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Core-shell Molecular Imprinted Polymer Colloids

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Molecular imprinted core-shell colloidal polymer particles have been prepared in which the shells were formed, in aqueous media, in the presence of organic templates. The most selective system involved the use of an unsaturated alkyl phosphate as the electrostatic binding motif. Systems that were able to selectively differentiate between caffeine and theophylline and the Gly-Gly sequence in tripeptides are described. Radiotracing experiments showed that all of the caffeine template could be removed following extraction.

Keywords: Core-shell polymers; Molecular imprinting; Peptide; Radiotracer; Leaching

INTRODUCTION

Recently, we have shown that it is possible to produce colloidal core-shell polymer particles that contain molecular imprints of caffeine; i.e. core-shell molecular imprinted polymers (CS-MIPS) [1]. Selective extraction and saturation binding experiments showed that these materials were able to differentiate between caffeine and the structurally similar molecule theophylline. Since the pioneering work of Wulff [2,3] and Mosbach [4,5] molecular imprinting methodology has been proposed in several different formats. Many of the subsequent materials were prepared by solution/precipitation polymerisations that yielded blocks of polymer, which were then ground to give irregular particles. More recently other polymerisation methods have been reported including: polymerisations of dispersions in water [6]; polymerisations of dispersions in fluorocarbons [7]; membrane synthesis at an aqueous/water interface [8]; and polymerisations at the surface of silica [9].

Polymer colloids have also attracted interest as substrates for imprinting technology. The first usage of polymer colloids in this area appears to be attributable to Yu *et al.* who introduced the use of imprinted polymer colloids for the selective extraction of metals [10]. Yoshida *et al.* also later examined similar systems imprinted with the amphiphilic amino acid tryptophan methyl ester [11]. The polymers could re-adsorb the template but no selectivity was reported. Also, the system was reported to be unsuitable for the recognition of more hydrophobic molecules, which become buried in the particle's interior during the polymerisation. This is of course a consequence of the one-step nature of the polymerisation. Other workers have since employed core-shell emulsion polymerisation in order to locate the template at the particle surface. Tovar *et al.* have used mini-emulsion techniques to do this [12] and Whitcombe *et al.* rely on the formation of hydrophobic indents in the shell to imprint cholesterol [13,14]. Recently [1], we also employed core-shell methodology, but as well as hydrophobic recognition we used the phosphate binding system employed by Yu *et al.* in their metal binding experiments [10]. Using this system we were able to extract caffeine from a mixture of caffeine and a close analogue, theophylline. One feature of these colloidal surface imprinted systems, which is potentially attractive, is that it should be much easier to remove the last remnants of the template. Conventional MIPs often suffer from the drawback of leaching due to entrapment of the template within the tightly cross-linked network. Slow release of this material allows

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it to continuously diffuse through the system [15] and several strategies to reduce this bleeding are under investigation. For example, Elwanger *et al.* have compared various extraction procedures that were based on the use of microwave irradiation; supercritical fluids; thermal annealing or Soxhlet procedures [16]. CS-MIPs on the other hand locate the template at the surface making it more accessible to the extraction procedures. Also, electrostatic binding in water, the preferred medium for emulsion polymerisation and recognition of biological entities, is considerably weaker than in organic media. Thus, we considered that our CS-MIPs should release the template more effectively than the conventional systems and this may make them suitable for trace analysis. The radiotracer technique employing ^{3}H- and ^{14}C-labelled compounds provides a direct, accurate and sensitive method to probe the fate of molecules in chemical and biological systems [17] and the technique has been applied previously to measure the ratios of amino acids that are bound and free when in contact with molecular imprinted polymers [18].

EXPERIMENTAL

Materials

Styrene (Aldrich) and divinylbenzene (Aldrich) were purified by washing with 10% sodium hydroxide (× 3), to remove inhibitors; washing with distilled water (× 3) and drying over anhydrous magnesium sulphate. The divinylbenzene was distilled under vacuum and the styrene used directly. Sodium dodecyl sulphate was "specially pure" grade (BDH chemicals). Oleyl phenyl hydrogen phosphate was prepared from oleyl alcohol (Aldrich) and phenyl phosphorodichloridate (Aldrich). 4-Morpholine-ethanesulphonic acid (Aldrich) and 4,4′-azobis-4-cyanovaleric acid (Aldrich) were used as purchased. De-ionised water was obtained from a *Millipore* (Milli-Q) purification system at resistivity 18.2 MΩ cm. [8-^{14}C]Caffeine (Sigma) was supplied as an ethanol solution (50 μCi) and the specific activity was 53 mCi mmol^{-1}(1.86 MBq). A working solution was formed by evaporating the solvent under a stream of nitrogen and dissolving in distilled water (500 μl).

