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# Molecularly imprinted polymers by suspension polymerisation in perfluorocarbon liquids, with emphasis on the influence of the porogenic solvent

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## Abstract

Methacrylic acid (MAA)–trimethylolpropane trimethacrylate (TRIM) copolymer beads imprinted with *tert*-butoxycarbonyl-L-phenylalanine (Boc-L-Phe) have been prepared by suspension polymerisation in a perfluorocarbon liquid. The suspension polymerisation procedure has been adapted to work with a number of different porogenic solvents, and with UV initiated or thermal initiated polymerisation. The resulting polymers have been evaluated as stationary phases for the chromatographic separation of Boc-D,L-Phe. Polymer beads of average diameter 8–25  $\mu\text{m}$  packed into 100 $\times$ 4.6 mm chromatography columns were used with a mobile phase of dichloromethane–acetic acid (99:1, v/v) at flow-rates up to 5 ml  $\text{min}^{-1}$ . A polymer prepared with 1,2-dichloroethane as porogenic solvent (average bead diameter 15.1  $\mu\text{m}$ ) was capable of the baseline separation of 100  $\mu\text{g}$  Boc-D,L-Phe at a flow-rate of 0.5 ml  $\text{min}^{-1}$ . © 1997 Elsevier Science B.V.

**Keywords:** Molecular imprinting; Stationary phases, LC; Enantiomer separation; Porogenic solvents

## 1. Introduction

Molecular imprinting [1] is a form of template polymerisation which has been used to produce artificial binding sites in macroporous polymer particles (usually acrylate, acrylamide or styrene based), that exhibit selective rebinding of the imprint or template molecules used in their fabrication. The specific binding properties of the polymers have often been evaluated by high-performance liquid chromatography (HPLC), and imprinted polymers

have been shown to be useful chiral stationary phases for enantioseparations [2].

Most imprinted polymers have been prepared by bulk polymerisation yielding polymer monoliths which are then ground and sieved to produce particles of the desired dimensions. This process is wasteful and time consuming. For chromatographic applications, uniform sized macroporous beads are desirable [3]. A few reports [4–7] have described methods for producing imprinted polymers in bead form, which are less laborious to prepare and better suited to chromatography. Two of these approaches, the formation of thin layers of imprinted polymer on the surface of organic polymer [4] or silica [5,6] beads give materials with only low rebinding capacities. The third approach involves suspension poly-

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merisation using water as a dispersing medium [7]. Unfortunately, water, which is commonly used as a dispersing solvent in this way, can weaken the hydrogen bond and ionic interactions which are known to be involved in non-covalent imprinting [8]. Thus, non-polar solvents are generally used in molecular imprinting, and the presence of water is usually avoided. Another method for preparing imprinted stationary phases for chromatography makes use of an in situ polymerisation technique [9] to prepare solid polymer rods containing continuous pores [10]. However, this technique also requires fairly polar porogenic solvents (cyclohexanol and 1-dodecanol), and it is unclear what is the capacity of such a stationary phase.

Suspension polymerisation has recently been shown to result in molecularly imprinted polymer beads with good chromatographic and recognition properties when a perfluorocarbon liquid is used as the dispersing solvent [11]. Such liquids have the advantages of being immiscible with most organic solvents and chemically inert so that, unlike water, they do not interfere with the non-covalent interactions involved in the formation of recognition sites. The method described was for the preparation of methacrylic acid (MAA)–ethyleneglycoldimethacrylate copolymer beads with diameters ranging between 1 to 100  $\mu\text{m}$ , molecularly imprinted with the amino acid derivative Boc-L-Phe. Perfluoromethylcyclohexane was employed as a dispersing phase, and chloroform as porogenic solvent. Acetone and toluene could also be used as porogenic solvents, though more damaged and aggregated beads resulted and the recognition properties of these polymers were not assessed.

The goal of the present work was to improve the previously described method so that it could be used in conjunction with different porogenic solvents, and with thermal initiated polymerisation. Our motivations were several: Many interesting print molecules are insoluble in chloroform; many print molecules strongly absorb or are unstable in UV light so thermal initiated polymerisation is necessary; UV initiation in the laboratory gives poor control over the polymerisation temperature, thus, thermal initiation is expected to give more reproducible results; solvents other than chloroform have been shown to give polymers with better recognition properties in some cases [12].

## 2. Experimental

### 2.1. Materials

Perfluoro-1,3-dimethylcyclohexane and polyfluoroalcohol ( $\text{C}_n\text{F}_{2n+1}\text{SO}_2\text{N}(\text{C}_2\text{H}_5)\text{C}_2\text{H}_4\text{OH}$ , where  $n \approx 7.5$ ) were from Fluorochem (Old Glossop, UK). Poly(ethylene glycol) (MW 2000) monomethyl ether (PEG2000MME) was from Fluka (Buchs, Switzerland). Trimethylolpropane trimethacrylate (TRIM), 1,1'-azobis(cyclohexanecarbonitrile), Brij 30 [polyoxyethylene(4)lauryl ether], Brij 35 [polyoxyethylene(23)lauryl ether], Brij 72 [polyoxyethylene(2)stearyl ether] and Brij 78 [polyoxyethylene(20)stearyl ether] were from Aldrich (Steinheim, Germany). MAA, 1,1'-azobis(isobutyronitrile) and triethylamine were from Merck (Darmstadt, Germany). 2,2'-Azobis(2,4-dimethylvaleronitrile) was from Waco (Osaka, Japan). Boc-L-Phe (*tert*-butyloxycarbonyl-L-phenylalanine), Boc-D-Phe and Boc-D,L-Phe were from Nova Biochem (Läufelfingen, Switzerland). Acryloyl chloride was from Janssen Chimica (Geel, Belgium). Solvents were of HPLC grade and dried before use, chloroform was additionally treated with basic alumina (M. Woelm, Eschwege, Germany) to remove ethanol.

