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Molecularly imprinted polymers on silica: selective supports for high-performance ligand-exchange chromatography

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Abstract

Thin coatings of molecularly imprinted, metal-complexing polymers have been grafted to activated silica beads suitable for high-performance liquid chromatography (HPLC). Propylmethacrylate-activated silica particles were coated by copolymerization with a metal-chelating monomer, Cu^{2+}–[N-(4-vinylbenzyl)-imino]diacetic acid, a metal-coordinating (imidazole) template, and ethylene glycol dimethacrylate. After extraction to remove the template and re-loading with metal, the composite materials re-bind the templates with which they were prepared and exhibit selectivities comparable to bulk-polymerized imprinted materials. The strong Cu^{2+}–imidazole interaction, desirable for creating a high-fidelity imprint, leads to excessive retention in elution chromatography. By replacing the copper in the imprinted metal-complexing polymers with weaker-binding Zn^{2+}, these novel ligand-exchange supports can effect partial to complete chromatographic separation of their bis-imidazole templates from other, highly similar imidazole-containing substrates. This "bait-and-switch" approach can significantly enhance the performance of molecularly imprinted materials. Scatchard plots of equilibrium binding data show a significant degree of heterogeneity in the imprinted binding sites of material prepared with a bis-imidazole template, but not with a mono-imidazole template. The best chromatographic separations are observed with small sample sizes, where the substrates occupy the strongest (highest-fidelity) sites.

1. Introduction

We have proposed that the unique spatial distribution of metal-coordinating groups on a target molecule can provide the basis for its selective binding by materials with complementary distributions of metal ions [1]. Bis-metal "receptors" designed to test this concept exhibit high affinity as well as selectivity for complementary distributions of imidazole moieties on a series of bis-imidazole compounds [2,3]. The difficulty of orchestrating the precise placement of multiple functional groups in a synthetic receptor or directly in a chromatographic support underlies our interest in systems that can self-assemble to form specific recognition sites. Thus we have been developing methods of template polymerization, or "molecular imprinting", wherein the target molecule assists in the preparation of a metal-complexing polymeric receptor by serving as a template to impart the critical structural information [4,5]. The template can direct the arrangement of complementary functional groups in polymeric binding cavities, by forming a monomer–template assembly that is captured in a highly crosslinked macroporous

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polymer [6,7]. After extraction of the template, these materials exhibit selectivity for the substrates with which they were prepared. Molecularly imprinted polymers have shown promise as selective adsorbents for chromatographic separations [6] and as "antibody mimics" for radioassays of small molecules [8]. This approach is also an attractive route to materials to recognize proteins and other biological macromolecules for which the structural information needed for "rational" receptor design is lacking.

Although template polymerization is conceptually attractive for preparation of highly selective adsorbents, successful demonstrations of chromatographic separations involving these materials are still relatively few. One problem has been that efforts to optimize the imprinting process (preparation of the polymer) can conflict with the material's chromatographic performance. For example, the relatively high degree of crosslinking needed to capture a specific arrangement of functional monomers in the solid polymer can hinder the diffusion of substrates in the particles, resulting in band spreading and poor peak resolution. Access of very large substrates to binding sites can be severely impeded. Furthermore, bulk-polymerized templated polymers which are ground and sieved to prepare column packings may not exhibit the mechanical stability and uniformity required for rapid, high-resolution chromatographic separations. The presence of high concentrations of copper ions, for example, in the polymerization mixture can limit crosslinking efficiency and therefore the rigidity of the material.

One approach to alleviating these problems is to graft copolymerize the monomer–template assemblies and crosslinker to a reactive support with the desired physico-mechanical properties. This laboratory, for example, has investigated poly(trimethylolpropane trimethacrylate) (TRIM) as a reactive surface for template polymerization [9]. Silica-based supports, widely used in chromatography, are available in a broad range of bead sizes and pore diameters. Silica can be readily modified using commercially available silanizing reagents to produce a variety of stationary phases, including reverse-phase and ion-exchange supports. Reactive groups for template polymer grafting can be introduced in a similar manner. Mosbach and coworkers have reported the separation of dyes on porous silicas coated with molecularly imprinted polymers [10], while Wulff et al. [11] have coated silica particles with thin layers of imprinted polymers for chiral separations. In the current work we have used a similar approach to coat macroporous silica particles suitable for high-pressure liquid chromatography (HPLC) with metal-complexing molecularly imprinted polymers [12,13].

The binding selectivity exhibited by a molecularly imprinted polymer depends on the fidelity of the imprinting process–that is, how well the arrangement of functional groups captured during polymerization complements the arrangement of functional groups on the template. Weak interactions between template and monomers during imprinting can result in the random placement of a significant fraction of the functional groups in the polymer. This randomization of binding functions dramatically reduces selectivity. Therefore, optimizing the polymerization conditions to favor formation of the non-covalent monomer–template complexes (e.g., lower temperature, appropriate solvent) is highly desirable [6,14,15]. We reasoned that a monomer–template assembly held together by strong metal-to-ligand interactions would also minimize dissociation during polymerization. Unfortunately, very strong interactions with the template can interfere with the material's subsequent chromatographic performance, since tightly bound substrates experience very long retention times and excessive band spreading.

