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Molecularly imprinted composite materials *via* iniferter-modified supports

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Wide pore silica (D_P = 100 nm) and gel-type or macroporous (12% nominal crosslinking density) Merrifield resins were modified with iniferter groups for grafting of crosslinked molecularly imprinted or non-imprinted polymer layers through quasi-living polymerisation. Prior to iniferter coupling, the silica supports were premodified by silanisation with *p*-(chloromethyl)phenyl trimethoxysilane. The iniferter groups were then introduced by reacting the resin-bound chloromethyl groups with sodium *N*,*N*-diethyldithiocarbamate. It was shown that the coupling yield, measured as the conversion of the chloromethyl groups, could be varied between 5 and 85% through kinetic control, with the fastest conversions observed for the macroporous resins. This allows the density of radical generating groups to be finely adjusted. Ultraviolet light-initiated copolymerisations of ethyleneglycol dimethacrylate and methacrylic acid in toluene resulted in grafting of 0.2–1.9 g of polymer per gram of support, where the grafted amount increased with reaction time, iniferter content and monomer concentration. The dry-state texture of the composite beads prepared from the gel-type resin depended strongly on the amount of grafted polymer. According to the scanning electron micrographs, the beads with the lower grafted amounts (0.4 g polymer per g support) were deformed exhibiting a peculiar folded structure, whereas the beads containing more grafted polymer (1.6 g polymer per g support) were spherical, with an appearance similar to the precursor particles. None of these materials exhibited permanent porosity. Only the composites obtained from the porous precursor particles also exhibited porosity after grafting. Among these, the silica-based composites also showed recognition for their templates when assessed in the chromatographic mode, whereas no imprinting effects could be demonstrated for the polystyrene-supported materials.

Introduction

Molecular recognition elements in the form of molecularly imprinted polymers (MIPs) (Fig. 1) can today be rapidly synthesised against a large number of target molecules.[1–5] Considering these materials as inexpensive robust alternatives to antibodies, the potential number of applications could be very large and encompass analytical sample pretreatment,[6] chemical sensing,[3] drug delivery,[7] catalysis,[8] separations[9] and, finally, screening elements or reactors in drug discovery.[10]

Although the feasibility of these applications has been demonstrated, commercial applications are few. This can be partly attributed to difficulties in generating high affinity binding sites while simultaneously controlling the porous properties, morphology or other structural features of the polymers. One method to decouple the imprinting step from the generation of a particular morphology is the use of grafting techniques.[11–18] These are divided into "grafting" to[11–14] and "grafting" from[15–18] techniques, the latter refering to polymerizations initiated from a preformed polymer or material surface.

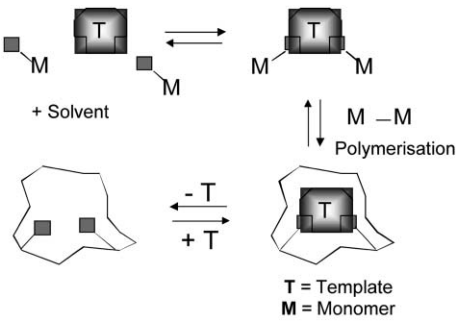

Fig. 1 The principle of molecular imprinting.

Using immobilised azoinitators, we recently proposed a semicontinuous process for the synthesis of MIP composite materials with improved kinetic properties.[15] Due to the one-point attachment of the initiators however, solution polymerisation and resulting gelation was difficult to avoid. One solution to this problem is to use initiatiors where one of the radicals formed upon decomposition is unable to initiate polymerisation. This is the case in living radical polymerisation using benzyl-*N*,*N*-diethyldithiocarbamate iniferter species[19] (Fig. 2), which has found important applications in the manufacturing of micropatterned or biocompatible surfaces.[20,21] In a previous report, the use of an iniferter-modified membrane surface to graft a molecularly imprinted polymer layer was described.[16] However, the use of this concept for the preparation of porous composite beads has no precedent.

