General Method for Chiral Imprinting of Sol−Gel Thin Films Exhibiting Enantioselectivity

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Chirally imprinted sol−gel thin films were fabricated through the molecular imprinting technique. The films were spin coated on glass plate substrates. Enantioselective discrimination of these films was observed toward three different templates. In each case a different enantiomer pair was selected as a template and a sol mixture was tailored accordingly. By extracting the template molecule a defined chiral cavity was created. The enantiomer pairs were (*R*)- and (*S*)-propranolol, (*R*)- and (*S*)-2,2,2-trifluoro-1-(9-anthryl) ethanol, and D- and L-3,4-dihydroxyphenylalanine (D- and L-dopa). Selective adsorption properties of the resulted films toward the imprinted molecules were measured by radioactive and fluorescence analysis. In all cases, preferred adsorption of one enantiomer was revealed. This preference was due to configuration match between the cavity and the adsorbed molecule. Nonspecific adsorption was remarkably low and was measured for reference nonimprinted films.

Introduction

Preparation of chiral discriminative polymeric materials by the molecular imprinting technique has been extensively researched.[1−9] The imprinted polymers are usually used as chiral stationary phase (CSP) in high-performance liquid chromatography (HPLC)[10−14] and in thin-layer chromatography (TLC).[15] Most of the studies have focused on ground bulk organic polymers, which were packed in a column. However, chiral recognition and separation which is based on bulk polymers suffer from some limitations: (i) long diffusion periods, (ii) unavailability of inaccessible cavities in the bulk, resulting in low specific capacity and, (iii) enantiomeric resolution which is governed not only by the success of the imprinting process, but also by the chromatographic procedure (eluent composition, flow rate, column length, etc.). Molecularly imprinted organic-polymers thin films have generally been shown to be devoid of these disadvantages and therefore have offered an attractive alternative.[16−19] In an earlier report in this journal[20] we opted for the sol−gel (SG) materials alternative, motivated by several potential advantages it offers over the organic-polymer thin films. These advantages include simple preparation at room temperature, which this materials methodology entails, rapid condensation followed by solvent evaporation during the coating process, and the availability of a large library of functional trialkoxy-silanes needed for successful imprinting.[21,22] Indeed, in that study,[20] which compared two thin film polymeric systems, organic (acrylic) and metal oxide inorganic (SiO_2 based SG), for imprinting of racemic propranolol (**1**, Figure 1) the latter proved to offer higher affinity to the imprinting molecule, higher selectivity (low nonspecific binding), and faster kinetics of adsorption. Specific alkyl trialkoxy silane functional monomers were used (to be described below) with which the complementary types of chemical interactions to the template were introduced into the film, which was uniform, thin, and porous.

Whereas the bulk of the study (detailed in ref 20) was devoted to the imprinting of racemic **1**, in a Note-Added-

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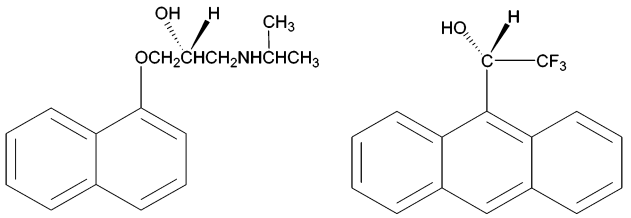
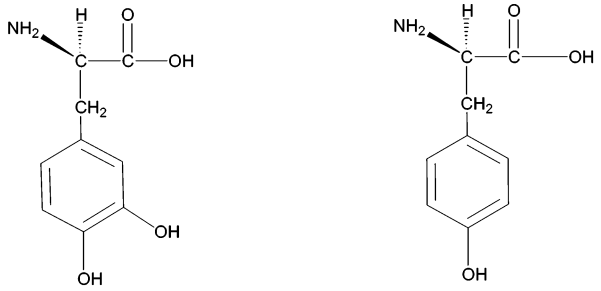


(R)-**1** (S)-**2**



D-**3** D-**4**

Figure 1. Chemical structure of template molecules: **1**, propranolol; **2**, 2,2,2-trifluoro-1-(9-anthryl) ethanol; **3**, dihydroxyphenylalanine; and **4**, tyrosine.