The strength and kinetics of metal coordination interactions can be tailored for a specific application by replacing the metal ion used during imprinting with others better suited for the chromatographic separation. We have exploited this ability to substitute the binding function in molecularly imprinted metal-complexing polymers to prepare efficient chromatographic supports. The strong Cu^{2+}–imidazole interaction used previously to prepare the templated polymers [4,5] produces excessive retention times in elution chromatography of bis-

imidazole substrates. Removing the copper ions and replacing them with weaker-binding Zn^{2+}, however, yields adsorbents capable of separating closely related bis-imidazole substrates. This "bait-and-switch" approach can significantly enhance the performance of molecularly imprinted materials.

2. Experimental

Silica (LiChrosphere 1000, 10 μm particles, 1000 Å pores) was the generous gift of E. Merck. ServaGel was obtained from Serva (Paramus, NJ, USA). Ethylene glycol dimethacrylate (EGDMA), azobisisobutyronitrile (AIBN), 3-(trimethoxysilyl)propylmethacrylate and 1-benzylimidazole were obtained from Aldrich and used without further purification. Ammonium peroxysulfate (APS) was obtained from Biorad and triethylamine from EM Science. 1,4-(Bis-imidazol-1-ylmethyl)benzene (**5**), 1,3-(bis-imidazol-1-ylmethyl)benzene (**6**) and 4,4′-(bis-imidazol-1-ylmethyl)biphenyl (**7**) were all prepared as described in Ref. [5], and copper(II)[N-(4-vinylbenzyl)imino]diacetate · $2H_2O$ (CuVBIDA, **1**) was prepared as described in Ref. [5], except that the copper was loaded at pH 9.0 instead of 7.0.

2.1. Derivatization of silica particles

The LiChrospheres were washed with distilled H_2O and boiled in 5% HNO_3. The particles were filtered on a fine-fritted filter, washed extensively with distilled H_2O, and dried at 150°C for 24 h. A 1-μm vacuum was applied to the oven-dried silica in a three-neck flask for 30 min, after which the vessel was sealed. Toluene, dried over sodium and distilled, was added to the silica under vacuum. To this was added 3-(trimethoxysilyl)propylmethacrylate (50% w/w silica) and a trace (ca. 1 mg) of triethylamine. This mixture was refluxed for 15 h under nitrogen. The silica was isolated by filtration over a fine-fritted filter and was freed of any residue by successive washing with toluene, acetone, and ether. The silica was then dried under aspirator vacuum for 24 h.

The modified silica was assayed for the presence of vinyl groups by titration with bromine water [10]. A known weight of silica was suspended in distilled H_2O, and dilute bromine water was added until a slight yellow color persisted. The residual HBr was titrated with NaOH to obtain the total number of unsaturated bonds accessible to the solution. When this procedure was repeated using underivatized silica, no residual HBr was observed.

2.2. Polymer coating processes

For high-temperature (70°C) polymerization, 2.0 g of propylmethacrylate-derivatized silica was placed in ca. 8 ml of methanol. To this suspension, 0.38 g of EGDMA, 20 mg of CuVBIDA and 0.5 equivalents (or 1 equivalent for **4**) of the template were added. After mixing for 1 h, the reaction vessel was placed under aspirator vacuum to degas the mixture and allow the solvent to penetrate the silica pore spaces. AIBN (4 mg) was then added. The vessel was thoroughly mixed, sealed, and held at 70–75°C for 48 h with no agitation. The vessel was opened and maintained at 70°C until enough solvent had evaporated to leave a thick slurry in the reaction vessel. The coated silica was washed at least three times by suspension in methanol followed by centrifugation.

In the low-temperature (40°C) procedure, 2.0 g of derivatized silica was placed in enough 80% aqueous methanol to cover the surface to a depth of approximately 1–2 mm (ca. 3 ml). To this was added 0.38 g EGDMA, 20 mg CuVBIDA and 0.5 equivalents (or 1 equivalent for **4**) of the template. After mixing for 45–60 min, this mixture was sonicated for 20 min to allow penetration of solvent into the pore space. APS (4 mg) was then added in a small amount (20 μl) of water, and the vessel was sealed and degassed under vacuum. The reaction mixture was then placed in constant-temperature bath at 40°C for 48 h without agitation. The coated silica was then suspended in methanol and centrifuged, a process repeated three times.