Instrumentation

A Packard Tri-Carb 1500 liquid scintillation analyser was used in conjunction with Ecoscint A (National Diagnostics, Hull UK) as scintillant and glass scintillation vials. Centrifugation was carried out using a Mistral 2000 bench-top centrifuge fitted with a swing-bucket rotor. Centrifugation cartridges (Vivaflow 20, polyether sulphone, MWCO 10,000, 20 ml) were from Sartorius (Surrey UK). Particle sizes were measured with a Coulter N4 SD sub-micron particle size analyser.

Preparation of Divinylbenzene-cross-linked Polystyrene Core Particles

An aqueous solution of 4-morpholineethane-sulphonic acid monohydrate (0.6398 g, 3.00 mmol, 50 mM) in de-ionised water (52.5 ml) was prepared and then purged with nitrogen for 15 min. Sodium dodecyl sulphate [source BDH, "specially pure"] (1.50 g, 5.20 mmol) was added then the mixture was stirred until dissolution, sonicated for 10 min at room temperature then adjusted to pH 6.0 by the addition of 1 M NaOH. The solution was stirred at 300–350 rpm under a nitrogen atmosphere with the temperature maintained at 60°C. Styrene (4.0173 g, 38.57 mmol) and divinylbenzene (5.0217 g, 38.57 mmol) were then added dropwise to the mixture over 30 min with stirring. The polymerisation was initiated by the addition of one portion of 4,4′-azobis(4-cyanovaleric acid) (0.5460 g, 1.95 mmol) and stirring was continued for 16 h to give an emulsion of core particles.

Surface-template Emulsion Polymerisation with Caffeine and Theophylline

A mixture of ethylene glycol dimethacrylate (0.4520 g, 2.28 mmol) and oleyl phenyl hydrogen phosphate (0.4080 g, 0.96 mmol) in de-ionised water (7.5 ml) was added to the emulsion at 60°C and stirring was continued for 30 min. The template, caffeine or theophylline, (1.923 mmol, 2 eq) was added and stirring was continued for a further 30 min. Then the second stage of polymerisation was initiated by the addition of 4,4′-azobis(4-cyanovaleric acid) (0.5460 g, 1.77 mmol) in one portion and heating to 60°C. The reaction was continued for 105 min at 60°C and was then quenched by lowering the temperature to 0°C in an ice–water bath. Blank particles were also prepared by repeating the polymerisation in the absence of either template.

Surface-template Emulsion Polymerisation Templated with [8-^{14}C]Caffeine

A sample of the core emulsion (5.25 ml) was stirred rapidly at 60°C under a nitrogen atmosphere in a three-necked round bottom flask fitted with a condenser. A mixture of ethylene glycol dimethacrylate (0.0452 g, 0.228 mmol) and oleyl phenyl hydrogen phosphate (0.0408 g, 0.096 mmol) was added to the emulsion as a suspension in water (1.00 ml) and stirring was continued for 10 min. A sample of caffeine (0.0373 g, 0.192 mmol, 2 eq) was

then added in one portion followed immediately by the aqueous [8-^{14}C]caffeine solution (250 μl, 25 μCi) and stirring was continued for 30 min. The surface polymerisation was initiated using 4,4′-azobis (4-cyanovaleric acid) (0.0546 g, 0.177 mmol) and stirring continued for 105 min and then the reaction was quenched in ice–water.

Preparation of Solid Resins

The resins were obtained by controlled coagulation of the latex (2.0 ml aliquots) by the addition of acetone (1.1 ml). The solid particulate mass obtained was then slurry packed into solid-phase extraction cartridges, and then washed with a 2:1 acetone–water mixture (40 ml); phosphoric acid (aqueous 1 mol dm^{-3}) (40 ml), methanol (40 ml); equilibrated with phosphoric acid (aqueous 1 mol dm^{-3}) (2.0 ml); washed with de-ionised water (2.0 ml) and allowed to dry under vacuum. Phosphorus incorporation was measured by ICP-AES following concentrated nitric acid digestion of the resins [1], and amounted to 13.3% for caffeine-templated resins, 16.1% for theophylline-templated resins and 14.3% for the blank (non-templated resins).