in-Proof we reported a chirally imprinted SG thin film capable of discriminating between (*R*)-**1** and (*S*)-**1**. As we show here, that preliminary observation turned out to be what we believe is the first general methodology for preparation of chirally imprinted SG thin film. It is general in the sense that it demonstrates how to tailor the SG mixture of monomers to the various moieties of the imprinting molecule. Thus, after thoroughly extending the (*R*)-**1**/(*S*)-**1** study, we demonstrate the methodology on two additional chiral pairs: the successful chiral imprinting with (*R*)- and (*S*)-2,2,2-trifluoro-1-(9-anthryl) ethanol, ((*R*)-**2** and (*S*)-**2**), and with D- and L-3,4-dihydroxyphenylalanine (D- and L-Dopa respectively, D-**3** and L-**3**). With the latter we moved also toward showing that D- and L-chiral cavities can be used for the discrimination of related molecules to the imprinting one, namely L-tyrosine. Each of the three template molecules required the tailoring of a specific matrix by choosing suitable functional silicon alkoxide monomers to create a well-defined cavity from the structural and interactive points of view (Figure 2), as detailed below. We showed for all three templates that this cavity formation, which is a negative transcription of the template molecule, preserved its configuration when the template molecule was removed, and that the resulting cavity was sensitive enough to recognize either the chiral template again or a close derivative of it even after four subsequent adsorption cycles, and that the formed cavity was capable of discriminating the template from its enantiomer. Thus, the films we report exhibit chiral cavities with enantioselective properties.

It is of relevance to this report to mention other studies where enantioselectivity between pairs of enantiomers was achieved with SG materials.[23−27] The earliest report of chirally imprinted chromatographic silica is apparently due to Curti et al. in 1952,[28] followed by studies of Erlenmeyer et al. and Beckett et al. during 1960−1966.[29−31] More recent studies include the preparation of 80-microns-thick imprinted titania layers for specific discrimination of three chiral carboxylic acids derivatives,[32] and titania films for the discrimination of some chiral carbobenzyloxy amino acids derivatives.[33] Finally, we mention several studies aimed at introducing chirality into SG materials. These include the entrapment of chiral molecules,[34−38] the chiral imprinted silica particles for catalysis,[39,40] the use of chiral gelators which resulted in helical silicas (on a micron scale)[41−44] and the use of chiral trialkoxy silanes for enantioselective adsorption[45] and for catalysis.[46−48]

Experimental Details

Reagents. Tetramethoxysilane (TMOS) 99+%, tetraethoxysilane (TEOS) 99+%, phenyl trimethoxysilane (PTMOS) 97%, methyl trimethoxysilane (MTMOS) 98%, 3-aminopropyltriethoxysialne (APTES) 99%, (*R*)- and (*S*)-propranolol hydrochloride (**1**-HCl), (*R*)- and (*S*)-2,2,2-trifluoro-1-(9-anthryl)-ethanol (**2**), D- and L-3,4-dihydroxy phenylalanine (D- and L-Dopa, **3**) were purchased from Aldrich. ^{3}H-(*S*)-Propranolol ((*S**)-**1**), 21 Ci/mmol, was from NEN. L-[3,5-^{3}H]-tyrosine (L*-**4**) 49 Ci/mmol was from Amersham Pharmacia Biotech. Ecoscint H (National Diagnostics, Atlanta, GA) was used as scintillation cocktail. Basic phosphate buffer (BPB, 10 mM, pH 7.6) and acidic phosphate buffer (APB, 10 mM, pH 4.0) were prepared from KH_2PO_4 and K_2HPO_4.

Propranolol Imprinted Sol−Gel Film (SG-1). The HCl salts of (*R*)-**1** and (*S*)-**1** were first converted to their free amine form by dissolving 1.0 g of **1**-HCl in 10 mL of 10% NaOH and

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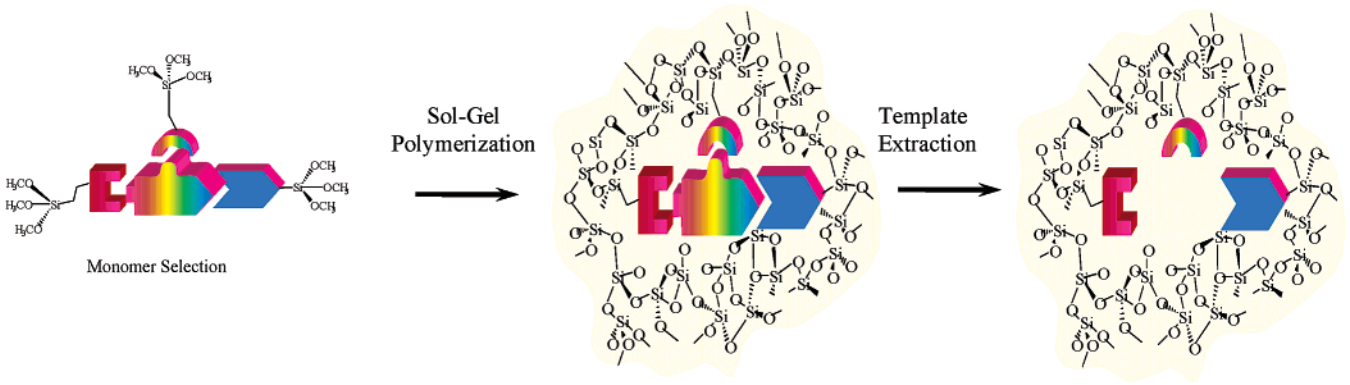

Figure 2. Scheme of formation of chiral cavity in a sol−gel matrix.