2.3. Evaluation of coating efficiency

Dried, coated silica was sieved and subsequently suspended and decanted in methanol to remove any bulk polymer that forms at the solution–vessel interface. Polymerization efficiency was determined by weighing the total solids and subtracting the weight of bulk polymer. Total particle coating efficiency was determined by subtracting the weight of bulk polymer. For further quantitation of coating efficiency, templates were extracted from the coated silica particles with 20 m*M* HCl in 95% methanol, with stirring at 40°C for 48 h. After removal of solvent under vacuum, the template was quantified by ^{1}H NMR of the residual dissolved in $CDCl_3$, using α,α'-dibromo-*p*-xylene as an internal reference [13]. For quantitation of coating efficiency with respect to incorporation of copper, the metal was removed by stirring at 40°C with 0.10 *M* aqueous EDTA, pH 7.0, followed by a second treatment with 25 m*M* 1,4,7-triazacyclononane in methanol. The copper content of the residuals obtained after drying was quantified from the UV absorbance (800 nm) in 0.1 *M* EDTA, pH 7.0, with reference to a standard curve prepared from solutions of known copper concentration [5,13].

2.4. Scanning electron microscopy

All micrographs were obtained using a Cam-Scan Series 2 electron microscope. The silica particles were dried extensively under vacuum and placed on conducting carbon tape. Gold coating was applied and the particles were loaded into the staging area. Images were acquired at working distances of 5–35 mm using both a backscattering and secondary emission detector.

2.5. Chromatography and equilibrium binding studies

Before use as a packing material, the polymer-coated beads were suspended in methanol and sonicated for 20 min to fully disperse the particles. Following template extraction, the material was equilibrated with 10 m*M* sodium phosphate in 80% aqueous methanol, apparent pH 7.0. Approximately 1 g of polymer-coated silica was suspended in 50% aqueous sucrose and placed in a steel packing reservoir attached to a 50×4.6 mm I.D. stainless steel column. The column was packed under a constant pressure of 2.55×10^6 Pa. After packing, the column was washed extensively with methanol (at least 100 ml). Void fractions were measured from the retention of a small tracer under non-binding conditions (e.g. **8**, or **4** using columns with all metal removed).

To load the polymer-coated particles with Zn^{2+}, the columns were first washed with 100 ml of 10 m*M* diethylenetriamine in 100% methanol to remove all of the copper. The columns were then washed with 100 ml of 20 m*M* $ZnCl_2$ in methanol, followed by 100% methanol until a steady detector baseline was reached. When zinc acetate was used as a mobile phase competitor, the columns were washed with 50 m*M* zinc acetate until a steady baseline was reached. The chromatographic separations were carried out at 65°C, flow-rate of 0.5 ml/min, with UV detection at 220 nm. For each stationary phase, the final zinc acetate concentration was adjusted so that the template eluted between 5 and 10 column volumes (30–50 m*M*).

The equilibrium-binding studies were carried out using ServaGel (30 μm, 500 Å) derivatized with propylmethacrylate and coated using the 40°C process (total monomer 50% w/w with silica, monomer composition: 5% **1**, 30% methylmethacrylate, 65% EGDMA). The coated silica was extracted with 20 m*M* HCl in 95% methanol with stirring at 40°C for 48 h, and then washed two times with methanol. Material equilibrated with 10 m*M* N-morpholinosulfonic acid (MOPS) in 90% methanol, apparent pH 7.0, was washed two times with methanol and dried under vacuum. Aliquots of the materials were suspended in methanol solutions of varying substrate concentration, mixed for 48 h at 25°C, and centrifuged. Substrate concentrations in the supernatants were determined from the absorbance at 240 nm, relative to a standard curve.

3. Results and discussion

3.1. Preparation of molecularly imprinted polymer-coated silica

To provide anchoring sites for the grafted polymer coating, 10 μm porous silica particles (LiChrosphere 1000, 1000 Å pores) were modified with propylmethacrylate using a silanizing reagent, 3-(trimethoxysilyl)-propylmethacrylate. The resulting anchoring group is shown in Fig. 1 (**3**). Bromine titration of the resulting vinylic groups indicated 34 μeq/g silica had been achieved, or ca. 50% surface coverage, based on the manufacturer's reported specific surface area of 17.5 m^2/g and 40 $Å^2$ per immobilized propylmethacrylate group. This coverage is comparable to that reported by Nörrlow et al. [10].

Two coating processes were studied in an effort to prepare molecularly imprinted materials suitable for HPLC applications [13]. In the first process, the polymerizations were performed by mixing the functional monomer, copper(II)[N-(4-vinylbenzyl)imino]diacetate · $2H_2O$ (**1**), one of the template molecules shown in Fig. 2 (**4–7**),

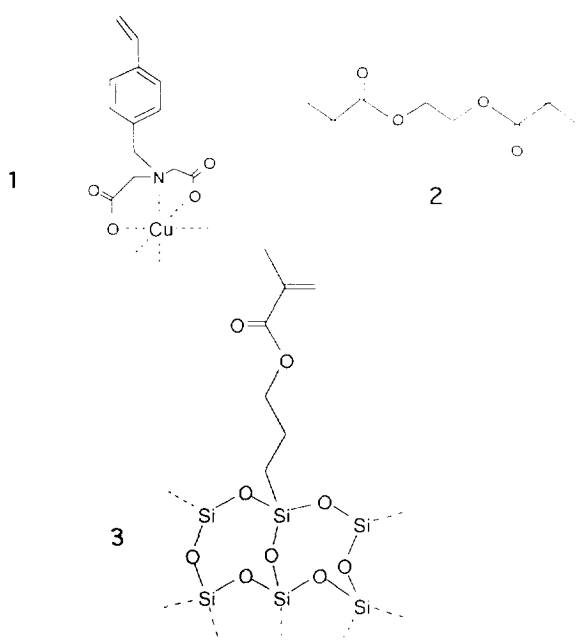

Fig. 1. Monomers used for molecular imprinting.