In this study we demonstrate that iniferter-modifed support materials can be used to prepare molecularly imprinted composites exhibiting molecular recognition properties. The iniferters were coupled to readily available polystyrene- or silica-based supports; this was followed by grafting of copolymers of methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EDMA) using D- or L-phenylalanine anilide [D(L)-PA] as template and toluene as solvent (Fig. 2). Of particular interest was the use of a hydrophobic polystyrene-based surface to graft a hydrophilic imprinted polymer layer. Some reports have indicated that such interfaces can stabilise imprinted sites, even when using polar, water-containing monomer/template systems.[18]

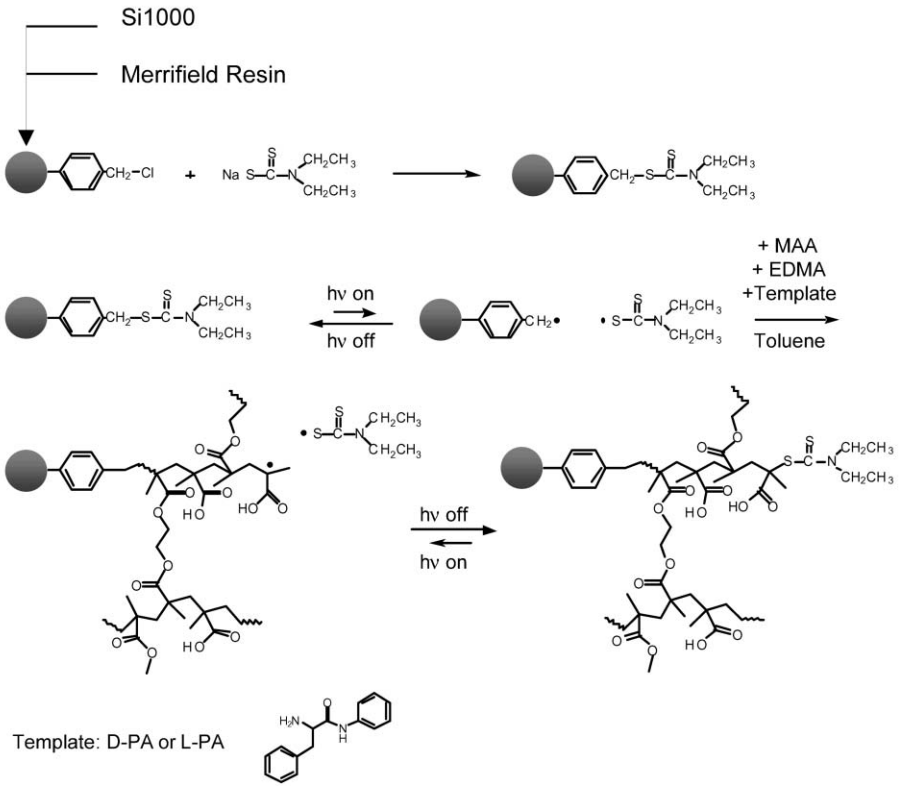

Fig. 2 Reaction used to modify silica- (*e.g.* Si1000) or polystyrene- (*e.g.* Merrifield resin) based supports with a dithiocarbamate iniferter and use of the resulting supports for grafting of imprinted copolymers of methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EDMA) using D- or L-phenylalanine anilide [D(L)-PA] as template. The supports were modified in one (polystyrene-based supports) or two steps (silica-based supports), as described in the Experimental section.

Experimental

Chemicals

Gel-type non-porous Merrifield particles were purchased from Acros (Geel, Belgium), the macroporous particles were kindly provided by Professor D. C. Sherrington (University of Strathclyde, UK) and the silica (Si1000) was a gift from Merck K.G. (Darmstadt, Germany) (see Table 1 for characteristics). Hexamethyl disilazane was obtained from Janssen Chimica (Geel, Belgium) and used without further purification. Tetrachloro- and dichloromethane were purchased from Merck K.G (Darmstadt, Germany) while *p*-(chloromethyl)-phenyl trimethoxysilane was obtained from ABCR (Karlsruhe, Germany). Sodium diethyldithiocarbamate trihydrate was bought from Fluka Chemie AG (Buchs, Switzerland) and used without further purification. The templates D- and L-phenylalanine anilide (D- or L-PA) were synthesised according to literature procedures.[22] Dry ethanol came from C. Roth GmbH (Karlsruhe, Germany) and was stored over molecular sieves (3 Å) before use. Methanol was purchased from Merck KG (Darmstadt, Germany). Ethyleneglycol dimethacrylate (EDMA) and methacrylic acid (MAA), obtained from Sigma Aldrich Chemie GmbH (Deisenhofen, Germany), were treated as follows prior to use: EDMA was purified by extraction with 10% NaOH, washing with brine, drying over anhydrous magnesium sulfate and subsequent distillation under reduced pressure. MAA was purified by distillation under reduced pressure. Dry grade toluene, dichloromethane and THF were purchased from Fluka Chemie AG (Buchs, Switzerland) and were stored over molecular sieves (3 Å). HPLC grade acetonitrile was obtained from Acros (Geel, Belgium), while *N*,*N*′-dicyclohexylcarbodiimide, 3-aminoquinoline and 1-hydroxybenzotriazole were obtained from Fluka Chemie AG (Buchs, Switzerland).