20 mL of chloroform. The free base **1** was extracted into the organic phase, which was separated and dried over $MgSO_4$, and then the solvent was evaporated. The monomer mixture for the thin film preparation consisted of 3.0 mL (20.3 mmol) of TMOS, 0.37 mL (1.98 mmol) of PTMOS, 0.3 mL (2.1 mmol) of MTMOS, 3.0 mL of ethoxyethanol, 1.0 mL of H_2O, and 1.0 mL of 0.1 M HCl. The final total water/total silane ratio was 3.4:1. After 5 h of mixing at room temperature, 1.0 mL of this solution was mixed with a solution of 5.0 mg of (*R*)-**1** or (*S*)-**1** dissolved in 25 μL of 0.1 M HCl and 50 μL of ethoxyethanol. The original solution (without the enantiomers) was used for nonimprinted SG films. Glass plates (BDH, thickness no. 1, 12-mm diam.) were spin coated (EC101D, Headway Research Inc.) by placing 30 μL of the sol on the plate and spinning for 20 s at 4000 rpm. All plates were allowed to polymerize and dry overnight in a covered Petri dish. The thickness of the film was 735 ± 3 nm, as determined with a Filmetrics F-20 reflectance spectroscopy instrument.

To verify that the imprinted and nonimprinted film were of a similar porosity, we examined a TMOS-only based film that contained no functional monomers for its ability to selectively bind propranolol. The film bound nonselectively less than 0.1 nmol of either enantiomers of propranolol.

2,2,2-Trifluoro-1-(9-anthryl)ethanol Imprinted Sol−Gel Film (SG-2). TEOS (3.0 mL, 13.5 mmol), 0.2 mL (1.1 mmol) of PTMOS, and 3.0 mL of ethanol were mixed. A 0.1-mL portion of concentrated HCl, 0.2 mL (0.84 mmol) of APTES, and 1.0 mL of H_2O were added dropwise in this order (water/silane ratio 3.8:1). The mixture was stirred at room temperature for 20 h. For imprinting, 2.0 mL of this sol was mixed with 200 μL of 0.1 M solution of (*S*)-**2** in ethanol and stirred for an additional 4 h at RT. Spin coating and drying processes were similar to those reported above for SG-**1**. The resulting film was 636 ± 2 nm thick. To confirm that the TEOS-imprinted and reference film are of similar porosity, another control experiment was carried out, in which the above-mentioned TEOS-based monomer mixture was imprinted with (*S*)-naproxene, which is very similar in size to the anthracene derivative, while **2** was used as the binding probe. We have found that no (*S*)-2 or (*R*)-2 bound to the naproxene-imprinted film, although the porosity is similar.

Dopa-Imprinted Sol−Gel Film (SG-3). A 2.0-mL (13.5 mmol) aliquot of TMOS was mixed with 0.5 mL (2.67 mmol) of PTMOS, 2.0 mL of ethoxyethanol, 0.66 mL of 0.1 M HCl, and 0.66 mL of H_2O (water/silane ratio 3.4:1). After the mixture was stirred at RT for 2 h, 1.0 mL was mixed with 100 μL of ethanolic solution of D- or L-Dopa (0.1 M) for an additional 2 h. Spin coating and drying procedures were preformed in a manner similar to that for SG-**1**. The thickness of the film was 812 ± 2 nm.

Template Extraction. For extraction of the template in order to obtain the chiral cavities, all glass plates (including the nonimprinted) were rinsed repeatedly with 15 mL of methanol (for extraction of **1**) or ethanol (for extraction of **2** or **3**) for 24 h in a rotating shaker. After extraction, the amount of extracted molecules was determined from fluorescence measurements (PTI−Photon Technology International fluorimeter) at the suitable spectral regions (λ_{ex} = 288, 366, and 278 nm, and λ_{em} = 335, 431, and 308 nm, for **1**, **2**, and **3**, respectively). Extraction was continued until the fluorescence signal measured for the extracting solvent was negligible.