### 2.2. Synthesis of perfluorinated polymeric surfactants

Polyfluoroacrylate (PFAC-1) was prepared as follows: polyfluoroalcohol (39.5 mmol) was stirred in chloroform (40 ml) at 0–5°C. Acryloyl chloride (43.4 mmol) in chloroform (10 ml) and triethylamine (43.4 mmol) in chloroform (10 ml) were added cautiously and simultaneously over 5 min. The mixture was stirred for a further 20 min at 0–5°C, then at 20°C overnight. Chloroform was removed at the rotary evaporator until the suspension became cloudy. It was then filtered to remove most of the triethylamine and the remaining chloroform was removed at the rotary evaporator, yielding a viscous orange/brown liquid.

PEG2000MME acryloyl ester (acryloyl PEG2000MME), acryloyl Brij 30, acryloyl Brij 35, acryloyl Brij 72 and acryloyl Brij 78, were prepared similarly from the corresponding alcohols except the quantities were 10 mmol alcohol in 20 ml chloroform, 11 mmol acryloyl chloride in 5 ml and 11

mmol triethylamine in 5 ml, yielding waxy white solids.

Perfluorinated polymeric surfactants (PFPS) A–Å were prepared as follows: acryloyl PEG2000MME or acryloyl Brij (various quantities: See Table 1), PFAC-1 (various quantities: See Table 1) and 1,1'-azobis(isobutyronitrile) (2 mol%) were dissolved in 10 ml chloroform in a screw-top vial. Nitrogen was bubbled through the mixture for 5 min, whereupon the vial was sealed and polymerisation conducted at 60°C for 48 h. After this time the chloroform was

removed at the rotary evaporator yielding white to yellow solids of waxy to gluey consistencies.

### 2.3. Preparation of molecularly imprinted polymers

The apparatus for UV initiated suspension polymerisations was as described previously [11]. The polymerisation vessel was a borosilicate glass tube (50 ml volume, 15 cm height, 2.5 cm O.D.). The imprinting phase contained Boc-L-Phe (1 mmol), MAA (4 mmol), TRIM (4 mmol), solvent (4.0 ml toluene, 2.7 ml all other solvents) and 1,1'-azobis(cyclohexanecarbonitrile) (20 mg). The dispersing phase consisted of perfluoro-1,3-dimethylcyclohexane (20 ml), the amount of porogenic solvent found necessary to saturate the perfluorocarbon (e.g., 1.0 g toluene) and perfluorinated polymeric surfactant (25 mg). The phases were mixed by stirring at 2000 rpm for 5 min. Nitrogen gas was bubbled through the resulting suspension for 5 min. Polymerisation was then performed stirring at 500 rpm at room temperature under a slow stream of nitrogen. The UV source was a Camag lamp set at 356 nm, placed 10 cm from the polymerisation vessel. After 3 h, the polymer beads were separated by filtration and washed with acetone. The perfluorocarbon liquid was kept for redistillation and reuse. Large polymer aggregates were removed by sonication in 50 ml acetone followed by sedimenting for 1 min and decanting: The sedimented fraction was discarded. The remaining fraction was dried at the funnel, and then under vacuum.

Thermal initiated polymerisation was performed similarly, stirring at 600 rpm with the polymerisation vessel three-quarters immersed in a water bath at 50°C. 2,2'-Azobis(2,4-dimethylvaleronitrile) (20 mg) was used as initiator and in the case of toluene 50 mg PFPSW was used as surfactant, instead of the usual 25 mg.

### 2.4. Chromatography

Polymer beads were suspended in acetone (50 ml) and slurry packed into chromatography columns (100×4.6 mm) at 300 bar with acetone as solvent using an air driven fluid pump (Haskel, Burbank, CA, USA). The chromatographic setup comprised a Waters 501 pump, Waters 484 tunable absorbance

Table 1  
Composition of perfluorinated polymeric surfactants PFPSA–PFPSÅ

PFPS	PFAC-1 (mmol)	Acryloyl X (mmol)	Successful beads
A	6.67	PEG2000MME (0.368)	**
B	3.68	PEG2000MME (0.368)	*
C	3.68	PEG2000MME (0.368)	*
D	3.68	PEG2000MME (1.23)	*
E	6.67	PEG2000MME (0.667)	**
F	6.67	PEG2000MME (0.167)	*
G	6.67	Brij 30 (0.667)	
H	6.67	Brij 30 (0.368)	
I	6.67	Brij 30 (0.167)	
J	6.67	Brij 35 (0.667)	**
K	6.67	Brij 35 (0.368)	*
L	6.67	Brij 35 (0.167)	*
M	6.67	Brij 72 (0.667)	
N	6.67	Brij 72 (0.368)	*
O	6.67	Brij 72 (0.167)	
P	6.67	Brij 78 (0.667)	***
Q	6.67	Brij 78 (0.368)	**
R	6.67	Brij 78 (0.167)	*
S	13.34	PEG2000MME (1.334)	*
T	6.67	PEG2000MME (2.222)	*
U	6.67	PEG2000MME (6.67)	
V	13.34	Brij 35 (1.334)	***
W	6.67	Brij 35 (2.222)	****
X	6.67	Brij 35 (6.67)	**
Y	13.34	Brij 78 (1.334)	***
Z	6.67	Brij 78 (2.222)	**
Å	6.67	Brij 78 (6.67)	*