Iniferter coupling

Polystyrene-based supports. Gel-type (1% crosslinking) or macroporous chloromethylated polystyrene-based resin beads

Table 1 Characteristics of supports used for grafting of molecularly imprinted polymers prior to and after iniferter coupling[a]

Modified support[b]	%C	%S	S/m^2 g^{-1}	V_P/mL g^{-1}	–CH_2Cl[c]/mmol g^{-1}	DTC[d]/mmol g^{-1}
PS1	83.0	0	0	0	2.3	—
PS1-I	83.0	0.63	—	—	—	0.10
PS2	85.2	—	54	0.96	1.9	—
PS2-I	86.0	1.00	58	0.52	—	0.16
Si	1.10	0	33	0.53	0.13	—
Si-I	1.10	0.16	30	0.52	—	0.025

[a]The immobilisations were performed, as described in the Experimental section and in Fig. 2, in one (for polystyrene-based supports) or two steps (for the silica support) and the modified supports analysed by elemental microanalysis of carbon, sulfur and chlorine, and by nitrogen sorption. From the latter, the BET specific surface area (S) and pore volume (V_P) were calculated. [b]PS1 = Merrifield resin particles (particle diameter: 37–76 μm) containing 1% nominal crosslinking density. PS2 = macroporous polystyrene-*co*-divinylbenzene resin prepared from a monomer feed of 12% (w/w) divinylbenzene, 57.5% styrene and 30.5% vinylbenzylchloride. Si = wide pore silica support particles (average particle diameter: 10 μm) with an average pore diameter of 100 nm. The index I refers to the support after iniferter immobilisation. [c]Content of chloromethyl groups based on chlorine analysis. [d]Content of dithiocarbamate groups after immobilisation.

(4.5 g) were suspended in dry ethanol (10 mL). To this suspension was added sodium diethyldithiocarbamate trihydrate (1.36 g, 6 mmol) dissolved in dry ethanol (14 mL) with stirring at room temperature. The solution was then heated at 60 °C for 50 h under stirring. The particles were filtered out, washed with 100 mL of water and 100 mL of methanol and then dried at 40 °C under vacuum overnight.

Silica-based supports. *Silanisation.* To 10 g of rehydroxylated macroporous silica (LiChrospher Si 1000, particle diameter 10 μm, pore diameter 100 nm, BET surface area 33 $m^2 g^{-1}$) was added *p*-(chloromethyl)phenyl trimethoxysilane (2.4 mL, 2.7 mmol) in dry THF under stirring. The suspension was heated at 60 °C for 48 h and then filtered; the silica was washed with THF and dried at 40 °C under vacuum overnight.

Endcapping. Silica (9.5 g), modified as described above, was suspended in CCl_4 and hexamethyl disilazane (3.24 mL, 2.51 mmol) in CCl_4 was added slowly under stirring. This suspension was continuously stirred for a further 3 h at room temperature and, subsequently, the particles were filtered out, washed with CH_2Cl_2 and then dried at 40 °C under vacuum overnight.

Iniferter coupling. The endcapped silica was suspended in dry THF (20 mL) and sodium diethyldithiocarbamate trihydrate (61.7 mg, 0.27 mmol) dissolved in dry THF (2 mL) was added dropwise under stirring. The suspension was stirred for a further 3 h and then filtered. The particles were washed with THF and then dried at 40 °C under vacuum overnight.