Binding Experiments Analyzed by Fluorescence. The extracted SG-**1** or SG-**2** coated glass plates were incubated in 2.0 mL of a 1.0 μM solution of the proper enantiomer in basic phosphate buffer (BPB) for 24 h at 25 °C. The plates were then rinsed with BPB followed by extraction of the bound species into 3.0 mL of APB for SG-**1**, or ethanol for SG-**2,** carried out for 2 h at 25 °C. The concentrations of the extracted solutions were determined by fluorescence measurement at the wavelengths indicated above, using calibration curves. The fluorescence intensity of **3** is too weak for this type of analysis, and binding was determined as described next.

Binding Experiments Using Radioactive Labels. *Assay 1 for SG-1.* The template-extracted SG-(*S*)-**1** film and its nonimprinted reference film were incubated for 24 h at 25 °C in a 2.0-mL solution of 5.0 μL *S**-**1** in 100 mL solution of 10.0 μM of either (*S*)-**1** (a (*S* + *S**)-**1** solution) or (*R*)-**1** (a (*R* + *S**)-**1** solution). After incubation the films were rinsed thoroughly with BPB solution to remove nonspecifically adsorbed **1** molecules, and then mixed for 1 h in plastic vials containing 15 mL of scintillation cocktail. Measurement of β-emission (in disintegration per minute (DPM) units) of adsorbed **1** was carried out by a LKB Wallac 1214 β-counter. Four films were used for each data point.

Assay 2 for SG-3. The template-extracted SG-L-3 and SG-L-3 films and the nonimprinted film were incubated in a 2.0-mL solution of 50 nM L*-4 in BPB. After incubation, the plates were rinsed thoroughly with BPB and analyzed as in Assay 1.

Results and Discussion

Imprinting Process. In this study we examined the possibility of creating specific chiral cavities in thin films of sol−gel material. The sol−gel process is a convenient way to get transparent, smooth, homogeneous, and porous films with facile control of thickness. The molecular imprinting technique was used to create the chiral cavities that were subsequently used to discriminate between enantiomers. A basic requirement for successful molecular imprinting is the selection of suitable functional silane monomers that can create the molecular recognition site by interacting in noncovalent fashion with the template molecule and maintain their shape when extracted after polymerization (Figure 2).

Monomers Selection for Molecular Imprinting. Prior to the actual preparation of the imprinted film, a design stage is necessary. For each molecule a specific sol mixture was tailored which consisted of a combination of organosilane monomers according to the structure and features of the template molecule including

polarity, hydrophobicity, and acidity. By careful tailoring of specific functional monomers to the designed template, the resulting cavity will bear the negative three-dimensional structure and chemical functions situated in predefined locations. One should bear in mind that in addition to the silane residues, the imprinting process also uses the hydrophilic vicinal and geminal silanols (along with strongly adsorbed water molecules) and hydrophobic siloxanes, both of which are products of TEOS and TMOS hydrolysis and polycondensation. The question then is whether these two functional groups can stand alone for imprinting purposes. The early work[28−31] of preparation of what was known in the 1950s and 1960s as "special silicas" indeed relied on these two functionalities alone, but the scope of that approach was quite limited, and never caught up. Modern imprinting studies pointed to the importance of tailoring additional functionalities, more specifically selected to suit the templating molecule. Thus, although TMOS or TEOS were used as the main component (85% mol of silane mixture) the remaining 15% were other functional silane monomers that were selected as detailed next.

When inspecting template **1**, the functional groups on this molecule that one can use as anchors are the aromatic naphthyl group, the ether link, the alcohol residue, the amine, and the alkyl groups. Thus, the monomers selected were TMOS, providing the SiOH group needed for both hydrogen bonding (with the CH−OH residue) and, through the acidity (p*K*i of **1** = 9.2),[49] for the acid/base interaction with the amine; TMOS, again, providing the hydrophobic ether-like Si−O−Si bond (for interaction, e.g., with the Ar−O−R moiety); PTMOS, providing the phenyl group for $\pi-\pi$ interactions with the naphthyl residue; and MTMOS, providing added hydrophobicity through its methyl group. For instance, in the work portrayed in ref 20, 13 different combinations of methacrylic acid with different cross linking agents (ethylene glycol dimethacylate and trimethylol trimethacrylate) and concentrations, as well as other functional monomers (hydroxyethyl metacrylate and acrylamide) were considered. Similarly, for the preparation of propranolol imprinted sol−gel films, TEOS was considered as the main monomer, as well as other functional silanes such as butyl triethoxysilane as the hydrophobic element. Similar considerations led to the selection of monomers for **2** which included, in addition to TEOS and PTMOS, the basic APTES for interaction with the acidic hydroxyl residue of **2**. For the imprinting of **3**, two main functions were recognized: the phenyl ring and the chiral center that bears a typical amino acid carbon with the amine and carboxyl groups. Therefore, SG-**3** consisted of TMOS and PTMOS in order to promote $\pi-\pi$ interactions with the aromatic ring. Because **3** is a zwitterion, we did not use acidic or basic silane monomers in the sol mixture, and only PTMOS was employed as a functional monomer. Below we shall see that these specific monomers selections carried out their potential to discriminate between the two enantiomers through imprinting. To promote chiral selection, chiral additives such as chiral alcohol as cosolvent or chiral silane as functional monomer, were tested. For example, combinations consisting of (*R*)- and (*S*)-*N*-1-phenylethyl-*N*′-triethoxysilylpropylurea or (*S*)-2-methyl-1-butanol were examined, but proved to be not contributing to chiral recognition.