Competitive Binding from Mixtures of Caffeine and Theophylline

Solutions of equimolar mixtures of caffeine and theophylline (300 nmol dm^{-3}) were prepared in 0.1 M sodium phosphate buffer at pH 8.0 or de-ionised water. The caffeine-, theophylline- and blank-templated resins (50 mg each) were added to solid-phase extraction cartridges. The materials were swelled with 2:1 acetone–water (2.0 ml) for 10 min and then washed with water. A 1.5 ml aliquot of each of the solutions was added to a sample of the resin and these mixtures were agitated every 5 min over a 30 min period. The solutions were then drained under vacuum, filtered and the uptake of each component in the mixture was determined using LC analysis via the integration of peaks corresponding to caffeine and theophylline in the mixture both before and following application to the resin. The selectivity for either caffeine (on a caffeine imprinted material) or theophylline (on a theophylline-imprinted material) was represented by determining the ratio of the uptake, U (amount adsorbed/amount applied), of either component relative to the other competing component. That is, selectivity for caffeine, S_C, is given by:

$$S_C = \frac{C_B/C_A}{T_B/T_A} = \frac{U_c}{U_T}$$

where C_A = amount of caffeine applied; C_B = amount of caffeine bound; T_A = amount of theophylline applied; T_B = amount of theophylline bound.

Similarly, the selectivity for theophylline, S_T, is given as the inverse of this relationship.

Extraction of [8-^{14}C]Caffeine-CS-MIP

To a 20 ml centrifugation cartridge (MWCO 10,000) was added [8-^{14}C]caffeine surface-templated emulsion (2.0 ml). 2-Propanol (IPA) (1.1 ml) was added dropwise to the emulsion and the tube was gently agitated until the solids had precipitated out. The sample was allowed to stand for 10 min to ensure complete precipitation then centrifuged at 4500 rpm at room temperature, using a bench-top centrifuge with swing-bucket rotor, for 45 min. To the remaining damp solids was added 7:3 IPA–H_2O (5.0 ml), the suspension was agitated and centrifuged for 30 min. The procedure was repeated six times. The activity of each wash and solution was determined by taking 20 μl samples for use in liquid scintillation counting.

Elution of the [8-^{14}C]caffeine was continued by suspending the resin in 1 M H_3PO_4 (5.0 ml) and centrifuging at 4500 rpm at room temperature for 3.5 min. A total of 6 × 5.0 ml washes were carried out and 20 μl samples were taken for use in liquid scintillation counting. The final elution procedure was carried out using methanol (5.0 ml) as the eluting solvent for a total of 6 × 5.0 ml washes and centrifugation was carried out at 4500 rpm at room temperature. The activity of each wash was determined by taking 20 μl samples for use in liquid scintillation counting. The polymer was next swelled with 7:3 IPA–H_2O and allowed to stand for 16 h then a 20 μl sample was taken and used for liquid scintillation counting. The polymer was then suspended in water (10 ml) and then samples (20 μl) were taken after 1, 16 and 48 h. The radioactivity of this suspension was measured by adding Ecoscint A (10 ml) and comparing the measured counts to that of the background radiation. Another experiment in which Ecoscint A (10 ml) was added to the suspension immediately after resuspension in water was performed and the radioactivity of this material was also measured at 1, 16 and 48 h. Next a sample of the washed polymer (0.5440 g) was refluxed in conc. HNO_3 (5 ml) at 100°C. A sample of this suspension was taken and added to Ecoscint A (10 ml) and the suspension was shaken for 16 h. The radioactivity of the suspension was then measured. A summary of the elution protocol is shown in Table I.

