# A facile method for preparing molecularly imprinted polymer spheres using spherical silica templates

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A novel and facile preparation method for spherical molecularly imprinted polymer (MIP) beads is presented. Two types of beads were synthesized and investigated: (i) silica–MIP composites were obtained by filling spherical, porous $C_4$-coated silica beads with print molecule and monomers followed by polymerisation; (ii) spherical molecularly imprinted polymer beads were acquired mirroring the silica particles in size, shape and pore structure by removing the silica matrix from the silica–MIP composites. With regard to their chromatographic properties and yield of the materials both types of particle were more advantageous compared to irregularly shaped traditional MIPs. Also the work-up time to obtain imprinted spherical particles is greatly reduced compared to traditional methods using polymer monoliths, which have to be ground, sieved and sedimented. Generally, the described method may open new possibilities for synthesis of novel types of imprinted polymer formats such as membranes, bulk polymers, films or *in-situ* columns using appropriate support or sacrificial materials.

## Introduction

Molecular imprinting is a method that enables the formation of tailor-made recognition materials by co-polymerising suitable monomers in the presence of a desired print molecule.[1–3] The technique allows the direct production of stereo- and regio-selective matrices that are suitable for use as chiral stationary phases.[4,5] In contrast to the normally applied techniques using chiral stationary phases MIPs can be tailor-made and exhibit a high specificity for a given analyte molecule. Therefore, a major research and potential application area of molecularly imprinted polymers is their employment as separation materials in high-pressure liquid chromatography (HPLC). Particularly interesting is the preparation of imprinted chiral stationary phases.

To date, most MIPs employed in HPLC have been produced in the traditional way: by grinding and sieving bulk polymers, resulting in irregularly shaped materials with heterogeneous size and porosity. Although this technique has led to highly selective materials for a multitude of analytes, such particles are not well suited as packing materials for HPLC. For example, high backpressures and low mass transfer kinetics are usually observed, and a substantial part of the material is lost in the preparation process. Beaded materials, on the other hand, can overcome these problems and several studies comprising imprinted polymer beads have been published. For example, molecularly imprinted polymers have been prepared by filling support particles (usually derivatised with polymerisable groups on the surface) with a traditional imprinting mixture followed by polymerization.[6–10] MIP beads have also been prepared by suspension,[11] emulsion,[12] precipitation polymerization[13] protocols and swelling techniques,[14] often with superior characteristics in chromatography and analysis.

In this study we are presenting a new, facile preparation method for preparing uniformly shaped, spherical MIP beads (Fig. 1). We have adapted existing methods[8,15] into a MIP preparation protocol in which mesoporous silica beads are filled with a pre-polymerisation imprinting mixture that is subsequently polymerised (Fig. 1). In this way, a composite material consisting of silica and MIP is obtained maintaining the shape of the silica support (silica–MIP composites). In a following step, the silica can be dissolved and removed, resulting in spherical beads only consisting of molecularly imprinted polymer (spherical MIP beads). Compared to the silica–MIP composites the porosity of such polymer beads is enhanced in terms of pore volume and surface area. Those two novel materials were investigated with respect to their physical and chromatographic properties and are compared to 'traditional' irregularly shaped MIP particles. As a chiral model compound (−)-isoproterenol (a beta-adrenergic agonist) was used throughout this study (Fig. 2).
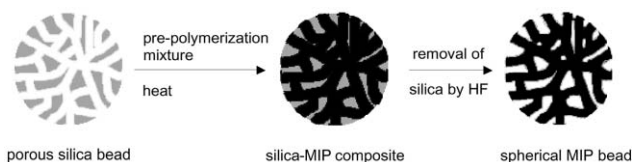


**Fig. 1** Schematic presentation of the preparation of silica–MIP composites and spherical MIP beads.
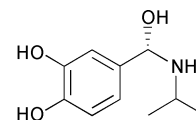


**Fig. 2** Structure of the print molecule (−)-isoproterenol.

## Experimental

### Materials

**Silica.** Commercially available silica particles (Kromasil, 13 μm mean diameter, $C_4$-phase, 0.7 ml $g^{-1}$ pore volume, 195 Å pore size (determined by BET nitrogen absorption)) were provided by Eka Chemicals AB, Separation Products (Bohus, Sweden).