Finally, we note that because the different monomers tend to hydrolyze at different rates, premixing and partial prehydrolysis were necessary before adding the template and before the onset of the polycondensation. Generally, TMOS was preferred to be the main component in the initial sol mixture as its hydrolysis is faster. But when APTES was added to the sol mixture, the condensation was accelerated and therefore TEOS was employed as the main silane component. However, one should note that for SG-**2** the template molecule was added to the sol mixture after 20 h of monomer premixing, because of TEOS slow hydrolysis.

Template Extraction, Binding, and Recognition Measurements. One of the basic assumptions of the imprinting process is the feasibility of extraction of the template molecule after the polymerization is completed. In practice, the extraction step proved to be far from trivial and became a rather tedious step. Because the sol−gel matrix underwent some shrinkage during the drying step, the template molecules remained entrapped and required repeated solvent extractions until the resulting imprinted matrix was free of the template.

Selective adsorption measurements were carried out to estimate the efficiency of chiral discriminating ability of the SG imprinted films. The evaluation of the amount of adsorbed molecules onto the templated films was then determined in two ways: (a) by fluorescence analysis of extracted template molecules from the imprinted cavities; and (b) by direct radioactive assay of the films after adsorption. When the adsorption was detected by fluorescence, pure enantiomer solutions were employed, whereas in the radioactive measurements radio-labeled *S**-**1** enantiomer was mixed with either nonlabeled *S*-**1** or *R*-**1** as described above. For radioactive detection of **3**, pure enantiomer solution of L*-**4** was used. In all cases, control experiments were carried out according to the customary procedure in the literature[22,50−53] by performing the same experiment on nonimprinted films, which were prepared from the same solution without the template molecule.

Propranolol Enantioselective Sol−Gel Films. The chiral discriminative recognition ability of the extracted, two enantiomeric SG-**1** films was evaluated by two methods: the (indirect) fluorescence method and the direct radioactive assay. By the fluorescence method, the (*S*)- and (*R*)-imprinted films were exposed to solutions of either (*R*)-**1** or (*S*)-**1**. Both specific and nonspecific adsorption take place at this stage. The adsorbed **1** (both specific and nonspecific) was then liberated by extraction into APB, and its amount was determined by fluorescence. Figure 3 shows that the selection of SG-monomers, as described above, has achieved the goal. The (*S*)-imprinted film recognizes (*S*)-**1** better than it recognizes (*R*)-**1** (0.22 ± 0.02 nmol of (*S*)-**1** vs 0.14 ± 0.01 nmol of (*R*)-**1**); and the (*R*)-imprinted film recog-

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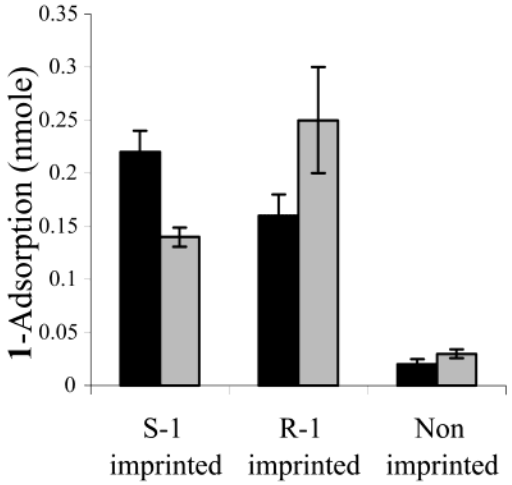

Figure 3. Fluorescence assay for (*R*)-**1** (gray bars) and (*S*)-**1** (black bars) adsorption to (*R*)-**1** and (*S*)-**1** imprinted SG thin films ($\lambda_{ex} = 288$ nm, $\lambda_{em} = 335$ nm) after incubation period of 24 h at 25 °C, in 1 μM pure enantiomer solution in BPB (10 mM, pH 7.6) and extraction into APB (10 mM, pH 4). Nonimprinted films serves as a control ($n = 4$).