Liquid Scintillation Counting

A liquid scintillation analyser was used in conjunction with Ecoscint A as scintillant and measurements were carried out in glass scintillation vials. The total radioactivity (R_S), of the 2.0 ml sample of the latex, which was precipitated with IPA, is thus 286.15 kBq

TABLE I Summary of elution protocol and centrifugation times for the elution of [8-^{14}C]caffeine from surface molecularly-imprinted polymer particles

Wash number sequence	Eluting solvent	Volume eluting solvent/ml	Centrifugation time/minutes
1	IPA	1.1	45
2–7	7:3 IPA–H_2O	30	30,20,10,5,5,5
8–13	1 M H_3PO_4	30	3.5
14–19	MeOH	30	2.5

as calculated using Eq. (1), where V_E is the volume of the as-prepared core-shell emulsion, which contained 930 kBq radioactivity (R_T) and V_S is the volume of the latex precipitated with IPA.

$$R_S = (V_S/V_E) \times R_T = 2.0/6.5 \times 930$$

$$= 286.15\,\text{kBq} \quad (1)$$

To account for solvent loss due to evaporation in the centrifuge and adsorption to the polymer, the samples (5.0 ml) from the centrifugation/elution of the precipitated polymer colloid were each weighed to give a value, W_{wash}. A weighed 20 μl sample of the wash, $W_{20\,\mu l}$, was then added to a scintillation vial containing 10.0 ml Ecoscint A,and the activity, $R_{20\,\mu l}$, was measured by liquid scintillation counting. The radioactivity (kBq) of the wash sample, R_{wash}, could then be determined according to the relationship:

$$R_{wash} = \frac{R_{20\,\mu l}}{W_{20\,\mu l}} \times W_{wash} \quad (2)$$

The activities were corrected for background and the accumulated activity was determined as a percentage of the original activity added, i.e. 286.15 kBq.

Surface-template Emulsion Polymerisation with Tripeptides

A mixture of ethylene glycol dimethacrylate (0.4520 g, 2.28 mmol) and oleyl phenyl hydrogen phosphate (0.4080 g, 0.96 mmol) in de-ionised water (7.5 ml) was added to the core emulsion at 60°C and stirring was continued for 30 min. The tripeptide template, either: Met-Leu-Phe acetate salt (0.4514 g, 0.96 mmol) or Tyr-Gly-Gly (0.2839 g, 0.96 mmol), was added and stirring continued for a further 30 min then the second stage of polymerisation was initiated by the addition of 4,4′-azobis(4-cyanovaleric acid) (0.5460 g, 1.77 mmol) in one portion and heating to 60°C. The reaction was continued for 105 min at 60°C and was then quenched by lowering the temperature to 0°C in an ice–water bath. Blank particles were also prepared by repeating the polymerisation in the absence of either template.

The resins were obtained by controlled coagulation of the latex by the addition of acetone and washed as described previously [1]. A weighed amount of the resin was swelled with 2:1 acetone–water mixture, then washed with water in preparation for competitive binding studies with the three peptides.

Competitive Binding of Tripeptides

An aqueous equimolar mixture of three tripeptides was prepared by dissolving the components in water with sonication for 30 min and adjusting the solution to pH 7.0 with 1 M NaOH to give a solution of Met-Leu-Phe (138 μg ml^{-1}), Tyr-Gly-Gly (100 μg ml^{-1}), Gly-Gly-His (91 μg ml^{-1}). An aliquot (2.0 ml) of this solution was added to the solid resin (100 mg) and the mixture was agitated periodically over 30 min. The solution was filtered through the column and the amount of each tripeptide present was determined by LC analysis. Analysis of the eluent was achieved by LC-MS using an aqueous/acetonitrile gradient. Gly-Gly-His eluted at 1.12 min, Tyr-Gly-Gly at 3.92 min and Met-Leu-Phe at 12.90 min. The chromatographic conditions were: Column-Waters Novapak C-18; 0–20% acetonitrile (0.01% TFA)/10 mM aq. ammonium acetate; elution profile-Waters curve 8, 10 min then 20% acetonitrile/aq. ammonium acetate (0.01% TFA), 5 min; detector PDA 190–320 nm; injection volume 20 μl, flow rate 1 ml min^{-1}.

RESULTS AND DISCUSSION

Polymer Characterisation

Table II gives the particle sizes of the materials using the polymerisation process in water. The emulsion polymerisation data clearly show that particle diameters are significantly lower when a template molecule is incorporated, caffeine or theophylline in

TABLE II Particle sizes of imprinted emulsion polymer particles

Template	Diameter/nm
Caffeine	49
Theophylline	40
None	30 (57%), 262 (43%)

this case, than in their absence. In the results shown in Table II, it should also be noticed that the non-imprinted colloid displays a bimodal particle size distribution, whereas, the imprinted particles have mono-modal particle size distributions. This latter observation is further evidence for the enhanced colloidal stability and surface reorganisation arising from the location of the template at the particle surface. Elemental analysis showed that typically 15–17% of the added phosphorus was incorporated in the particle shells.