**Other chemicals.** $(\pm)$-Isoproterenol·HCl and $(-)$-isoproterenol·HCl were purchased from Sigma (St. Louis, USA). $(-)$-Isoproterenol, trifluoromethacrylic acid (TFMAA), methacrylic acid (MAA) and divinylbenzene (DVB) were obtained from Aldrich (Steinheim, Germany). DVB was of technical grade (80%) and the inhibitor was removed using basic alumina (Merck, Darmstadt, Germany) prior to use. Solvents used for polymer preparation were of anhydrous quality and were purchased from Labscan (Dublin, Ireland). Hydrofluoric acid (HF), acetone (p.a.) and acetonitrile (MeCN) (HPLC quality) were obtained from Merck.

### Methods

**Preparation of silica–MIP composite beads.** A pre-polymerisation mixture was prepared in the following way: initiator AIBN (95 mg, 0.58 mmol), print molecule $(-)$-isoproterenol (396 mg, 2.5 mmol) and functional monomer TFMAA (1.05 g, 10 mmol) were weighed in a screw-capped glass vial, dissolved in MeCN (4.5 ml) and DVB (6.7 ml, 50 mmol) and sonicated. After complete dissolution the solution was cooled and then sparged with $N_2$ for 2 min. In order to obtain discrete silica–polymer particles and to prevent sticky particle agglomerates the amount of pre-polymerisation mixtures added to the silica was slightly lower (0.65 ml added per g silica) than the BET pore volume of the silica (0.7 ml $g^{-1}$ silica). Thus 3.25 ml of the pre-polymerisation mixture was added to 5 g silica and shaken vigorously. The mixture was then gently stirred with a spatula until all the pre-polymerisation mixture penetrated the silica pores. Finally, the mixture was sonicated to remove any entrapped air bubbles. A free-flowing material was then obtained. It was flushed with $N_2$, sealed and allowed to polymerise in an oven at 65 °C for at least 16 h. After polymerisation was completed, silica–MIP composite beads were obtained.

**Preparation of spherical MIP beads.** In order to prepare spherical MIP beads the above described silica–MIP composite beads were prepared first. The obtained composite material was transferred into a screw-capped polypropylene tube and acetone (10 ml) was added in order to increase the wettability of the hydrophobic particles. This suspension was then cooled in a water–ice bath and 20 ml of 40% aqueous HF was added in several portions while shaking the tube. The suspension was allowed to incubate overnight on a rocking table at room temperature to completely dissolve the silica matrix of the composite. After dissolution of the silica, the suspension was diluted with 100 ml deionised $H_2O$, filtered on a glass filter funnel (7 μm pores) and washed extensively with 2 L of deionised $H_2O$ (containing 20% acetone) until neutrality.

**Preparation of traditional MIP particles.** The polymers used in this case were of the same composition as the pre-polymerisation mixtures used to prepare the silica–MIP composite beads and the spherical MIP beads, except for the amount of porogen. The amount of porogen used here is according to standard methods[16] and corresponds to 4/3 of the volume of the monomers and cross-linkers. After polymerisation, the bulk polymer was crushed, repeatedly ground in an electrical mortar, wet sieved and sedimented in acetone to remove fine particles. Granular particles in the approximate size range of 10–25 μm were obtained.

**Physical characterization and elemental analysis.** Scanning electron microscopy and analysis of the pore characteristics (BET nitrogen adsorption) were done by the Department of Chemical Engineering, Lund Institute of Technology on an ISI-100A and on a Micromeritics ASAP 2400, respectively. Flash combustion elemental analysis coupled to gas chromatography was done by MikroKemi AB (Uppsala, Sweden).

**Chromatographic evaluation.** Polymer particles were slurried in water (25% MeCN) and then slurry-packed into stainless steel columns (250 mm × 4.6 mm id) using an air-driven fluid pump (Haskel, Burbank, CA, USA) and water (25% MeCN) as the pushing solvent. The packed columns were washed on-line on a Beckman HPLC system (comprising a solvent module 126 and diode array detector 168) using MeCN (20% acetic acid) to remove the print molecule until a stable base line was achieved.