Photografting of poly(methacrylic acid-*co*-ethyleneglycol dimethacrylate). Iniferter-modified support beads (PS1: 2.0 g; PS2: 1.2 g; Si: 1.46 g) were suspended in a solution of EDMA (PS1: 0.81 g, 4.1 mmol; PS2: 3.52 g, 17.8 mmol; Si: 2.14 g, 11.3 mmol), MAA (PS1: 0.060 g, 0.6 mmol; PS2: 0.31 g, 3.6 mmol; Si: 0.20 g, 2.3 mmol) and toluene (PS1: 20 mL; PS2: 8 mL; Si: 2.7 mL) containing, in the cases of the imprinted resins, the templates L-PA or D-PA (PS1: 32 mg, 0.13 mmol; PS2: 217 mg, 0.90 mmol; Si: 50 mg, 0.21 mmol). After sealing with a rubber septum and mixing, the solution was degassed for 5 min by purging with nitrogen. The flask was then placed in a thermostatted water bath at 20 (PS1) or 15 °C (PS2, Si) at a distance of 5 cm from a high-pressure mercury vapour lamp (Philips, HPK 125 W). The subsequent grafting polymerisation was performed for 24 (PS1) or 2.5 h (PS2, Si). Thereafter, the beads were filtered off, washed with 60 mL of toluene and dried at 40 °C under vacuum overnight.

Characterisation techniques. *Infrared spectroscopy and elemental analysis.* Transmission infrared spectra (KBr) were obtained using an FT-IR spectrometer of the Matson 2030 Galaxy Series. Elemental microanalyses were performed after careful drying of the samples using a Heraeus CHN-rapid instrument.

Nitrogen sorption measurements. For the determination of the specific surface area (S), the average pore diameter (D_P) and the specific pore volume (V_P) the samples (50 mg) were degassed at room temperature and the volume of adsorbed nitrogen *versus* pressure measured at 77 K by nitrogen porosimetry (Quantachrome Autosorb 6B). The specific surface areas were evaluated using the BET method, the specific pore volumes following the Gurvitch method and the average pore diameter using the BJH theory.

Scanning electron microscopy. For the preparation of samples for scanning electron microscopy, the materials were fixed on a carbon support and covered with gold at 5×10^{-5} Torr in a Bal-Tec SCD 050 sputter machine with a distance between support and gold target of 5 cm and a current of 30 mA.

Fluorescence microscopy. The polymer-modified silica (0.04 g), HOBt (9 mg) and DCC (14 mg) were mixed in dry CH_2Cl_2 (20 ml) and stirred for 30 min, before a solution of 3-aminoquinoline (3AQ) (41 mg, 1.1 eq. based on the theoretical amount of COOH groups on the polymer) in CH_2Cl_2 was added dropwise. The solution was stirred for 48 h, the silica filtered out and then washed consecutively with CH_2Cl_2 and ethyl acetate. The silica was dried under vacuum at 40 °C. The particles were suspended in dichloromethane and a few drops of the slurry were transferred onto a microscope slide for analysis using a Leica IM 1000 fluorescence microscope.

Chromatographic evaluation of silica-based materials. The particles were slurried in methanol–water (80 : 20) in a small flask and sedimented to remove fine particles. The slurry was then used to pack HPLC columns (25 × 4 mm) at a maximum pressure of 300 bar using a compressed gas-driven slurry packer. The columns were then fitted to an HPLC system (Hewlett Packard 1050 instrument) and conditioned with pure acetonitrile as mobile phase. The apparent separation factor ($\alpha = k'_L/k'_D$) was calculated from the retention factors (k') of the two enantiomers (L- or D-PA), estimated from the peak maxima obtained after separate injections of solutions (10 μL, 1 mM) of the two enantiomers or the racemate using acetone as a void marker. The UV detection wavelength was 255 nm for both enantiomers.

Results and discussion

In the grafting of polymers from solid supports, the density of initiating groups,[23] the surface wetting of the monomer mixture[24] and monomer partitioning effects are important factors controlling the density and homogeneity of the grafted layers. In order to establish the influence of these factors on the grafting of imprinted polymers, we included resins of different surface polarities and modified these with different amounts of dithiocarbamate iniferters. Initially, we chose chloromethylated polystyrene resins, since these are commercially available and can be modified in one step (Fig. 2). The Merrifield-type resins are of the gel-type, containing typically only 1% cross-linking density (Table 1). Thus, they are of limited interest as supports for imprinted polymers aimed at high uptake capacity combined with compatibility with different solvents. However, by grafting of a network polymer on the surface of these resins in the swollen state, we anticipated that they would be stabilised, resulting in permanently porous structures. In order to test this concept, we first established that the substitution of the chloromethyl groups with *N*,*N*-diethyldithiocarbamate could be precisely controlled through the time of reaction (Fig. 3).