The quantities of PFAC-1 (polyfluoroacrylate monomer) and acryloyl PEG2000MME (poly(ethylene glycol) 2000 monomethyl ether monoacrylate ester) or acryloyl Brij shown were used for the synthesis of each surfactant. The resulting surfactants were then employed in the preparation of MAA–TRIM copolymer beads imprinted with Boc-L-Phe using toluene as porogenic solvent and perfluoro-1,3-dimethylcyclohexane as dispersing solvent. The number of stars (\*) indicates the quality of the beads produced: No stars corresponds to no beads, purely aggregated polymer lumps and irregular fragments.

detector, Rheodyne 7125 injection valve with a 20  $\mu\text{l}$  sample loop (all from Millipore, Sunbyberg, Sweden) and a Kipp and Zonen chart recorder (Scandinavata, Sollentuna, Sweden). Packed columns were washed on-line with methanol–acetic acid (7:3, v/v, 200 ml) at 0.1–0.5  $\text{ml min}^{-1}$ , then equilibrated in the mobile phase, dichloromethane–acetic acid (99:1, v/v) at 0.5  $\text{ml min}^{-1}$  until a stable baseline was achieved. Samples (10  $\mu\text{g}$  enantiomer, 20  $\mu\text{g}$  racemate) were injected and eluting species detected at 254 nm. Void volumes were determined by injection of dichloromethane–acetone (50:1, v/v).

### 3. Results and discussion

#### 3.1. Suspension polymerisation using toluene as porogenic solvent

The choice of porogenic solvent is critical in most molecular imprinting procedures: The solvent should be as non-polar as possible in order to maximise the strength of hydrogen bond and ionic interactions between the print molecule and monomer, but the print molecule should remain readily soluble. In addition, the swelling of imprinted polymers in different solvents will affect the recognition [12]. Ideally, polymerisation and characterisation should be performed in the same solvent. We chose to concentrate initially on toluene as a porogenic solvent and *tert.*-butoxycarbonyl-L-phenylalanine (Boc-L-Phe) as an imprint species since this molecule has been used extensively as a template both in conventional bulk polymerisation [13] and suspension polymerisation [11]. MAA was employed as functional monomer and TRIM as cross-linking monomer since this particular combination has been observed to result in imprinted polymers exhibiting particularly high capacities in HPLC [14]. Perfluoro-1,3-dimethylcyclohexane was used as dispersing solvent instead of perfluoromethylcyclohexane, as used previously [11], on account of its higher boiling point, which should reduce the loss by evaporation.

Initially, it was attempted to produce imprinted polymer beads by surfactant-free suspension polymerisation, and then with some commercially available fluorinated surfactants: However, stable suspensions were never attained, with the result being

aggregated lumps of polymer. Thus, we prepared a perfluorinated graft copolymer surfactant by the copolymerisation of acryloyl PEG2000MME and PFAC-1, as previously described [11]. This surfactant gave better results, though still with extensive aggregation of the resulting polymer beads. Thus, a large number of new perfluorinated polymeric surfactants were prepared, incorporating more hydrophobic side chains than the original poly(ethylene glycol), in the hope that these might interact better with the toluene phase and, thus, give more stable suspensions.

The perfluorinated polymeric surfactants PFPSA to PFPSÅ were not all synthesised initially, rather the process was iterative: First PFPSA to PFPSF were synthesised, used in suspension polymerisation and the results used to design a new group of surfactants, PFPSG to PFPSR, and so on. The results are shown in Table 1. Using PFPSW a good yield of regular shaped polymer beads was obtained.

#### 3.2. Combination of different surfactants with different porogenic solvents

It was attempted to prepare imprinted polymers using PFPSA or PFPSW as surfactants with a variety of different porogenic solvents. In some cases the polymerisation resulted only in aggregated polymer lumps or irregular fragments, but in many cases a good yield of regularly shaped beads was obtained (Table 2). When chloroform or dichloromethane were used as porogenic solvent, either surfactant gave equally satisfactory results. When acetone, 1,2-dichloroethane or methanol were used as porogenic solvent, better beads were obtained using surfactant PFPSA. When toluene or tetrahydrofuran were used as porogenic solvent, better beads were obtained using surfactant PFPSW. With acetonitrile, dimethylformamide and heptane, neither surfactant gave satisfactory beads.

#### 3.3. Thermal initiated polymerisation

The preparation of imprinted polymer beads was attempted using thermal initiated polymerisation. Preliminary attempts using chloroform as the porogenic solvent were unsuccessful, apparently

Table 2

Preparation of beads, imprinted with Boc-L-Phe, using UV initiation, perfluorinated polymeric surfactants PFPSA or PFPSW and various porogenic solvents

Solvent	bp (°C)	Beads using PFPSA	Beads using PFPSW
Acetone	56	++	+
Acetonitrile	81	–	–
Chloroform	61	++	++
Cyclohexanol	155	–	–
Dichloroethane	83	++	+
Dichloromethane	40	++	++
Dimethylformamide	153	–	–
Heptane	98	–	–
Methanol	65	+	–
Tetrahydrofuran	66	+	++
Toluene	111	+	++

++ : good yield (>75%) of regularly shaped polymer beads.

+ : Some regularly shaped polymer beads (25–75%).

– : Aggregated polymer lumps or irregularly shaped fragments, <25% good beads.

because the chloroform evaporated at the temperature required (50°C) and escaped from the reaction vessel. Only lumps of aggregated polymer and irregular fragments were produced. Comparison of the boiling points of the solvents used in Table 2 suggested that thermal initiated polymerisation was most likely to be successful using 1,2-dichloroethane as porogenic solvent and PFPSA as surfactant, or toluene as porogenic solvent and PFPSW as surfactant. The success of the thermal initiated polymerisation was found to be dependent on a judicious balancing of the stirrer rate and polymerisation temperature: If the stirrer rate was too high or the temperature too low, polymerisation did not occur; if the stirrer rate was too low or the temperature too high, only lumps of aggregated polymer and irregular fragments were produced. A good compromise was found at a stirrer rate of 600 rpm and temperature of 50°C. Polymerisation was performed under these conditions using either toluene or 1,2-dichloroethane as porogenic solvent, and in each case a good yield (>75%) of regular shaped polymer beads, imprinted with Boc-L-Phe, was obtained.