Selective Binding from Mixtures of Caffeine and Theophylline

Table III gives data on the extraction of caffeine or theophylline by the emulsion polymerisation prepared particles. The data show the expected result only for the caffeine imprinted particles that were exposed to a mixture of caffeine and theophylline in de-ionised water. That is a caffeine-imprinted particle adsorbs more caffeine from solution than theophylline and the uptake value for caffeine is approximately twice that achieved on a non-imprinted particle. However, the theophylline-imprinted particles also remove caffeine from solution in preference to theophylline. Our previous paper gives the binding data for these two situations [1]. Table III also illustrates the detrimental effect of carrying out these competitive experiments in media of higher ionic strength, in this case phosphate buffer. In these conditions all of the particles adsorb caffeine in preference to theophylline and the adsorbance is now dominated by hydrophobic interactions, which are enhanced as the ionic strength of the medium increases. This latter result also indicates that whilst the imprinting process, in water, clearly has an effect in reorganising the surface of the particles (the imprinted particles have different particle sizes and adsorb different amounts of caffeine than blank particles) the nature of the medium also plays an important role. Effects associated with interactions between the medium and the target molecule may thus be sufficient to dominate the system.

TABLE III Uptake and selective binding in competitive binding experiments

Template	Extraction medium	U_C	U_T	S
Caffeine	Water	0.94	0.51	1.84
Theophylline	Water	0.57	0.48	0.84
None	Water	0.56	0.51	1.10
Caffeine	Buffer	0.83	0.51	1.63
Theophylline	Buffer	0.87	0.57	0.66
None	Buffer	0.88	0.47	1.87

Radiotracer Study of Template Leaching

Leaching of the template during trace analysis is one of the major obstacles to be overcome in MIP technology. During our work in this area we noticed that, using LC-MS to analyse the washings from CS-MIP flocculates, leaching from these materials is minimal [1]. In order to further investigate this aspect we employed radiotracer technology. The strategy in this study was to use a radiolabelled template and to determine the radioactivity of both the extracts and the final material once it was nominally free of template. [^{3}H]Caffeine can be easily synthesised using a one-pot exchange reaction with HTO in a sealed tube at elevated temperature [19]. However, whilst the rate of tritium–hydrogen exchange at the imidazole ring system is low for [8-^{3}H]caffeine at room temperature and neutral pH, under the conditions used in our study (pH 6.0, 60°C) the exchange rate is significant. Thus radioactivity would be lost during the surface-template polymerisation stage through back exchange. The alternative, more stable candidate, [8-^{3}H]caffeine is commercially available. It is a medium range β-emitter, has a long half-life and can be purchased at an activity that is sufficiently high for very accurate liquid scintillation counting experiments.

The polymerisation process proceeded well and generated a stable polymer latex. The colloid was stable and showed little signs of coagulation on standing. The synthetic procedure produces particles with nominal (i.e. the shell thickness estimated by assuming that all of the mass added to the system coats the particles and is polymerised on the shell) shell thickness of 2 nm, which is the previously optimised dimension for maximum selectivity observed in these systems [1].

Figure 1 shows the progress of the removal of the template after three elution procedures. The data are presented as the percentage of template removed expressed as the cumulative percentage of

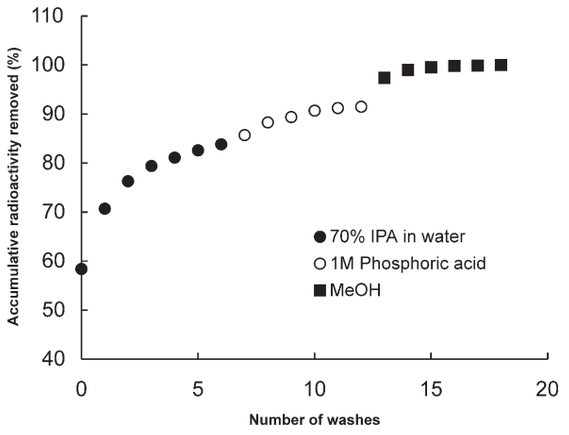

FIGURE 1 Extraction of [8-^{14}C]caffeine from the CS-MIP.