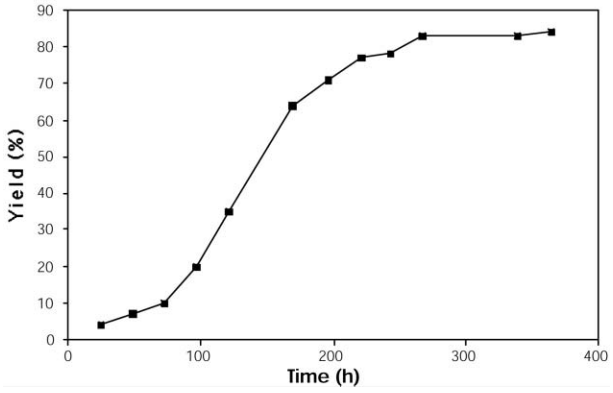

Fig. 3 Conversion of chloromethyl groups *versus* time during coupling of sodium diethyldithiocarbamate (12 mmol) to PS1. For other conditions, see Experimental section.

Table 2 Synthesis conditions and characterisation of molecularly imprinted composite materials

Composite[a]	%C	%S	S/m^2 g^{-1}	V_P/mL g^{-1}	Grafted content[b]/g g^{-1}	Separation factor,[c] α
PS1-L	78.2	0.41	0	0	0.26	1
PS1-N	79.2	0.31	0	0	0.20	1
PS2-L	77.8	0.21	26	0.26	0.46	1
PS2-N	78.6	0.38	28	0.36	0.40	1
Si-L	22.2	0.12	27	0.26	0.58	2.4
Si-N	21.8	0.12	50	0.29	0.57	1
Si-D	19.9	0.29	45	0.38	0.49	0.35

[a]The materials were prepared using the iniferter-modified silica supports described in Table 1 suspended in a mixture of MAA and EDMA in toluene, according to the polymerisation procedures described in the Experimental section. The presence of templates, L-PA or D-PA, is indicated with L or D in the names of the composites. Absence of template is indicated by N (non-imprinted). [b]The grafted content is given as weight of grafted polymer per gram of support material. [c]The HPLC runs were performed using columns (25 × 4 mm) packed with the composite particles, separate injections of D- and L-PA (10 nmol) and using acetonitrile as mobile phase. The apparent enantiomer separation factor (α) was calculated from the retention factors (k') as $\alpha = k'_L/k'_D$.

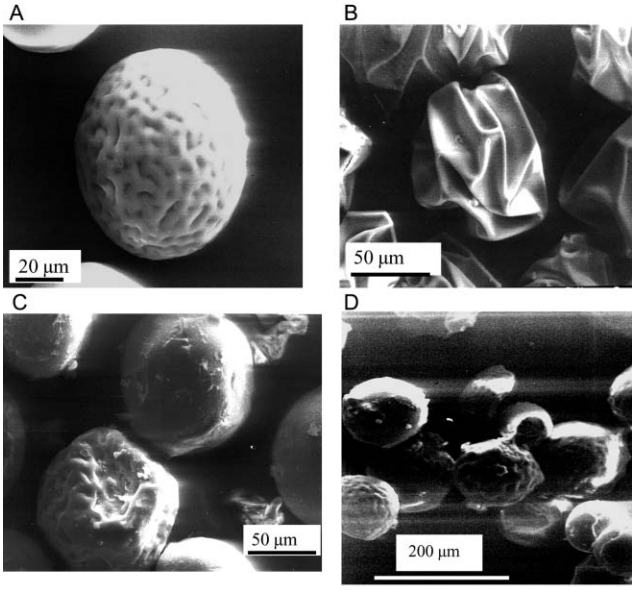

Fig. 4 Scanning electron micrographs of iniferter-modified gel-type (PS1) polystyrene resin supports prior to (A) and after (B–D) grafting of a poly(MAA-*co*-EDMA) layer. The resins used contained 0.10 (B), 0.14 (C) and 0.18 mmol g^{-1} (D) of immobilised iniferter. The amount of grafted polymer estimated by carbon analysis after these modifications were 0.47 (B), 1.55 (C) and 1.90 g polymer per g support (D).