Due to the highly acidic functional monomer TFMAA being incorporated into the polymer a rather high acidity in the mobile phase was necessary to elute the analyte from the column. Also, a certain amount of organic modifier (acetonitrile) was needed to suppress hydrophobic non-specific interactions and to achieve good wettability properties because the polymeric material is rather hydrophobic. Thus, all chromatographic characterizations were performed using an aqueous mobile phase (25 mM sodium citrate, pH 3.0) containing 10% MeCN at a flow rate of 1 ml $min^{-1}$ and chromatograms were recorded at 280 nm. All chromatographic elutions were run in isocratic mode. Injections of 20 μl of 2 mM racemic isoproterenol HCl (40 μmol) dissolved in the mobile phase were done in order to evaluate the enantioselectivity towards its imprinted print molecule isoproterenol. Chiral discrimination data (α-values) were calculated according to standard chromatographic theory using sodium nitrite as a non-retained void marker. The number of theoretical plates was calculated based on the analysis of the non-imprinted enantiomer $(+)$-isoproterenol. In order to determine the capacity of each stationary phase, breakthrough curve analysis was performed by injecting 20 ml of a 1 mM $(-)$-isoproterenol HCl solution (dissolved in mobile phase) onto the column.

## Results and discussion

### Design of the composite system

Silica particles are excellent materials as HPLC stationary phases. They can be dissolved and removed with appropriate reagents and that makes them very suitable both as a support and sacrificial material for the method presented. Three major factors were considered when designing the imprinted polymer system: (i) the form-giving silica support particles should not interfere with the components of the imprinting mixture, (ii) the silica particles should be an appropriate 'host' for the incorporation of polymer in terms of compatibility and (iii) the components of the resulting polymer (*i.e.* the polymer monomers and cross-linkers) should withstand the harsh hydrofluoric acid treatment. Since the surface silanol groups of bare silica are potent in disturbing the essential, non-covalent print molecule–monomer interactions that are crucial to the imprinting effect, hydrophobic $C_4$-coated silica was chosen. The coverage of the particles is very high (0.82 mmol $C_4$ $g^{-1}$) with minimal accessible residual silanol groups on the silica surface. Further hydrophobisation of remaining silanol groups by end-capping with *e.g.* trimethylchlorosilane was therefore not necessary; the support particles could be used as obtained. Additionally, the hydrophobic properties of the $C_4$-silica surface will provide

good wettability of the pre-polymerisation mixture onto the silica surface, which will result in a homogeneously filled silica particle. It should also be mentioned that silica with polymerisable groups on the surface was not necessary to anchor the polymer inside the silica. The extensively branched inner structure of the silica is sufficient to retain the polymer within the pore structure of the silica particles simply by mechanical entrapment. Comparatively large silica particles with a mean diameter of 13 μm were chosen to facilitate filtration and to speed up sedimentation. A pore size around 200 Å and a BET pore volume of 0.7 ml g$^{-1}$ appeared to be a good compromise between porosity of the silica particle (55%), resulting particle stability, pore volume, and surface area of the polymeric materials after silica removal. Silica particles with larger pore sizes (e.g. 1000 Å) would lead to polymer particles with an unfavourably small surface area and silica with a lower pore volume would result in polymeric materials with very brittle structures. Silica with a C$_4$-phase and 200 Å pores is also readily available from commercial suppliers. TFMAA and DVB were chosen as the functional monomer and cross-linker, respectively, because the resulting polymer has been shown to be resistant to the harsh HF treatment which is necessary for removal of the silica support.[17] In order to facilitate analysis and further demonstrate the selectivity, a chiral print molecule, (−)-isoproterenol, was chosen to produce an enantioselective polymer. Chiral print molecules are highly useful tools in MIP development, since the physical properties of the enantiomers are identical in a non-chiral environment, and chiral discrimination is a direct measure on the outcome of the imprinting process.