### 3.4. Physical properties of imprinted polymers

The porogenic solvent and method of polymerisation initiation are known to affect the physical

properties of polymers prepared by suspension polymerisation [15]. One of these properties is the extent of polymerisation: Inefficient initiation or the use of a porogenic solvent which can act as a chain-terminating agent, may result in incomplete polymerisation. Not all monomer will be incorporated in the polymer and the polymer may be soft. In the present work, gravimetric analysis showed that the mass of polymer produced was in each case  $100 \pm 5\%$  of the expected mass if all monomer was incorporated. In addition the polymers were all sufficiently rigid to be used as chromatography matrices without collapsing even at flow-rates up to  $5 \text{ ml min}^{-1}$  for a  $100 \times 4.6$  mm column. Thus polymerisation was assumed to be essentially complete in each case.

Another property which may vary is the bead diameter. Non-imprinted polyTRIM beads have been prepared by suspension polymerisation in water with diameters in the range 10 to  $150 \mu\text{m}$  [16]. However, the affect of varying the porogen on the size of beads was not reported. In the present work, polymers prepared with different porogenic solvents exhibited different average bead diameters (Table 3), the largest beads being obtained using acetone [P(ACN-A)] or tetrahydrofuran [P(THF-W)], and the smallest using chloroform [P(CHL-W)]. The surfactant also influenced the bead size: For chloroform and dichloromethane, smaller beads were obtained using PFPSW as surfactant than using PFPSA [P(CHL-W), P(CHL-A), P(DCM-W), P(DCM-A)]. Thermal initiation gave rise to smaller beads than UV initiation [P(DCE-A-Δ), P(DCE-A), P(TOL-W-Δ), P(TOL-W)]. This may be a temperature effect: However, for the beads prepared with toluene as porogenic solvent it might also be due to the use of a larger quantity of surfactant in P(TOL-W-Δ) [11]. SEM pictures of some of the beads are shown in Fig. 1a and Fig. 1b. It can clearly be seen that the beads produced with toluene [P(TOL-W)] have a wider size distribution, but are on average larger and are more often misshapen than the beads produced with chloroform [P(CHL-A)].

The most dramatic effect of the porogenic solvent is on the surface area and the size and size distribution of pores in the polymer. These properties depend on the solubility of the growing polymer chains in the solvent. Data are not available on the porous properties of conventional, non-imprinted

Table 3

Physical properties of Boc-L-Phe imprinted polymer beads prepared using different porogenic solvents and perfluorinated polymeric surfactants

Polymer	Porogenic solvent	Surfactant PFPS	Average bead diameter <sup>a</sup> (μm)	Surface area <sup>b</sup> (m <sup>2</sup> g <sup>-1</sup> )	Average pore diameter <sup>b</sup> (nm)
P(ACE-A)	Acetone	A	25.6	125	10
P(CHL-A)	Chloroform	A	9.6	0.8	24
P(CHL-W)	Chloroform	W	8.1	nd	nd
P(DCE-A)	Dichloroethane	A	15.1	2.5	15
P(DCE-A-D)	Dichloroethane <sup>c</sup>	A	14.3	nd	nd
P(DCM-A)	Dichloromethane	A	12.7	0.7	18
P(DCM-W)	Dichloromethane	W	8.5	nd	nd
P(THF-W)	Tetrahydrofuran	W	21.4	2.4	18
P(TOL-W)	Toluene	W	14.2	127	24
P(TOL-W-D)	Toluene <sup>c</sup>	W	9.2	nd	nd

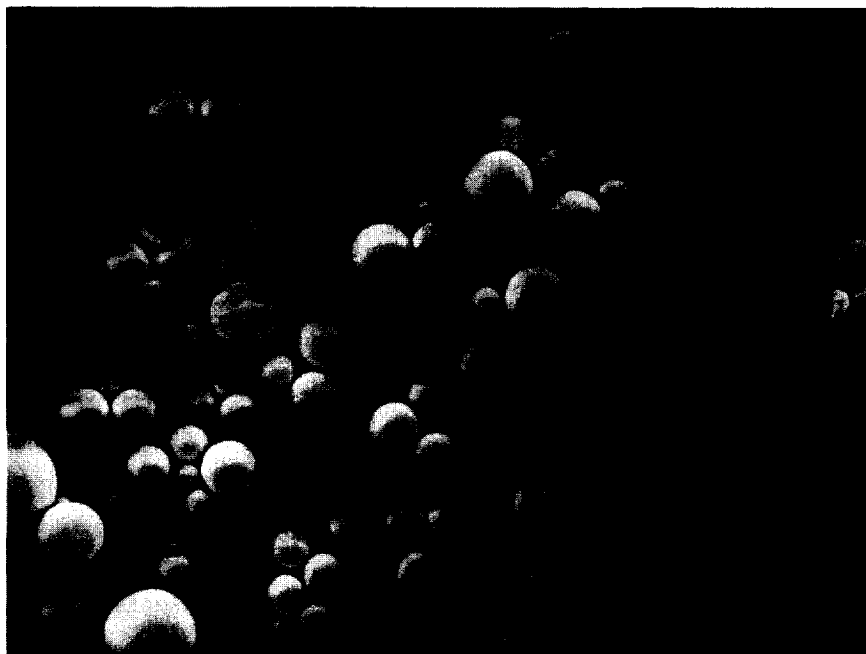
<sup>a</sup> The average bead size of each polymer produced was determined by estimating the sizes of 50–100 beads under the microscope.

