe, D.; Davies, S.; Turner, N. A.; Vulfson, E. N. Biotechnol Bioeng 1998, 60, 419.
30. Ansell, R. J.; Mosbach, K. Analyst 1998, 123, 1611.
31. Haupt, K. ACS Symp Ser 1998, 703, 135.