**Preparation of MIP beads/particles**

Two methods of introducing the polymer into the pores of the silica particles were evaluated. In the first 'wet-method' the C$_4$-silica particles and the pre-polymerisation mixture where agitated vigorously for several hours in aqueous solution. By this treatment, the hydrophobic polymerisation mixture accumulates inside the hydrophobic pores of the silica particles, and subsequent polymerisation is accomplished upon heating of the mixture.[15] This method is straightforward, but several aspects make it not generally suitable for application with molecularly imprinted polymers. Most importantly, the system becomes saturated with water, which interferes seriously with the interaction between the functional monomers and the print molecule. In all cases where this method was used, no imprinting effect could be observed. Another drawback is that a portion of the functional monomer (MAA was tested) remains dissolved in the water-phase, thus altering the resulting polymer composition.

Attempts to lower the solubility of the functional monomer in the aqueous phase by adding poly(vinyl alcohol) and/or brine were unsuccessful. Likewise, increasing the initial concentration of MAA in the reaction mixture did not improve the imprinting effect. Exchanging the water phase for liquid perfluorocarbons, previously shown to be suitable for the production of molecularly imprinted polymer beads,[11] also gave unsatisfactory results and was not explored further. In the light of these findings, synthesis of beaded MIPs with the aid of water or liquid perfluorocarbon suspensions did not present a promising alternate route for our polymer.

As a final means of producing silica polymer composites, a method in the dry state ('dry-method'), without the use of a liquid phase, was developed. This method was similar to a previously reported method where trimethylolpropane tri-methacrylate composites were produced.[8] Here, the appropriate amount of a pre-polymerisation mixture was added to the dry silica particles, shaken vigorously and finally gently stirred with a spatula. By this process, the pre-polymerisation mixture penetrated the pores of the silica and, after stirring, the
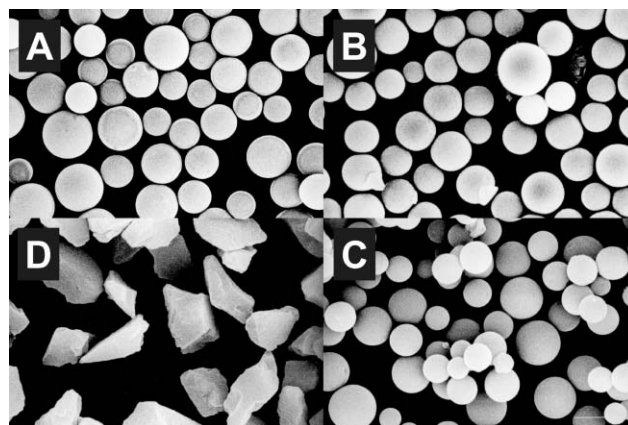


**Fig. 3** SEM of the materials, magnification × 1000: A. silica particles, B. silica–MIP composite particles, C. spherical MIP particles all having an approximate mean diameter of 13 μm, D. traditional MIP particles, approximate size 10–25 μm.

entire polymer accumulated inside the silica particles, which then showed a free-flowing behaviour. This indicated that no external pre-polymerisation mixture was present to make the particles 'sticky'. Upon heating the monomers and cross-linkers co-polymerised inside the silica particles, and discrete single silica-polymer composite beads were obtained (Fig. 3b). Due to the ease and simplicity of the dry-method, where no suspending continuous phase was needed, it was the method of choice for all other polymers produced.

The spherical MIP beads (Fig. 3c) were prepared from composite particles by dissolution of the silica matrix. Three different dissolution reagents were examined: concentrated aqueous solution of tetramethylammonium hydroxide, hydrofluoric acid, and concentrated sodium hydroxide in methanol.[15,18] Of these, the concentrated solutions of tetra-methylammonium hydroxide or sodium hydroxide dissolved the silica, but slower and less efficiently than did hydrofluoric acid. Concentrated hydrofluoric acid showed the best results in terms of dissolution speed and ease of handling. It was purchased and used as a 40% aqueous solution. Due to the hydrophobicity of the silica polymer composites, some acetone was added to the composites in order to increase the wettability with the aqueous HF solution. Following extensive washing by water/acetone, silica-free MIP beads were obtained that completely retained the size and form of the sacrificial silica phase (Fig. 3b). Elemental analysis showed that over 99% of the silica could be removed from the composite material. Moreover, nitrogen (which mainly originates from the print molecule) was not observed in the MIPs tested (detection limit ca. 0.3%). Even after the harsh HF treatment, the spherical MIPs (Fig. 3c) retained both the shape and size of the host silica (Fig. 3a). In contrast to traditional MIP particles (Fig. 3d), both the composite particles (Fig. 3b) and the spherical MIP beads (Fig. 3c) proved to be spherically shaped. In addition, the particle yield was at least 90% for the spherical MIP and nearly 100% for the silica–MIP composite particles, in contrast to the yield of the traditional MIPs, which was only 25%. A high yield of material is especially important for imprinting compounds that are expensive or not available in big quantities.