<sup>b</sup> Surface areas and pore diameters were determined by nitrogen adsorption using a Micromeritics ASAP 2400 covering pores between 17 and 3000 Å. Samples were degassed at 120°C and an 80-point pressure table was used with a 45 s equilibration time. Surface area was determined from a BET plot and total pore volume by single point measurements. Average pore diameter was calculated as 4×total pore volume/surface area.

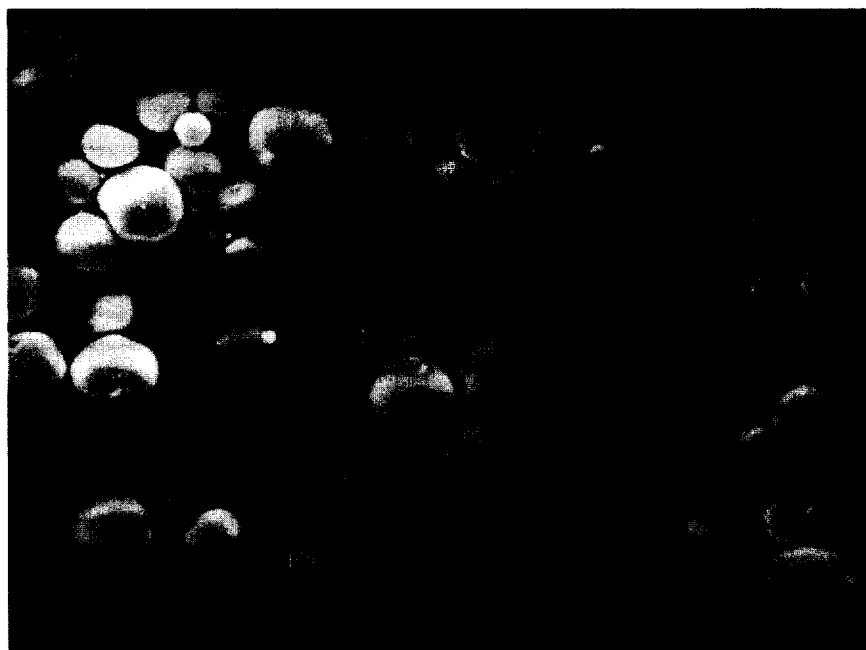
<sup>c</sup> Prepared using thermal initiation.

TRIM-MAA copolymers. We can, however, compare our data with results obtained for non-imprinted polyTRIM polymers, both monolithic polymers [17] and beads formed by suspension polymerisation in water [16]. For monolithic polyTRIM with toluene as porogenic solvent, at an initial monomer concentration of 30% (v/v), polymerised at 20°C, the BET surface area was 472 m<sup>2</sup> g<sup>-1</sup> and the average pore diameter (determined by mercury adsorption porosimetry) was 7.7 nm. For a polymer prepared identically except with 1,2-dichloroethane as porogenic solvent, the surface area was 1.0 m<sup>2</sup> g<sup>-1</sup> and the average pore diameter was 4.6 nm. Similar pore diameters were obtained with chloroform (4.9 nm) and dichloromethane (4.7 nm). Intermediate surface areas and pore diameters were observed with some other porogenic solvents [17]. The surface areas of polyTRIM particles prepared by suspension polymerisation in water, at initial monomer concentrations of 25% (v/v) and 90°C, were also found to vary significantly with the porogenic solvent: Values of 470 and 7.0 m<sup>2</sup> g<sup>-1</sup> were obtained for toluene and isooctane, respectively [16]. In the present work, P(TOL-W), polymerised with an initial monomer concentration of 29% (v/v), using toluene as porogen at 20°C had a surface area of 127 m<sup>2</sup> g<sup>-1</sup>

and P(DCE-A), polymerised with an initial monomer concentration of 37% (v/v), using 1,2-dichloroethane as porogen at 20°C had a surface area of 2.5 m<sup>2</sup> g<sup>-1</sup> (Table 3). This trend is very similar to that for the polyTRIM monoliths [17]. The differences in actual values, particularly comparing P(TOL-W) to the non-imprinted polyTRIM monolith or beads made in toluene, may be due to the inclusion of MAA, the polymerisation process or the presence of the imprint species. The trend in surface areas P(DCM-A)≈P(CHL-A)<P(THF-W)≈P(DCE-A)≪P(ACE-A)≈P(TOL-W) also agrees with results observed for molecularly imprinted bulk MAA-ethylene glycol dimethacrylate copolymers produced with different porogenic solvents [12] (surface area for polymer made with dichloromethane≈chloroform≪tetrahydrofuran<benzene). The difference in average pore diameters between our polymers and the polyTRIM monoliths prepared with different porogenic solvents [17] may be due to the measuring technique (nitrogen adsorption rather than mercury porosimetry). The calculated pore diameters are also slightly higher than those calculated using nitrogen adsorption for molecularly imprinted bulk MAA-ethylene glycol dimethacrylate copolymers produced with different porogenic solvents (5.2, 9.1 and 12.3