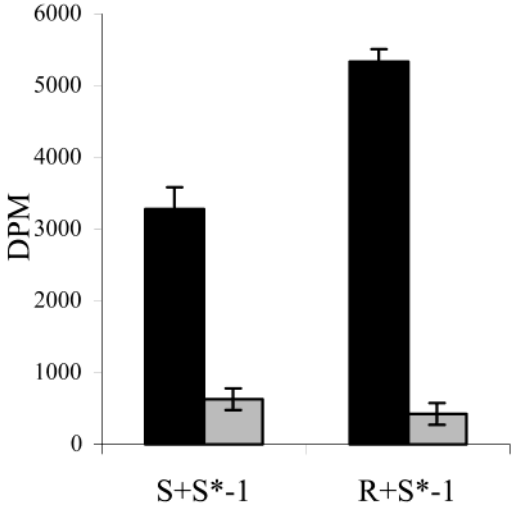

Figure 4. ^{3}H-(*S*)-Propranolol (*S**) and (*S*)-**1** or (*R*)-**1** (*S* + *S**, *R* + *S**, respectively) binding to (*S*)-**1** imprinted (black bars) and control nonimprinted films (gray bars). Incubation conditions: 24 h at 25 °C, in 2 nM radiolabeled ^{3}H-(*S*)-propranolol with either (*R*)-**1** or (*S*)-**1** solution in BPB (10 mM, pH 7.6) ($n = 4$).

nizes (*R*)-**1** better than (*S*)-**1** (0.25 ± 0.05 nmol of (*R*)-**1** vs 0.16 ± 0.02 nmol of (*S*)-**1**). Note that the behavior of the (*S*)-imprinted and (*R*)-imprinted films nearly mirror each other, thus confirming the authenticity of the observation. The adsorption equilibrium discrimination ratio is therefore about 1.6. Although this enantioselectivity value is not high compared to other chiral imprinting studies,[1] we bring this number into context by recalling that in chiral chromatography (a nonequilibrium process) an α (relative retention) factor of 1.1 already suffices for carrying out successful baseline enantiomeric separation.

Another interesting observation is that the imprinted **1**-films adsorb not only the imprinted enantiomer but the opposite enantiomer as well (Figure 3). The nonspecific adsorption, which originates from all of the interactions with all functional surface groups described above, contribute to the adsorption on the nonimprinted films of a negligible amount of 0.020 ± 0.005 nmol and 0.030 ± 0.004 nmol for (*S*)-**1** and (*R*)-**1**, respectively. And yet, as seen in Figure 3, the amount of (*R*)-**1** that was adsorbed on the (*S*)-imprinted film and the amount of the (*S*)-**1** adsorbed onto the (*R*)-film, by far surpass the nonspecific adsorption values. We have therefore an intermediate situation here which can be rationalized as follows. During the imprinting process, the imprinting molecule attracts around it all of the various functional moieties from the various monomers, to maximize the noncovalent interactions that are possible in all binding mechanisms ($\pi-\pi$, H-bonds, etc.) Therefore, the formed cavity is different from a general, nonimprinted adsorption site in the film in that one does not expect to have in such a general site the collection of all needed functionalities in that right proportion. Thus, when an (*R*)-**1** molecule approaches an (*S*)-**1** imprinted surface, it is within the (*S*)-cavities that the (*R*)-molecule will find a rich collection of relevant adsorption moieties with which it can interact, at least in part, and with a less-than-ideal geometric fit. The bottom line is that in addition to the discriminative power of the films, they also act as very good adsorption materials for either one of the enantiomers, compared to the nonimprinted films. To verify these results, we also performed radioactive analysis, which is quite different from the fluorescence analysis.

