Molecular Imprinting in Thin Films of Organic−Inorganic Hybrid Sol−Gel and Acrylic Polymers

Sharon Marx* and Zvi Liron

Department of Physical Chemistry, Israel Institute for Biological Research, Ness-Ziona, 70450 Israel

Received November 28, 2000. Revised Manuscript Received March 28, 2001

The molecular imprinting technique was applied on a model compound, propranolol, using two polymeric systems, acrylic and hybrid organic−inorganic sol−gels. The polymers were applied as thin films on glass substrates. The preparation of thin films of imprinted acrylic polymers required the development of a new polymerization system. The binding properties of the two polymers toward propranolol were characterized by radioactive and fluorimetric assay procedures. The acrylic system was found to have high uptake toward propranolol, but this was accompanied by a high degree of nonspecific binding. The sol−gel system had lower uptake, but remarkably lower nonspecific binding (<10%). The K_d of the sol−gel matrix to propranolol is 80 ± 6 nM, a value that is common in biological systems. The binding was found to be solvent sensitive—with high affinity and specificity in aqueous solution, which was completely lost in organic solvents. The uptake kinetics of the acrylic polymer was significantly slower than the sol−gel polymer, reaching saturation after 10 h, relative to <1 h for the sol−gel polymer. Imprinting of the sol−gel film with enantiomerically pure (S)-propranolol resulted in its pronounced chiral recognition over the (R)-enantiomer.

Introduction

The development of imprinted polymer films for various purposes (other than selective solid support for chromatography) has attracted a lot of interest recently. The role of imprinting polymers as a receptor-mimetic has been established in areas such as assay replacement,[1] antibody mimics,[2] selective transport,[3] and biosensors.[4,5]

One of the most appealing applications developed in the area of molecular imprinting is the use of the polymers as recognition elements in biosensor devices. Normally, a sensing element such as an antibody or receptor is immobilized on the surface of the sensor. A selective chemical signal, resulting from the binding process of the analyte to the recognition element, is subsequently transduced into an electrical signal, amplified, and converted to a measurable format. A characteristic of these devices is the close proximity between the sensing part and the transducing element. The possibility of substituting natural sensing elements with molecularly imprinted polymers has a number of potential advantages, similar to those found in the aforementioned antibodies. The use of imprinted films in sensor technology is quite advantageous for bioreceptors because the polymer possesses high physical and chemical stability with respect to immobilized biomaterials (enzymes and antibodies) as the recognition part. Also, when the target molecule has no biomaterial available as the recognition component, the imprinted polymer provides an excellent (and often the only) solution.[6−8] Bulk imprinted polymers have been employed in sensors as porous materials[9,10] but this design suffered from long diffusion times of the target molecule into the polymer. In recent years, several groups have dealt with the application of imprinted films as recognition layers applied on various transduction systems, for example, piezoelectric,[11,6,12] amperometric,[13,14] SPR,[15] fluorimetric,[16,17] and field effect transistors (FET).[18]

* To whom correspondence should be addressed. E-mail: marx@iibr.gov.il. Fax: +972−89381743.

(1) Haupt, K.; Mayes, A. G.; Mosbach, K. *Anal. Chem.* **1998**, *70*, 3936−3939.
(2) Sellergren, B. *Trends Anal. Chem.* **1997**, *16*, 310−319.
(3) Mathew-Krotz, J.; Shea, K. J. *J. Am. Chem. Soc.* **1996**, *118*, 8154−8155.
(4) Claycomb, R. W.; Delwiche, M. J. *Biosens. Bioelectron.* **1998**, *13*, 1173−1180.
(5) Haupt, K.; Mosbach, K. *Chem. Rev.* **2000**, *100*, 2495−2504.
(6) Lee, S.-W.; Ichinose, I.; Kunitake, T. *Langmuir* **1998**, *14*, 2857−2863.
(7) Dickert, F. L.; Besenbock, H.; Tortschanoff, M. *Adv. Mater.* **1998**, *10*, 149−151.
(8) Sasaki, D. Y.; Alam, T. M.; Assink, R. A.; Daitch, C. E.; Ashley, C. S.; Brinker, C. J.; Schoeniger, J. S.; Roe, D. C.; Pohl, P. I.; Rush, D. J.; Shea, K. J. *Sandia Techn. Rep. SAND97−1156* **1997**, 1−22.
(9) Kriz, D.; Kempe, M.; Mosbach, K. *Sensors Actuators B* **1996**, *33*, 178−181.
(10) Kriz, D.; Ramstrom, O.; Svensson, A.; Mosbach, K. *Anal. Chem.* **1995**, *67*, 2142−2144.
(11) Kugimiya, A.; Takeuchi, T. *Electroanalysis* **1999**, *11*, 1158−1160.
(12) Lee, S.-W.; Ichinose, I.; Kunitake, T. *Chem. Lett.* **1998**, 1193−1194.
(13) Wang, J.; Pamidi, P. V. A.; Rogers, K. R. *Anal. Chem.* **1998**, *70*, 1171−1175.
(14) Makote, R.; Collinson, M. M. *Chem. Mater.* **1998**, *10*, 2440−2445.
(15) Lai, E. P. C.; Fafara, A.; VanderNoot, V. A.; Kono, M.; Polsky, B. *Can. J. Chem.* **1998**, *76*, 265−273.
(16) Turkewitsch, P.; Wandelt, B.; Darling, G. D.; Powell, W. S. *Anal. Chem.* **1998**, *70*, 2025−2030.
(17) Jenkins, A. L.; Uy, M.; Murray, G. M. *Anal. Chem.* **1999**, *71*, 373−378.