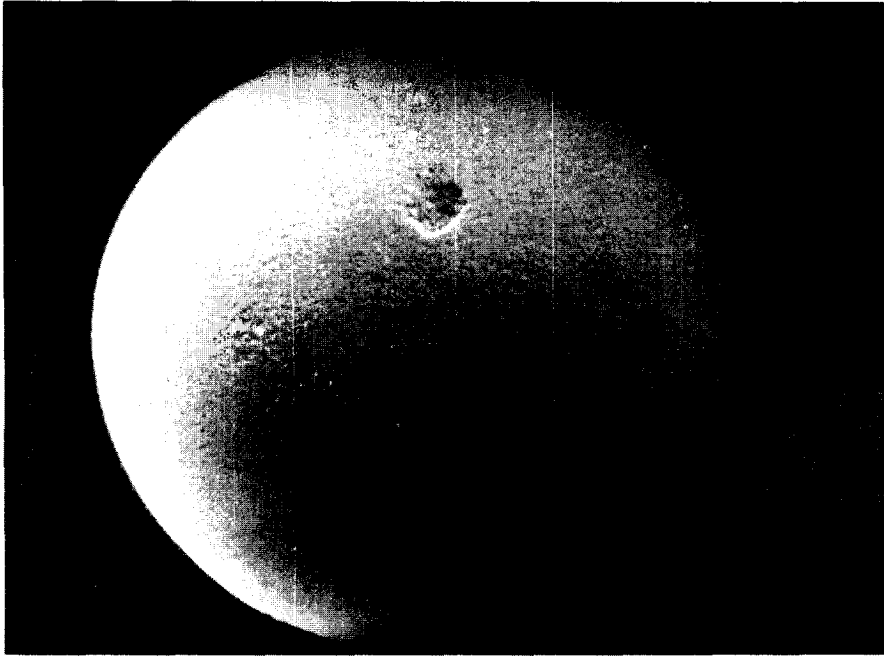


(a)

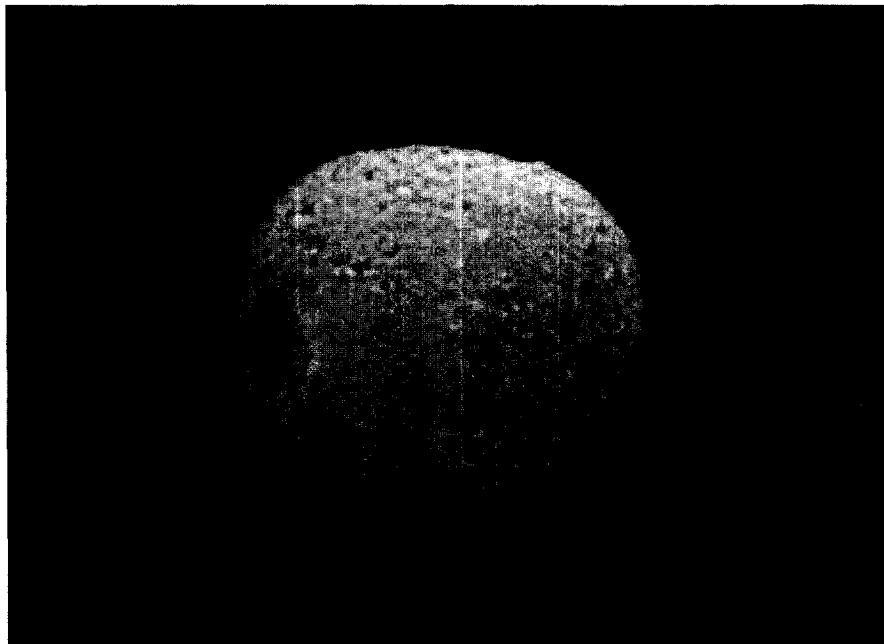


(b)

Fig. 1. Scanning electron micrographs of molecularly imprinted beads prepared by suspension polymerisation (a,c) P(CHL-A), (b,d) P(TOL-W). Beads were placed on aluminium pegs and sputter coated with 15 nm of gold using a polaron E5150 coater. The images were obtained using an ISI 100A SEM at 25 kV. Magnifications were 500 $\times$  (a and b) and 5000 $\times$  (c and d).



(c)



(d)

Fig. 1. (continued)



nm for polymers made with tetrahydrofuran, chloroform and acetonitrile, respectively) [12]. However, the figure for P(CHL-A) is in good agreement with that for a bulk TRIM-MAA copolymer molecularly imprinted with benzyloxycarbonyl-L,L-dialanine methyl ester using chloroform as porogenic solvent, previously shown to have a surface area of  $4.2 \text{ m}^2 \text{ g}^{-1}$  and average pore diameter of 28 nm [14]. Fig. 1c and Fig. 1d are SEM pictures of beads at higher magnifications: The surface of a bead of P(TOL-W) is clearly more irregular and contains larger pores than that of a bead of P(CHL-A).

It should be noted that the bead sizes, surface areas and pore sizes measured here and in the references reflect the properties of the dry polymer: In the mobile phase used for chromatography the polymers would be expected to swell and the surface areas and pore diameters in particular may change. An alternative method for the determination of pore size distributions which would reflect these properties in the solvent is size-exclusion chromatography [18].

### 3.5. Chromatographic performance of imprinted polymers

Each of the polymers was evaluated as a stationary phase in the chiral separation of Boc-D,L-Phe by HPLC. The separation provides a measure of the

integrity of the recognition sites: Since Boc-L-Phe was imprinted, it should be retained longer on the polymers than Boc-D-Phe. The porogenic solvent affects the chromatographic results for several reasons: The less polar the solvent, the stronger the interactions responsible for forming the specific recognition sites, thus the better recognition properties expected; when analysis is conducted in a solvent different from the porogenic solvent, the swelling of the polymer particles may be different, leading to poorer recognition; the physical properties of the polymer particles may be important, for instance a higher surface area might be expected to give faster exchange kinetics or a higher capacity; finally, the solvent may affect the efficiency of the polymerisation process, if polymerisation is slow the imprint molecule might not be "frozen" so effectively in the forming polymer and the recognition properties might not be expected to be optimal.