**Physical characterization**

Although it is the imprinted polymer that is responsible for the chiral separation of the analyte, the physical properties of the stationary phase material also contribute to the separation characteristics. Thus, the pore volume, pore size and surface area influence the separation performance of the stationary phases. For a proper comparison of the chromatographic properties of the different particle types we attempted to

**Table 1** Pore properties of the silica support and the MIP stationary phases

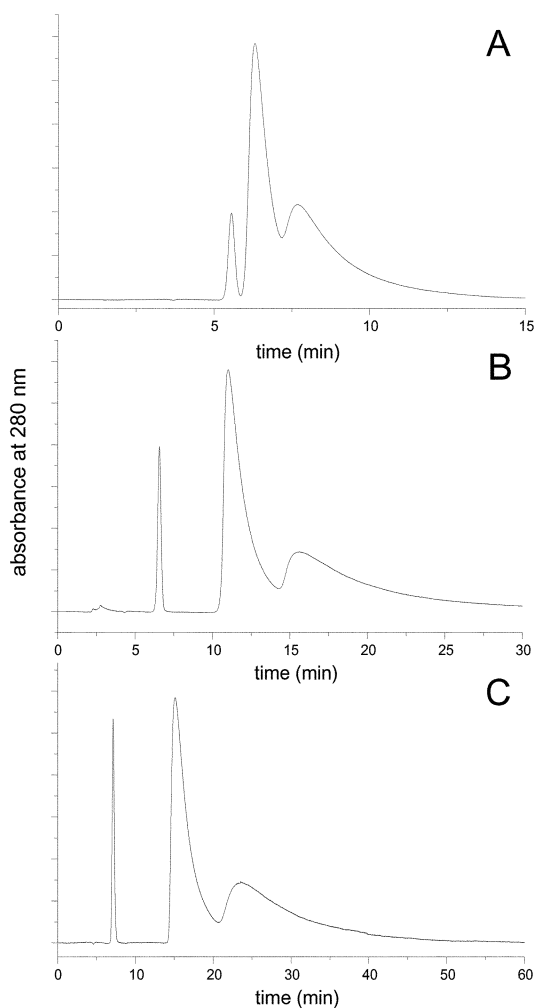|  | Silica | Traditional MIP | Silica–MIP | Spherical MIP |
|---|---|---|---|---|
| Pore-volume [ml g$^{-1}$] | 0.7 | 1.1 | 0.4 | 0.9 |
| Mean pore-size [Å] | 195 | 65 | 70 | 80 |
| Surface area [m$^2$ g$^{-1}$] | 145 | 665 | 225 | 460 |

produce materials with comparable physical properties, *i.e.* similar particle sizes, pore characteristics and polymer composition. The silica and MIP particles were characterized by BET nitrogen adsorption analysis and are listed in Table 1.

BET analyses show that all pore properties were enhanced for the spherical MIP by the dissolution of the silica matrix from the silica–MIP composite. There is a considerable increase of pore-volume and surface area from the silica–MIP composites to the spherical MIPs. This increase in pore-volume from composite to the spherical MIP is in approximate accord to the porosity of the silica material (55%) causing *ca.* 0.5 ml pores per gram dissolved silica. All imprinted materials had mean pore sizes of approximately 70 Å mainly formed by the porogenic solvent acetonitrile. The dissolution of the silica matrix contributed to the slightly higher mean pore size for the spherical MIP. Whereas the traditional and the spherical MIP had only one major pore size, the silica–MIP composite had a binodal pore size distribution. We assume that the 'gap' between polymer and silica surface is responsible for the additional pore size population.