the radioactivity, derived from the template that is dissolved in the extractant. The first extraction was the precipitation procedure and this removed 58% of the template. The next six extractions, with IPA–water mixture, removed over 80% of the template. The use of phosphoric acid then allowed us to remove over 90% of the template but it was necessary to supplement this procedure with a further extraction with methanol, presumably due to the limited solubility of the template in aqueous phosphoric acid. As can be seen from the figure, following this final methanol extraction all of the radioactivity could be removed and analysis of the polymer by liquid scintillation counting merely gave background level counts (100–200 dpm). The data indicate that at least two adsorption mechanisms are operative in these systems. The first of these are probably very weak electrostatic interactions between phosphate and the template. In this case most of the adsorption process would be dominated by hydrophobic interactions, which are disrupted by the IPA–water extractant. This mechanism is very similar to the system reported by Whitcombe *et al.* who use shape-specific cavities formed by imprinting of hydrophobic molecules without significant electrostatic interactions. In order to remove material that is more tightly bound to the phosphate groups it was necessary to break the electrostatic interactions by changing the acid strength of the medium but complete removal of these more tightly bound template molecules still required the use of a solvent (methanol) that was able to partially disrupt hydrophobic polymer–template interactions. It is probable that a continuum of interactions occur in these systems in which at one extreme the template is bound mainly by hydrophobic interactions and weak electrostatic interactions and at the other extreme the electrostatic interactions are stronger but hydrophobic interactions are still important. The hydrophobic interactions are probably derived from shape-specific cavities formed during the imprinting process and the strength of the electrostatic interaction is most likely dictated by the orientation of the template and phosphate groups during polymerisation.

Next, the particles were further extracted to examine the possibility that small amounts of [8-^{14}C]caffeine had remained trapped in the coagulated particles. Thus, the particles were swelled in 7:3 IPA–water mixture for 16 h and the following procedures carried out. Firstly, a sample of the polymer was suspended in water and the supernatant sampled at 1, 16 and 48 h and 14 day intervals by adding to Ecoscint A and determining the activity by liquid scintillation counting. The swelled particles were also added directly to Ecoscint A and liquid scintillation counting of the dispersion was again performed after 1, 16 and 48 h and 14 days. Each of these measurements resulted in radioactivity readings that were close to the background level, as shown in Table IV (entries 1 and 2, respectively). A further experiment involved the acid digest of the shell with nitric acid, which cleaves the phosphate ester and EGDMA ester linkages. Thus, any material, which had penetrated the core prior to shell polymerisation, should be released into solution following digestion of the shell. Liquid scintillation counting, in Ecoscint A, did not detect any radioactivity above the background from the digest solutions. Finally, the acid digest was found to contain solid polymer particles, the cores. They were mixed with Ecoscint A and the radioactivity examined after 1, 16 and 48 h and 14 days. The radioactivity of each of these fractions of the digest was not significantly above background, as shown in Table IV (entry 3).

Imprinted Tripeptides

Competitive binding studies were carried out on the particles imprinted with Met-Leu-Phe, Tyr-Gly-Gly and on blank (non-imprinted) particles using an aqueous 1:1:1 mixture of the tripeptides Met-Leu-Phe, Tyr-Gly-Gly and Gly-Gly-His at neutral pH. The uptake values are given in Table IV and the LC results, showing the relative amounts of each of the tripeptides contained in the eluant both before and following solid-phase extraction, are presented in Fig. 2. A chromatogram of a blank (water) sample showed that the presence of a broad UV absorbance at 6.23 and 6.25 min in the peptide samples was attributable an eluant gradient effect (photodiode array detector).