Both the retentions and the separations observed varied significantly with the porogenic solvent used in the polymers' preparation (Table 4). For P(ACE-A) and P(THF-W), retentions of Boc-L- and Boc-D-Phe were poor and no enantioseparation was observed. Acetone and tetrahydrofuran are relatively polar and disrupt the interactions required for efficient imprinting. Separation could possibly be observed by injecting smaller amounts of racemate. For the polymers prepared using chlorinated solvents,

Table 4  
Behaviour of Boc-L-Phe imprinted polymer beads, prepared using different porogenic solvents and perfluorinated polymeric surfactants, as stationary phases in HPLC

Polymer	Retention <sup>a</sup> $k'_L$	Retention <sup>a</sup> $k'_D$	Separation factor <sup>b</sup> $\alpha$
P(ACE-A)	1.8	1.8	1.0 <sup>c</sup>
P(CHL-A)	4.09	2.13	1.92
P(CHL-W)	2.69	1.40	1.92
P(DCE-A)	4.68	1.80	2.60
P(DCE-A-Δ)	3.83	1.65	2.32
P(DCM-A)	7.58	2.46	3.08
P(DCM-W)	4.39	1.64	2.68
P(THF-W)	2.0	2.0	1.0 <sup>c</sup>
P(TOL-W)	1.64	0.89	1.84
P(TOL-W-Δ)	1.85	0.90	2.05

<sup>a</sup> Capacity factor (retention) defined as:  $k'_{\text{sub}} = (t_{\text{R,sub}} - t_0)/t_0$ ;  $t_0$  was determined from the elution time of dichloromethane-acetone (50:1, v/v).

<sup>b</sup> Separation factor defined as:  $\alpha = k'_L/k'_D$ .

<sup>c</sup> No separation.

strong retentions and efficient separations were observed, with baseline resolution of 20  $\mu\text{g}$  Boc-D,L-Phe observed in each case. That these polymers have such small surface areas when measured in the dry state yet give efficient separations and high capacities clearly suggests that the pores swell significantly in the chromatographic solvent, increasing the accessibility of the imprinted sites; alternatively, the binding sites may be not only at the surfaces of the pores, and the ligand may be able to diffuse into and out of the polymer network.

The best separation was obtained on P(DCM-A): This may reflect that it is better to perform the analysis in the same solvent as the porogenic solvent [the mobile phase for HPLC was dichloromethane–acetic acid (99:1, v/v)]. However, sharper peaks were observed with P(DCE-A) and when larger quantities of Boc-D,L-Phe were injected clearly better resolution was obtained with this polymer than with P(DCM-A). At 0.5 ml  $\text{min}^{-1}$ , baseline resolution of 100  $\mu\text{g}$  Boc-D,L-Phe was observed on P(DCE-A), and even 1 mg could be resolved on the 100 $\times$ 4.6 mm column (Fig. 2). For P(DCM-A) the corresponding figures were 20 and 400  $\mu\text{g}$ . Thus, imprinting using 1,2-dichloroethane as porogenic solvent appeared to result in a larger number of good recognition sites. When chloroform or dichloromethane was used as porogenic solvent, the use of surfactant PFPSA resulted in longer retention times than for polymers prepared using PFPSW [P(CHL-A), P(CHL-W), P(DCM-A), P(DCM-W)]. For P(DCM-W), the separation also was worse than for P(DCM-A). The surfactant could affect the recognition properties in two ways: Some of the surfactant could remain associated with the surfaces of the polymer beads, or the surfactant could affect the efficiency of the polymerisation process.

For P(TOL-W) the retentions and separation were much smaller than for the polymers prepared using chlorinated solvents. These results were rather disappointing, since the less polar toluene would be expected to give rise to better recognition sites in the imprinting process. P(TOL-W) also had a much higher surface area than the polymers prepared using the chlorinated solvents. However, these effects could be outweighed by the difference in swelling when beads made using toluene are placed in dichloromethane, or polymerisation could be less

efficient in toluene, giving recognition sites of poorer quality than expected. Unfortunately it was not possible to evaluate the recognition properties of the polymers by HPLC using toluene as solvent, since the UV–visible absorption of toluene is such that it is not possible to detect Boc-L-Phe in the eluent. An alternative detection method such as refractive index monitoring would be required.

Thermal initiated polymerisation gave rather contradictory results: For the polymers prepared using 1,2-dichloroethane as porogenic solvent, retention times were shorter and separation less for the thermal initiated than for the UV initiated polymer [P(DCE-A- $\Delta$ ), P(DCE-A)]; for the polymers prepared using toluene as porogenic solvent, retention times were longer and separation better for the thermal initiated polymer [P(TOL-W- $\Delta$ ), P(TOL-W)]. In the case of P(TOL-W) and P(TOL-W- $\Delta$ ), the effect may be due also to the use of a larger amount of surfactant in the thermal initiated polymerisation, which gave smaller beads. The different behaviour of thermal and UV initiated polymers may also be due to differences in the efficiency of the imprinting process: It may be that thermal initiated polymerisation is more efficient when toluene is used as porogenic solvent, but vice versa for 1,2-dichloroethane.

#### 4. Conclusions

We have studied the use of suspension polymerisation in a perfluorocarbon dispersing phase for the preparation of TRIM–MAA copolymers molecularly imprinted with Boc-L-Phe, using different porogenic solvents. The results show that perfluorinated polymeric surfactants of slightly differing composition may be required for use with different porogenic solvents. It is possible to optimise the composition of the perfluorinated polymeric solvent for a given porogen, as has been done here for toluene.