Extracted (*S*)-**1** imprinted and nonimprinted films were exposed to solutions of two enantiomeric mixtures, (*S* + *S**)-**1** and (*R* + *S**)-**1** (at *S*/*S** and *R*/*S** ratios of ca. 5000), and the bound amount was evaluated by direct analysis of the films, as described in the Experimental Section. The results (Figure 4) were 3300 ± 300 DPM for (*S* + *S**)-**1** and 5330 ± 175 DPM for (*R* + *S**)-**1**. The value for (*S* + *S**)-**1** indicates only the *S**-**1** adsorption; in order to evaluate the total (*S* + *S**)-**1** adsorption, this value must be multiplied by 5000 because the cavity affinity to (*S*)-**1** and (*S**)-**1** is the same, and therefore the distribution between adsorbed (*S*)-**1** and (*S**)-**1** reflects their solution ratio. The higher value of adsorbed (*S**)-**1** that is obtained from the (*R* + *S**)-**1** mixture reflects the enantioselectivity of the (*S*)-film toward its own template. The enantioselectivity is 1.6, which is in remarkable agreement with the fluorescence experiment (same value). The nonspecific adsorption was measured by incubating nonimprinted films in the same solutions. This adsorption is low in comparison to the specific adsorption: 650 ± 150 DPM and 300 ± 150 DPM for (*S* + *S**)-**1** and (*R* + *S**)-**1** solutions, respectively.

As mentioned above, the chiral adsorption capacity of **1**-imprinted films can be derived from the ratio of the labeled and nonlabeled species in the incubation solution and from the *S**-**1** adsorption value from (*S* + *S**)-**1** solution. By translating the capacity to nmol units, the chiral capacity is 0.35 nmol, this value is compared to the fluorescence measurement results (0.25 nmol). The radioactive measurement is a direct method, because the total amount of the adsorbed species is detected, and therefore it is expected that the achieved value is higher than the value found in fluorescence results in which the detection was indirect and relied on extraction of bound species.

Enantioselective Adsorption Cycles with the (*S*)-Trifluoroanthrylethanol Sol−Gel Imprinted Film.

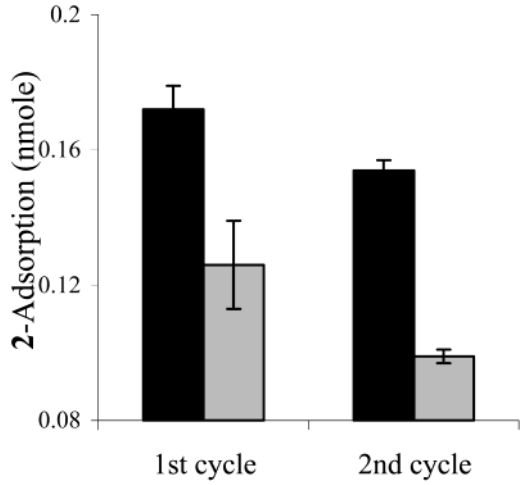

Figure 5. Fluorescence results of (*R*)-**2** (gray bars) and (*S*)-**2** (black bars) adsorption to (*S*)-**2** imprinted films ($\lambda_{ex} = 366$ nm, $\lambda_{em} = 408$ nm, 431 nm). In both cycles the films were incubated alternately in (*R*)-**2** and (*S*)-**2** solution. Films were incubated for 24 h at 25 °C, in 1 μM pure enantiomer solution in BPB (10 mM, pH 7.6) followed by extraction to ethanol and fluorescence assay ($n = 7$).

Evaluation of the enantioselectivity of the **2**-imprinted film was carried out by fluorescence spectroscopy, along lines similar to the analysis of the **1**-imprinted films. Two main issues were associated with this template: first, to test whether the chiral imprinting approach worked with a completely different molecular structure (compare **1** with **2**); and to test whether the same film kept its enantioselectivity in repeated cycles of recognition analysis. Thus, (S)-**2** imprinted film was first incubated in a solution of (R)-**2** and the adsorption determined as describe above. Then the film was cleaned and exposed to a solution of (S)-**2** and the adsorption determined again. This set of experiments was the 1st adsorption cycle, which was repeated again in reverse order (the films were first exposed to (S)-**2** solution) to give the 2nd adsorption cycle. In total the films were exposed to four pure enantiomer solutions alternately, which gave 2 adsorption cycles. As shown clearly in Figure 5 the chiral imprinting was successful, and the adsorption of (*S*)-**2** was found to be favored over (*R*)-**2** in both adsorption cycles; and it is evident that the film retains its enantioselectivity and that the order of adsorptions was insignificant. Coincidentally the enantioselectivity factor (1.4 in the first cycle and 1.5 in the second) is similar to the recognition factor for **1** (1.6). The measured adsorption values of (*S*)-**2** imprinted sites were 0.172 ± 0.007 nmol of (*S*)-**2** and 0.123 ± 0.013 nmol of (*R*)-**2** in the first adsorption cycle, and 0.154 ± 0.003 nmol of (*S*)-**2** versus 0.099 ± 0.002 nmol of (*R*)-**2** for the second cycle. Finally, the background nonspecific adsorption was again negligible: 0.008 ± 0.002 nmol of both enantiomers to nonimprinted film. As shown in Figure 5, the binding yield of the second cycle is a little smaller than that of the first cycle, however, the enantioselectivity is better. This may arise from slight deterioration of the film after several cycles of binding experiments.