The results in Table V and Fig. 2 show that tripeptide binding at pH 7.0 in water on the imprinted resins can be selective. The particles

TABLE IV Summary of background counts from liquid scintillation measurements of [8-^{14}C]caffeine extracted CS-MIPs

		Radioactivity/dpm in dispersing medium			
Entry	Dispersing medium (conditions)	1 h	16 h	48 h	14 days
1	Water (after swelling)	144	153	125	132
2	Ecoscint A (after swelling)	151	121	117	115
3	Ecoscint A (cores after digest)	149	116	150	178

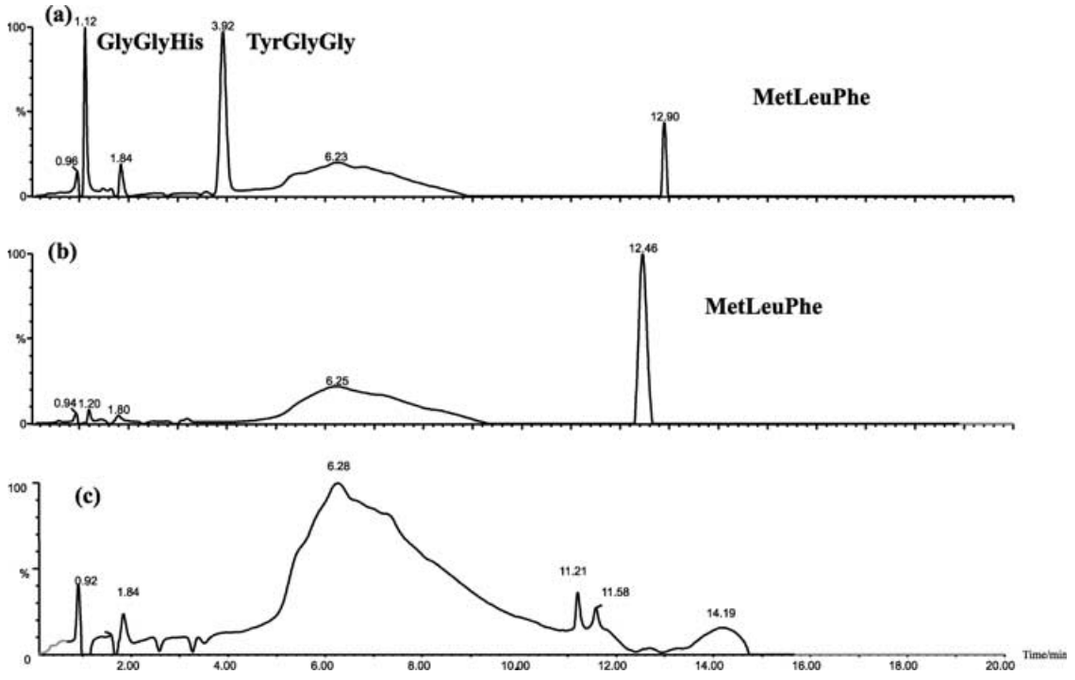

FIGURE 2 Chromatograms of (a) the mixture of tripeptides (b) the eluant after exposure of the mixture in (a) to the particles imprinted with Tyr-Gly-Gly and (c) a blank run (water sample), with no peptide components present.

imprinted with Tyr-Gly-Gly removed 100% of Tyr-Gly-Gly and Gly-Gly-His and exclude Met-Leu-Phe. This behaviour was very different to the blank particles, which show a preference for the Met-Leu-Phe peptide. Similarly, the particles imprinted with Met-Leu-Phe had a preference for adsorbing Met-Leu-Phe from the mixture. However, these particles did not exclude the two Gly-Gly containing peptides and the results were similar to the blanks.

CONCLUSIONS

CS-MIPs can be used to selectively extract caffeine from mixtures of caffeine and theophylline and tripeptides containing the Gly-Gly sequence from mixtures of tripeptides. Whilst enhanced uptake is observed in de-ionised water, polymers prepared by conventional emulsion polymerisation do not perform as well in phosphate buffer. It is likely that this result is due to the increased ionic strength of the medium, which favours non-specific hydrophobic adsorption of organic compounds to the polymer surface. The total removal of the template from molecular imprinted polymer systems has proved difficult and several strategies have been proposed to alleviate the consequences of the resultant slow leaching during applications such as trace analysis. One strategy that can facilitate the removal is to prevent template entrapment by using surface imprinting and here we show that the use of core-shell surface molecular imprinting methodology [1] at the surface of a polymer colloid can produce materials that are totally free of the template, as measured by a radiotracer technique.

TABLE V Uptake of tripeptides from peptide imprinted particles

Imprint	U_{MLP}	U_{TGG}	U_{GGH}
Met-Leu-Phe	1.00	0.78	0.71
Tyr-Gly-Gly	0.00	1.00	1.00
Blank	1.00	0.86	0.90

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