In order to optimise the preparation of any form of imprinted polymer for a given target compound, the amounts and types of monomers and cross-linkers, the porogenic solvent and method of initiation should be carefully optimised. When the polymer is prepared by suspension polymerisation, the composition and amount of surfactant, temperature and stirrer

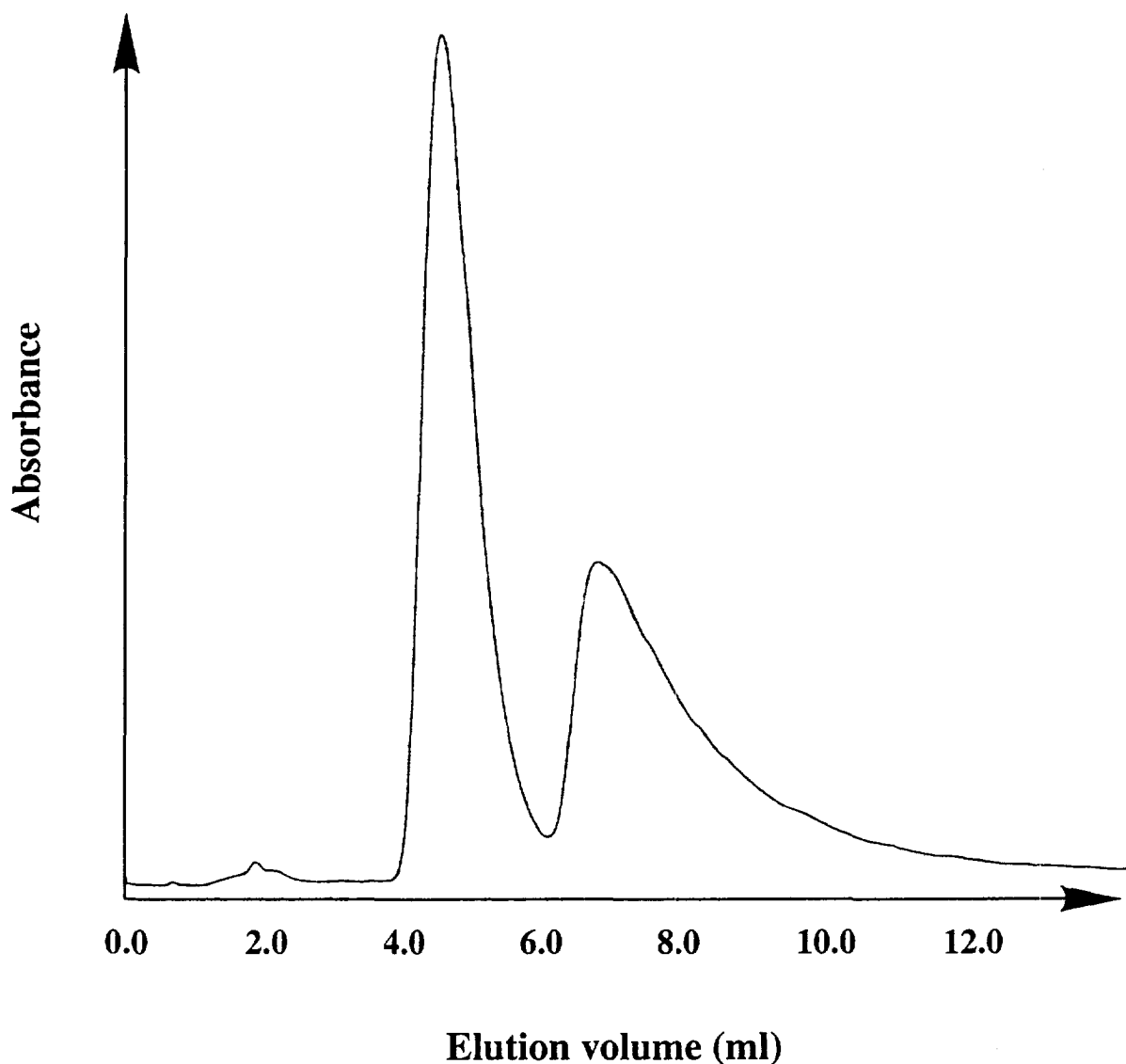


Fig. 2. HPLC traces showing separation of Boc-D,L-Phe on polymer P(DCE-A). 100×4.6 mm column, isocratic elution with dichloromethane–acetic acid (99:1, v/v) at 0.5 ml min<sup>-1</sup>. 100 μg racemate injected in 20 μl mobile phase.

speed also need to be studied. However, it is easier to carry out such an optimisation for polymers prepared by suspension polymerisation than for polymers prepared by bulk polymerisation since the former process is much more efficient and less time consuming. Additionally, for scale-up it is obviously beneficial to avoid the laborious grinding and sieving process associated with preparing imprinted polymers by bulk polymerisation. A small amount of

polymer was wasted here because of the need for size fractionation of the beads: With optimisation of the reactor suspension polymerisation can be expected to give a near-quantitative yield of useable polymer. Although additional reagents are required, the perfluorocarbon liquid and perfluorinated polymeric surfactant, the former can be reused after distillation. The resulting beads are much more useful for chromatographic purposes and can be used

at much higher flow-rates (we have used 100×4.6 mm columns at flow-rates up to 5 ml min<sup>-1</sup>).

The suspension polymerisation process in perfluorocarbon liquid has been extended to work with different porogenic solvents and with thermal initiation. The best recognition properties were exhibited by polymers prepared using 1,2-dichloroethane (following the method described in Section 2.3), which, to our knowledge, has not previously been used as a porogenic solvent in the preparation of molecularly imprinted polymers. We hope that these results will enable the preparation of molecularly imprinted bead polymers, specific for a wide range of compounds. With their improved flow properties and capacities relative to standard bulk imprinted polymers, these materials could be used as stationary phases in preparative scale chromatography.

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