Guided by our previous experience with grafting from azoinitiator-modified silica supports, we chose the supports containing low densities of dithiocarbamate groups (0.10, 0.14 or 0.18 mmol g^{-1}) for the initial investigations. Ultraviolet irradiation of the beads suspended in a mixture of methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EDMA) resulted in significant polymer grafting, as judged gravimetrically, by the decrease in the relative carbon content (PS1-L and PS1-N in Table 2 and Fig. 4) and from the infrared spectra by the increase in the intensity of the carbonyl stretching vibration at *ca* 1700 cm^{-1} (Fig. 5). The weight increase correlated with the amount of immobilised iniferter groups

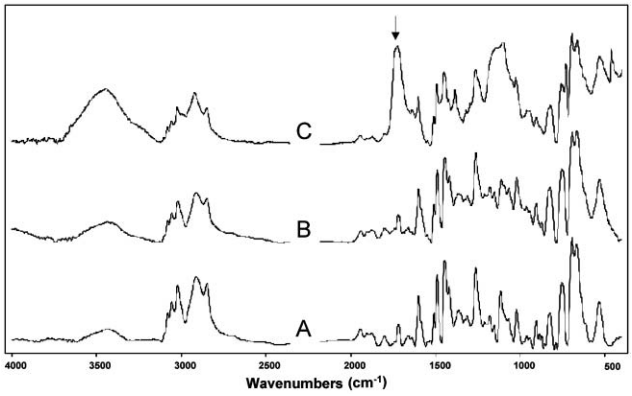

Fig. 5 Infrared spectra (KBr) of the Merrifield resin (PS1) before (A) and after (B) iniferter coupling and after grafting of an L-PA imprinted layer (C) (PS1-L). The amount of grafted ploymer estimated from carbon analysis was 0.26 g polymer per g support. The band corresponding to the carbonyl stretching vibration is indicated.

and varied from 0.2 g polymer per g support for the low iniferter content (0.10 mmol g^{-1}) to 1.9 g polymer per g support for the high iniferter content (0.18 mmol g^{-1}). The amount of grafted polymer had a pronounced influence on the dry-state morphology of the materials (Fig. 4). Low graft amounts resulted in deformed particles exhibiting a peculiar folded structure [Fig. 4(B)]. This is not irreversible, since in the swollen state, these particles again adopt a spherical shape (see supporting information). However, the grafting resulted in noticeble changes in the relative swelling of the particles in different solvents (Table 3). Thus, after grafting, an increase in swelling in polar solvents such as ethanol and acetonitrile was seen, whereas the swelling in chloroform was lower. In Fig. 4(C) and (D) particles containing higher amounts of grafted polymer are shown. Clearly, these do not exhibit the folded structure, but instead appear spherical like the original support bead. In spite of this stabilisation, the low surface area and pore volume (Table 2) showed that none of these materials exhibited permanent porosity. This may be due to complete filling of the voids in the swollen gel or a low crosslinking density of the

Table 3 Swelling of the composite and precursor materials in various solvents

	Swelling[a]/mL mL^{-1}							
Material	Water	Acetone	$CHCl_3$	THF	EtOH	Toluene	CH_3CN	Pentane
PS1	1.0	2.1	5.5	3.0	1.2	2.0	1.4	1.2
PS1-L	1.0	2.4	3.3	2.8	1.6	2.8	2.3	1.5
PS1-N	1.0	2.4	4.3	3.0	1.2	3.3	2.4	1.4

[a]The swelling is expressed as the ratio between the volume of a packed bed of swollen particles over the volume of a packed bed of the same particles in the dry state. They were performed by wetting a 1 mL packed bed of the particles in a 10 mL measuring cylinder with 5 mL of the corresponding solvent and reading of the swollen bed volume after 3 h.