Chiral Recognition of Tyrosine by Dopa Cavities. In the previous examples, a specific form of diastereoisomerism was tested where the diastereoisomers are adsorption complexes between a chiral cavity and a chiral adsorbate. Four combinations exist, (*S*)-cavity/(*S*)-adsorbate, (*R*)-cavity/(*R*)-adsorbate, (*S*)-cavity/(*R*)-adsorbate, and (*R*)-cavity/(*S*)-adsorbate, where the first pair of diastereomers and the last pair of diastereomers are each a pair of enantiomers. This diastereoisomerism is in fact the basis of enantioselectivity. However, as in chiral chromatography, there is nothing inherent in chiral imprinting that requires that the selector (the cavity, in our case) and the adsorbate will be of the same molecular structure, and the above diastereoisomeric complexes may be composed of a cavity formed by one molecule and an adsorbate that is another structurally similar molecule. Surprising as this may sound, chiral-imprinting studies have traditionally focused on recognition of the imprinting molecule and not on the recognition of related molecules. Thus, the next issue we set up to test has been this possibility of chiral recognition, which was carried out as follows.

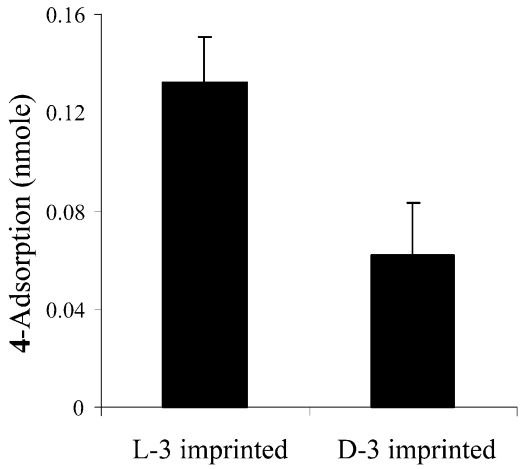

Figure 6. L-[3,5^{3}H]-Tyrosine specific adsorption to D and L-Dopa imprinted thin films. Incubation conditions: 24 h at 25 °C, in 50 nM radio-labeled L-[3,5^{3}H]-Tyrosine in BPB (10 mM, pH 7.6) ($n = 4$).

D and L-Dopa (**3**) were prepared as detailed in the Experimental Section. Then D-imprinted film and L-imprinted film were exposed to 50 nM of radio-labeled L-[3,5^{3}H]-tyrosine (L*-**4**) solutions. As shown in Figure 6, L-**3**-imprinted films adsorbed ca. twice the amount of L*-**4** compared to the D-**3**-imprinted film (0.13 ± 0.02 and 0.06 ± 0.02 nmol, respectively), with nonspecific binding of L*-**4** on the nonimprinted films of 0.028 ± 0.001 nmol. Thus, the diastereomeric distinction between the L-**3**(cavity)/L*-**4** and the D-**3**(cavity)/L*-**4** complexes has demonstrated that, in principle, chirally imprinted films may be developed into more general chiral selectors.

Conclusion

We have demonstrated the chiral imprintability of submicron composite sol−gel films, and their enantioselectivity under steady-state conditions. Three systems were tested, with which two key analytical tools were used − fluorescence and radio labeling − where in each system different aspects of the imprinting were tested. In the propranolol (**1**) system, all four possible diastereomeric adsorption complexes were compared as well as the two analytical methods. With the anthracene derivative (**2**) system, recyclability of the sol−gel films was demonstrated, and with Dopa (**3**) and tyrosine (**4**),

recognition of one molecule by the template cavity of another was demonstrated.

We showed that the sol−gel matrix is an excellent substance for tailoring specific film-adsorption and separation characteristics via molecular imprinting. The porosity, homogeneity, ease of fabrication, and stability are just some of its advantages. As reported in a previous study,[20] molecular imprinting of SG thin film is advantageous over acrylic polymer, not only because its facile preparation, but also due to better kinetics, low nonspecific adsorption, and high association constants. Adding to it the large library of existing trialkoxy-silane derivatives make the SG chiral-imprinting alternative for thin films an attractive approach. Further developments of this methodology are in progress and will be reported in subsequent publications.

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