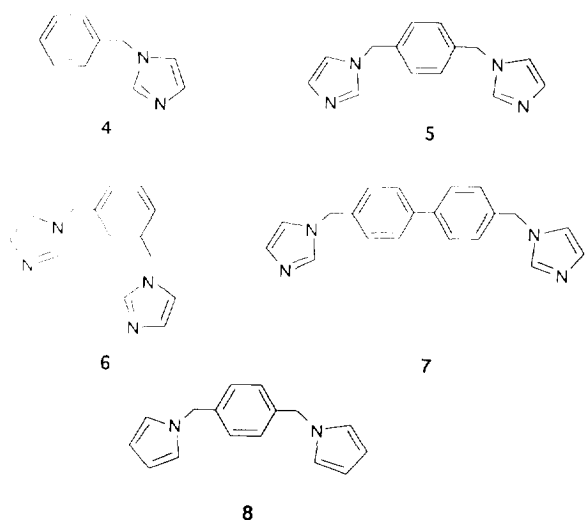

Fig. 2. Mono- and bis-imidazole substrates used for molecular imprinting and re-binding studies.

and ethylene glycol dimethacrylate (EGDMA, **2**) with derivatized silica suspended in 100% methanol. The total weight of the monomers was 20% of the weight of the silica (20% w/w coating). Polymerization was initiated with azobisisobutyronitrile (AIBN) and allowed to proceed at 70°C for 48 h. Under these conditions, 97% of the copper in the polymerization mixture was incorporated into the coating, and essentially all the polymer remained associated with the silica after washing and sieving. By the time polymerization was complete, all of the monomer was incorporated into polymer, and most of the solvent had evaporated. If this drying step did not take place, or if the silica was unmodified, the polymeric material did not associate with the silica to a significant extent. The copper-containing monomer **1** constituted 5 wt% of the total monomer added, giving the final material a copper content of approximately 35 μmol/g. For material templated with 1-benzylimidazole, 72% of the template and 88% of the included copper could be recovered by extracting the silica particles with solutions of EDTA and 1,4,7-triazacyclononane.

An alternative, lower-temperature coating method that did not require a drying step was also developed. In this process, the reagents

(EGDMA, **1**, and a template) were mixed with propylmethacrylate silica suspended 80% aqueous methanol. Polymerization was carried out at 40°C for 48 h after initiation with ammonium peroxysulfate (APS). The total weight of monomer added was 20% of the weight of the silica (20% w/w coating). Monomer **1** constituted 5 wt% of the total monomer added (35 μmol copper/g material). Less of the polymer associated with the silica during this process (73% versus 100%), and, on average, only 71% of the available copper was included in the polymer (versus 97% for the 70°C process). Extensive washing and extraction with solutions of EDTA and 1,4,7-triazacyclononane recovered 81% of the incorporated copper. Thus the total recoverable copper was about 20.3 μmol copper/g for these materials.

3.2. Scanning electron microscopy

The particles were examined by scanning electron microscopy (SEM) to compare the morphologies of their polymer coatings and investigate how they are affected by use as chromatographic packings. As shown in Fig. 3, an unmodified LiChrosphere particle is uniformly spherical, and the 1000 Å pores are clearly visible. After coating using the 70°C process, a typical particle (shown in Fig. 4a) has a surface layer that appears to be made up of packed, fused, polymer nanospheres. This structure is typical of highly crosslinked, porous polymeric beads [16]. A purely backscattering image (Fig. 4b) shows an open packing of these spheres, suggesting that the drying step required in this process serves to drive the polymer nanospheres from suspension in methanol to the silica surface, where they adhere to the beads either through covalent bonding or polymer chain entanglement. An image of this same material taken after use in the chromatography column (Fig. 4c) shows extensive loss of the polymer coating. During chromatography, accumulation of the stripped material on the column end frit caused a steady increase in backpressure over time, rendering the columns useless after a relatively short time.

Fig. 3. Scanning electron micrograph of LiChrosphere 1000 before derivatization. Particle washed with 5% nitric acid and oven-dried at 140°C.

The morphology of the material coated using the 40°C process, shown in Fig. 5a, differs markedly from that prepared using the higher-temperature process. The coating in this case appears as a continuous, convoluted surface with features in the 50–100 nm range, compared to the 300–500 nm features of the 70°C polymer. These and other images of populations of coated particles [13] show that the polymer coatings are very thin, on the order of 1 μm or even less. After prolonged use in a column, only a few particles show modest damage (Fig. 5b), far less than that sustained by the 70°C coating. This material could be used continuously for several hundred hours with essentially no change in performance. All the chromatographic separations were carried out on materials produced by the 40°C process.