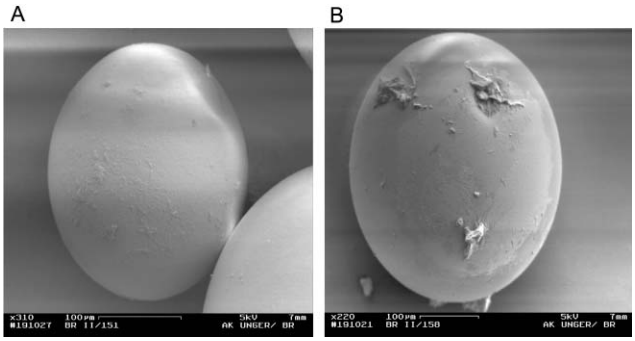

Fig. 6 Scanning electron micrographs of iniferter-modified macroporous (PS2) polystyrene resin supports prior to (A) and after (B) grafting of a poly(MAA-*co*-EDMA) layer. The amount of grafted polymer, estimated by carbon analysis, after these modifications was 0.97 g polymer per g support.

grafted polymer *per se*. The former explanation may be more plausible in view of the absence of strong vinyl bands in the infrared spectra (Fig. 5) and the fact that a reference polymer prepared from soluble iniferter exhibited a high surface area and pore volume. However, the absence of pores indicates that the morphology of the grafted polymer is strongly influenced by the polystyrene support.

Instead, we turned our attention to polystyrene-based resins of the macroporous type (see Table 1 for characteristics). These were modified in a similar manner as the Merrifield-type resins and significant grafting could be achieved in just 2.5 h (Fig. 6). As can be seen in Table 2, this resulted in a decrease in both surface area and pore volume, again indicating that the grafted polymer exhibits a very low porosity in the dry state.

None of the polystyrene-based composites exhibited imprinting effects when assessed in the chromatographic mode (Table 2). Originally we thought that this was due to the size of the beads (Table 1 and Fig. 6) or insufficient swelling in the mobile phase. However, even when using good swelling solvents (*e.g.* dichloromethane) as mobile phases or reducing the flow rate, the enantiomers of the template were weakly and similarly retained.

We finally attempted the grafting on wide pore silica, as we have previously successfully grafted imprinted films on these supports using immobilised azoinitiators.[15] The iniferter was here introduced in two steps and the surface hydrophobised by endcapping. In spite of a lower density of iniferter groups (see Table 1) high contents of grafted polymer could be achieved in a short time period (see Table 2). Interestingly, these composites exhibited pronounced imprinting effects and were capable of separating the template enantiomers with reasonable enantioselectivity (Table 2 and Fig. 7). The decrease in pore volume was here comparable to that observed for the macroporous polystyrene-based composites, but the surface area responded

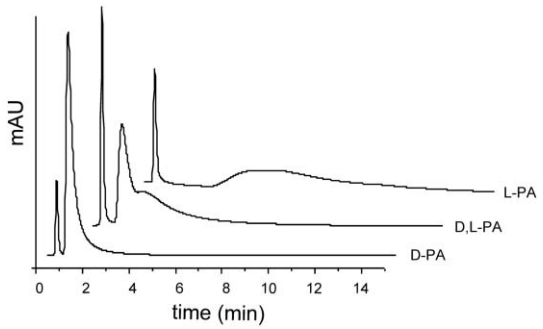

Fig. 7 Elution profiles obtained upon 10 nmol injections of PA as racemate or pure enantiomers on a column (25 × 4 mm) packed with silica-based L-PA-imprinted composite material. Mobile phase: acetonitrile. Flow rate: 1 mL min^{-1}.

differently. Here, no change or a slight increase in the specific surface area was observed upon grafting. This implies that the morphology of the grafted layer is different from those on the polystyrene-based supports. A porous structure of these grafts would lead to better accessibility to the imprinted sites which may in turn explain the selectivity observed in the chromatographic mode.

An advantage of using immobilised iniferters is the stability of the mobile dithiocarbamate radical. Since this radical is unlikely to initiate new chains, propagation in solution is minimal. This was confirmed by the NMR spectra obtained from the monomer solution after grafting. These indicated that MAA was incorporated slightly faster than EDMA in the polymer and that no oligomeric species was present in solution.

Conclusions

The nature of the support material is of crucial importance for successful grafting of molecularly imprinted polymer layers. Whereas templated sites appeared to be absent in composites prepared from polystyrene-based support materials, silica-based grafts were more successful in this regard. These exhibited enantioselectivity in the chromatographic mode and could be reproducibly prepared. Compared to the system based on immobilised azoinitiators, these systems exhibit the advantage that no or minimal propagation occurs in solution. This may open the way for continuous methods for the production of MIPs in beaded form. The living nature of the iniferter grafts may offer the additional possibility of consecutive grafting of multiple polymer layers.[25] These can consist of the same base polymer, but imprinted with different templates, or simply a linear polymer to introduce compatibility with a given matrix.

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