### Chromatographic evaluation

In the chromatograms (Fig. 4) the separation of a given amount of racemic analyte is performed fastest by the silica–MIP stationary phase (Fig. 4a) which is consistent with the low pore volume and hence with the low degree of interaction of the analyte with the stationary phase for these particles. Having both a twice as high surface area and pore volume the spherical MIP performs the separation of the same analyte solution in 30 minutes. For a comparison, the traditional polymer, which has the highest pore volume and surface area, requires 60 minutes. All imprinted stationary phases were further studied with respect to their chromatographic properties. Separation factors $\alpha$, plate numbers $N$ and capacity $B$ were determined and the backpressures gauged by the instrument are listed in Table 2.

All polymers exhibited chiral selectivity towards its print molecule and could separate (−)-isoproterenol from the non-imprinted optical isomer (+)-isoproterenol. The imprinted polymers kept their imprinting effect even after treatment with HF. In terms of enantioselectivity, the traditional material retained the analytes longest and therefore showed a higher $\alpha$ value than the silica MIP and the spherical MIP beads, which exhibited similar $\alpha$ values. A comparison of the theoretical plate numbers shows that the spherical MIPs have the highest and the traditional MIPs have the lowest number of theoretical plates, with the silica–MIP beads exhibiting an intermediate value. The flow rate was varied from 0.5 ml min$^{-1}$ to 2 ml min$^{-1}$, and the relationship between the values of $\alpha$ and $N$ for the three stationary phases remained unchanged. As expected, both the spherical particles (composites and spherical beads) displayed a higher chromatographic performance than the traditionally produced ground particles; the plate numbers were almost twice as high for the spherical MIPs than for the traditional particles. It should be stressed that at a given flow rate, both the spherical particles separated a given amount of analyte faster than the traditional MIP (Fig. 4a–c). Shorter separation times under the same conditions indicate an increased accessibility to the binding sites of the novel stationary phases. Also expected was the behaviour of the backpressures of the materials. They were lowest for the silica–MIP composites and highest for the



**Fig. 4** Chromatographic enantioseparation of racemic isoproterenol with various MIP stationary phases (A. silica–MIP composite, B. spherical MIP, C. traditional polymer). Acetone was used here as a reference marker. It should be noted here that the chromatographic conditions were not optimized for each stationary phase.

**Table 2** Chromatographic properties of the MIP stationary phases

|  | Traditional MIP | Silica–MIP | Spherical MIP |
|---|---|---|---|
| $\alpha$ | 1.6 | 1.3 | 1.3 |
| $N$/m$^{-1}$ | 1270 | 2050 | 2530 |
| $B$/µmol g$^{-1}$ polymer | 5.9 | 2.9 | 4.0 |
| Backpressure/MPa | 10.9 | 5.4 | 7.9 |

traditional MIPs. The high backpressure of the traditional particles is due to a wider spreading of the size of the particles and its irregularity. As the silica–MIP particles consist of a rigid silica core their mechanical stability is exceptionally high and they therefore display the lowest backpressures. The capacities of all the materials were rather similar and correlated to the porosity of the stationary phase.

Albeit the lower pore volumes and lower surface areas, both the spherical particles (silica–MIP composites and spherical MIPs) show a better chromatographic performance than the traditional particles.

### Conclusions

It has been shown that it is highly advantageous to produce spherical molecularly imprinted polymer beads by using common C$_4$-silica particles as a support material to obtain silica–MIP composites, or as a sacrificial support material to

obtain spherical MIP beads. The new approach and the novel materials offer the following features for the preparation of spherical imprinted particles: (i) the work-up procedure is simplified and drastically reduced, (ii) the silica–MIP composite material can be immediately used for HPLC packing, (iii) the yield of the materials is very high (>90%), (iv) the flow properties are improved displaying a) lower back-pressures b) higher mass transfers and c) higher plate numbers than the traditional ground particles, and (v) the enantioselectivity of MIPs is not affected. The preparation and evaluation of imprinted material for chromatographic and other applications can thus be done in a fast and easy way. Furthermore, the described methodology is not limited to using silica as support and sacrificial material. In our laboratory we have generated EDMA-based polymeric replicas of porous agarose templates.[19] Along this line, any appropriate support or sacrificial material can be envisaged for the synthesis of novel types of imprinted polymer formats such as membranes, films, *in-situ* columns and bulk materials.

## Acknowledgement

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