3.3. Chromatographic performance of molecularly imprinted polymer-coated silica

The polymer-coated silica beads were slurry-packed into 50 mm stainless steel columns (4.6 mm I.D.) and evaluated for their ability to separate a series of mono- and bis-imidazole substrates [imidazole and **4**–**7** (Fig. 2)] and a structural analog of **5** that does not have the coordinating nitrogens (**8**). Retention of the

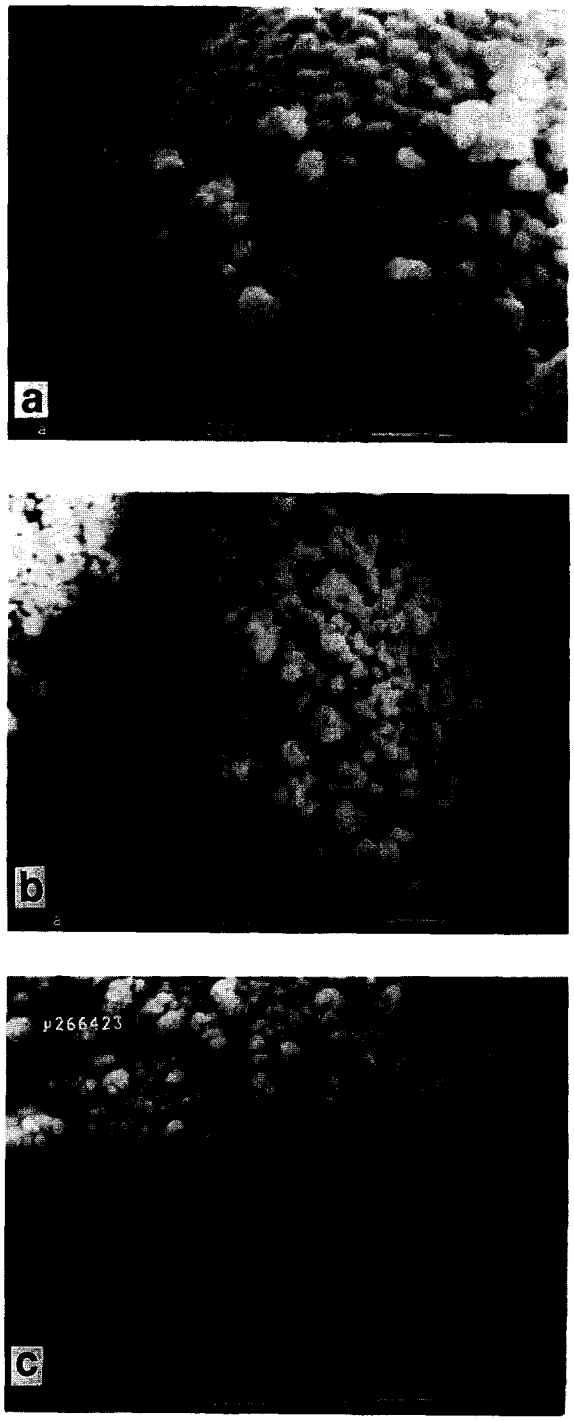

Fig. 4. Scanning electron micrographs of a LiChrosphere surface after polymer coating using the 70°C process (a); (b) backscattering detector only; (c) after prolonged use in a column.

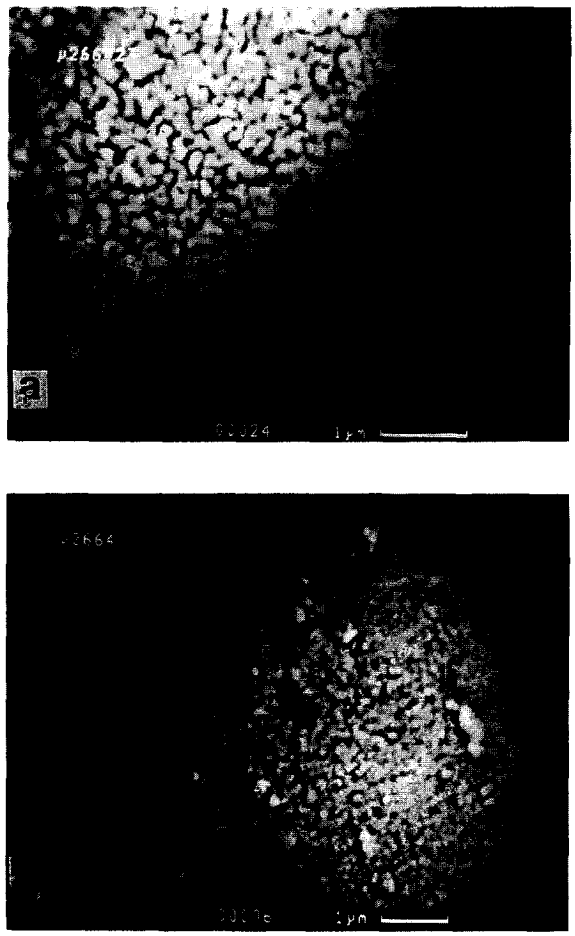

Fig. 5. Scanning electron micrographs of a LiChrosphere surface after polymer coating using the 40°C process (a); (b) after prolonged use in a column.

imidazole-containing compounds on materials loaded with Cu^{2+} was so strong that no peaks were observed during isocratic elution. Lowering the apparent pH of the mobile phase (50% methanol) to 4.1 by addition of acetic acid caused all the peaks to elute simultaneously (data not shown).

Substrate retention times were greatly reduced when the Cu^{2+} ions were replaced with Zn^{2+} by washing the column with diethylenetriamine followed by several column volumes of 20 m*M* $ZnCl_2$. Listed in Table 1 are the substrate retention volumes and chromatographic separation factors (α) on a column packed with poly-

Table 1
Elution volumes (V_e) and chromatographic separation factors ($\alpha_{i,j}$) for substrates on polymer-coated silica (LiChrosphere 1000) prepared using **5** as the template

Substrate	V_e (column volumes)	$\alpha_{5,j}$
8	0.7	$\rightarrow\infty$
Imidazole	6.8	>33
4	9.5	>23
5	>200	–

The Cu^{2+} has been replaced with Zn^{2+}. The 50 × 4.6 mm I.D. column was operated at 65°C, 0.5 ml/min 100% methanol, with a sample size of 10 μl of 0.4 m*M* solution. $\alpha_{i,j} = (V_e - V_o)_i/(V_e - V_o)_j$, where V_o is the column void volume.

mer prepared using bis-imidazole **5** as the template. The non-coordinating analog of the bis-imidazole template (**8**) elutes in the void volume, demonstrating that cavity formation around the template alone is not sufficient for binding: retention requires the metal–imidazole interaction. Retention of all the imidazole-containing substrates was still significant on the Zn^{2+}-loaded material: 5–10 column volumes for the mono-imidazole substrates (imidazole and **4**), while the bis-imidazole template **5** was retained so strongly that only the leading edge of the eluting peak was observed at ca. 200 column volumes.

The much greater retention of bis-imidazole **5** relative to 1-benzylimidazole **4** (23-fold) or imidazole (33-fold) indicates that the imprinted polymer re-binds its template in a fundamentally different manner. The strategic positioning of two metal ions in a binding cavity produced by the imprinting process would allow simultaneous coordination to both metals by the bis-imidazole template, greatly increasing the strength of its interaction relative to substrates that can bind by coordinating to only one metal ion. Rationally designed bis-metal (mercuric ion) complexes in fact exhibit as high as a 140-fold preference for binding a bis-imidazole over **4** in solution and can discriminate among closely related bis-imidazoles (**5** and **7**) with selectivities greater than 10 [3].

To further reduce the retention of the bis-imidazole substrates to easily measurable levels, zinc acetate was added as a mobile phase competitor. The resolving ability of the molecularly imprinted polymers under these conditions is illustrated in Fig. 6. Polymer-coated silica prepared using template **5** can separate bis-imidazoles **5** and **7** to baseline resolution (Fig. 6a). This column can also partially separate two bis-imidazoles with even more closely related structures, isomers **5** and **6** (Fig. 6b).

Results of a systematic study of retention volumes and chromatographic separation factors (α) for substrates **4–7** on materials prepared using the four different substrates as templates are presented in Table 2. Material prepared using the template that coordinates to a single

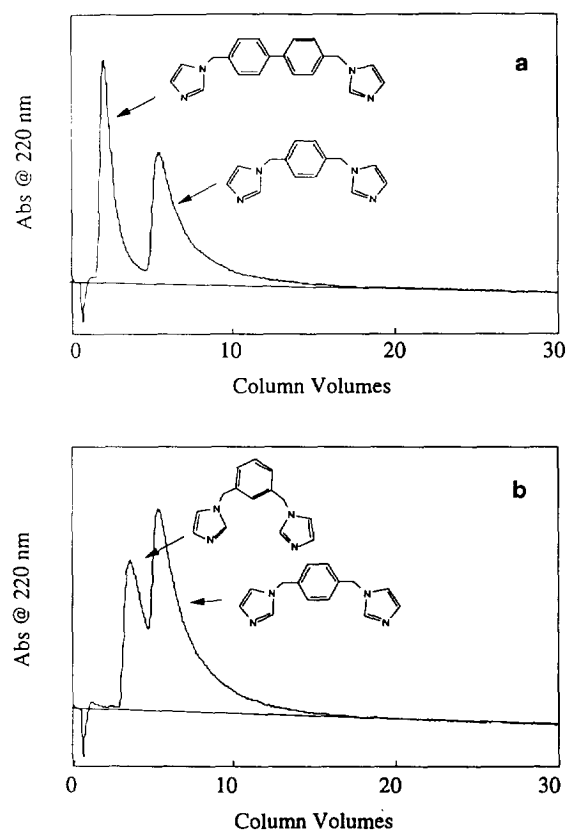

Fig. 6. Chromatographic separation of bis-imidazole substrates on polymer templated with **5**. Running conditions: 50 m*M* zinc acetate, 0.5 ml/min, 65°C; (a) 10 μl of substrates **5** + **7** (0.4 m*M* each in methanol); (b) 10 μl of substrates **5** + **6** (0.4 m*M* each in methanol).

Table 2
Capacity factors (k') and chromatographic separation factors ($\alpha_{i,j}$) for imidazole and substrates **4**–**7** on polymer-coated silicas (LiChrosphere 1000) prepared using **4**–**7** as templates

Substrate	Material prepared using:							
	4		**5**		**6**		**7**	
	k_i	$(\alpha_{4,i})$	k_i	$(\alpha_{5,i})$	k_i	$(\alpha_{6,i})$	k_i	$(\alpha_{7,i})$
Imidazole	0.75	1.16	0.69	8.1	1.5	5.8	1.7	6.6
4	0.87		0.69	8.1	1.7	5.1	1.8	6.3
5	2.9	0.30	5.6		6.6	1.4	7.7	1.5
6	2.6	0.33	3.5	1.6	8.7	–	6.3	1.8
7	2.7	0.32	1.5	3.9	2.9	3.0	11.3	–

Elution volumes (V_e) were measured on 50 × 4.6 mm I.D. columns, 0.5 ml/min 100% methanol, 65°C, with a sample size of 10 μl of 0.4 m*M* solution. The mobile phase contained zinc acetate in the following concentrations: experiments on **4**-templated material, 50 m*M*; **5**-templated, 50 m*M*; **6**-templated, 40 m*M*; **7**-templated, 30 m*M*. $k' = (V_e - V_0)/V_0$, $\alpha_{i,j} = (V_e - V_0)_i/(V_e - V_0)_j$, where V_0 is the column void volume.

metal, **4**, retains the three bis-imidazole substrates **5**, **6**, and **7** to very similar extents. As expected, the binding sites in this "random" material have no basis for discriminating among these compounds. The stronger retention of the bis-imidazoles relative to the template reflects the fact that these substrates have an additional metal-coordinating site (a statistical effect). (Zn^{2+}-acetate only affects the absolute retention of the bis-imidazoles on these materials, and not their elution order.)

As shown in Table 2, when the polymer-coated silica was prepared using bis-imidazole **5**, **6**, or **7** as the template, the template was always the most strongly retained. Thus polymer prepared with template **5** retained **5** more strongly than **6** or **7**, while polymer prepared with **6** retained its template more strongly than **5** or **7**. It is interesting to note that the material prepared with the largest template, **7**, is less selective than the materials made using the smaller bis-imidazoles **5** and **6**: for example, $\alpha_{7,6} = 1.8$ compared to $\alpha_{6,7} = 3.0$. This behavior mirrors that reported previously for competitive re-binding to bulk-polymerized molecularly imprinted materials [3,4]. The binding sites created by template **7** are apparently able to accommodate other, smaller substrates to a greater degree than the sites created by templates **5** or **6** can bind a larger substrate. This leads to a significant size-exclusion effect superimposed on the metal-ion binding.

3.4. Binding heterogeneity in molecularly imprinted polymers

The separations that can be achieved on molecularly imprinted polymers depend strongly on the sample size: the separation factors are highest for very small samples and decrease rapidly with increasing sample size. This behavior very likely results from adsorption to a heterogeneous population of binding sites formed in the imprinted polymer [15,17]. To investigate the nature of substrate binding to molecularly imprinted ligand-exchange materials, equilibrium re-binding studies were carried out on ServaGel particles coated with molecularly imprinted polymer (see Section 2). Heterogeneity is apparent for bis-imidazole re-binding these supports, as shown in the Scatchard plot in Fig. 7a. Polymer templated with bis-imidazole **5** exhibits a significant heterogeneity in re-binding its template. The binding affinity is highest at low loading and decreases dramatically as more sites become occupied. Interestingly, binding heterogeneity is *not* apparent when the same polymer re-binds 1-benzylimidazole (Fig. 7a). Similarly, the bind-

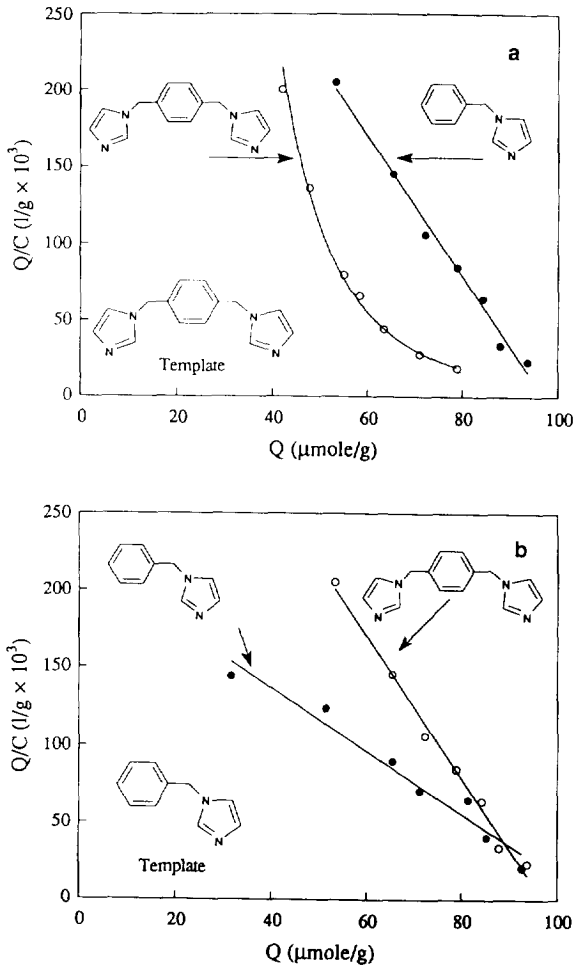

Fig. 7. Scatchard plot of equilibrium binding data for substrates **4** and **5** on molecularly imprinted polymer-coated silicas (ServaGel). Polymerization reaction included monomer at 50 wt% to silica, composition: 5% **1**, 30% methylmethacrylate, 65% EGDMA. Q = concentration of substrate bound (μmol/g), C = concentration of substrate in solution (mM); (a) **4** and **5** on polymer templated with **4**; (b) **4** and **5** on polymer templated with **5**.

ing sites in a "random" polymer [prepared using 1-benzylimidazole (**4**) as the template] appear homogeneous to both the mono- and bis-imidazole substrates (Fig. 7b).

This binding behavior supports the claim that the bis-imidazole-templated polymer recognizes its template by a mechanism different from 1-benzylimidazole (e.g. two-point binding versus one-point binding). While these data indicate that the template has influenced the organization of metal ions in the polymer, they also demonstrate that this imprinting process does not occur with perfect fidelity. While polymer prepared with **5** has a population of sites capable of supporting high-affinity binding, a large number of its sites apparently cannot. Substrate at low concentrations will tend to occupy only the high-affinity sites, leading to better separation for smaller samples.

4. Conclusions

Molecularly imprinted polymers grafted to derivatized silica particles provide composite materials suitable for high-performance ligand-exchange separations of closely related substrates. Strong interactions between the template and Cu^{2+} ions direct the strategic placement of the metal ions in the binding sites via template polymerization. Replacement of the Cu^{2+} with weaker-binding Zn^{2+} after polymerization optimizes the material for separations of metal-coordinating substrates. The ability to substitute metal ions and thereby "tune" the binding function is unique among molecular-imprinting systems. A comparison of molecularly imprinted materials to "rationally designed" metal-complexing receptors indicates that there is significant room for improvement in the binding and selectivities of imprinted materials [1]. These materials are still quite heterogeneous, even though the interactions stabilizing the monomer-template assembly during polymerization are strong relative to other electrostatic interactions commonly used for molecular imprinting [6–8]. Studies of the sources of this heterogeneity and methods for producing more homogeneous materials are underway.

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References

[1] S. Mallik, S.D. Plunkett, P.K. Dhal, R.D. Johnson, D. Pack, D. Shnek and F.H. Arnold, New J. Chem., 18 (1994) 299.
[2] S. Mallik, R.D. Johnson and F.H. Arnold, J. Am. Chem. Soc., 115 (1993) 2518.
[3] S. Mallik, R.D. Johnson and F.H. Arnold, J. Am. Chem. Soc., 116 (1994) 8902.
[4] P.K. Dhal and F.H. Arnold, J. Am. Chem. Soc., 113 (1991) 7417.
[5] P.K. Dhal and F.H. Arnold, Macromolecules, 25 (1992) 7051.
[6] K. Mosbach, Trends Biochem. Sci., 19 (1994) 9.
[7] G. Wulff, in T.T. Ngo (Editor), Molecular Interactions in Bioseparations, Plenum Press, New York, 1993, pp. 363–381.
[8] G. Vlatakis, L.I. Andersson, R. Muller and K. Mosbach, Nature, 361 (1993) 645.
[9] P.K. Dhal, S. Vidyasankar and F.H. Arnold, Chem. Mater., 7 (1995) 154.
[10] O. Nörrlow, M. Glad and K. Mosbach, J. Chromatogr., 299 (1984) 29.
[11] G. Wulff, D. Oberkobusch and M. Minarik, React. Polym., 3 (1985) 261.
[12] A preliminary report of this work appeared in: F.H. Arnold, S. Vidyasankar and S. Plunkett, Polym. Prep., 35 (1994) 996.
[13] S. Plunkett, Ph.D. Thesis, California Institute of Technology, 1995.
[14] B. Sellergren, Makromol. Chem., 190 (1989) 2703.
[15] B. Sellergren and K.S. Shea, J. Chromatogr., 635 (1993) 31.
[16] R.L. Albright, React. Polym., 4 (1986) 155.
[17] K.J. Shea and D.Y. Sasaki, J. Am. Chem. Soc., 113 (1991) 4109.