10.1021/cm000983z CCC: $20.00
Published on Web 07/31/2001

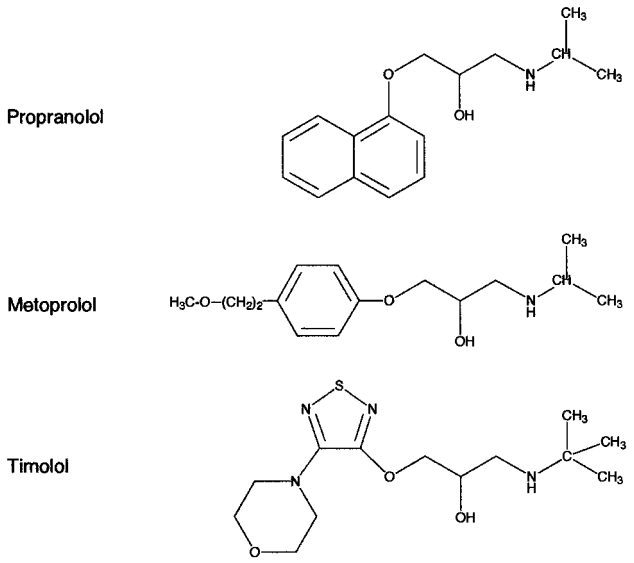

Figure 1. Chemical structure of the β-blockers used in this study

The major obstacle lies in transferring the imprinting procedure from bulk into thin film. This obstacle has been addressed by a number of investigators,[3,15,19,20] but the need to develop a general approach for imprinted thin films in the most popular matrix for molecular imprinting, the methacrylic acid−ethylene glycoldimethacrylate (MMA-EGDMA), and other matrixes still remains.

The fabrication of thin films of other matrixes of cross-linked imprinted polymers, like sol−gel,[21,22] and polyurethane[7] is well established, mainly because the polymerization occurs spontaneously. Radical polymerization of acrylic monomers is dependent not only on the initiating stimulus (light/heat) but also on the absence of radical quenchers, pressure, and solvent. In the present work, two polymeric systems were developed bearing molecularly imprinted sites toward the β-blocker propranolol. Andersson was the first to report on the imprinting of propranolol in bulk acrylic polymers,[23] and others have attempted the imprinting of propranolol or other β-blocker drugs (Figure 1) for chromatography[24−26] and solid-phase extraction.[27,28] The issue of propranolol imprinting in thin polymeric films has been less explored. Haupt and Kutner have achieved the fabrication of a 2-μm film of cross-linked acrylic polymer on a gold surface, with a somewhat cumbersome method.[29]

In this paper, we show the molecular imprinting of propranolol in thin films of two different polymer matrixes: the hybrid organic−inorganic sol−gel system[30] and the acrylic system. A novel facile method for the fabrication of cross-linked acrylic thin films of imprinted polymers is also reported. The sol−gel system was further explored to determine the association constant of propranolol to the sol−gel matrix, to study selectivity with other β-blockers, and to determine solvent and pH effects on propranolol binding.

Materials and Methods

Reagents. Methacrylic acid (MAA) and ethylene glycoldimethacrylate (EGDMA) were from Aldrich and were cleaned prior to polymerization by passing on an inhibitor-removing column (Aldrich). Azobisisobutyronitrile (AIBN) was from Polyscience. Trimethylol propane trimethcrylate (TRIM), tetramethyl orthosilicate (TMOS), phenyl thrimethoxysilane (PTMOS), methyltrimethoxysilane (MTMOS), and trimethoxysilyl propyl methacrylate were from Aldrich and were used as received. Propranolol hydrochloride (Aldrich) was transformed to the free amine form. ^{3}H-propranolol, 21 ci/mmol, was purchased from NEN. All other chemicals were of analytical grade, and solvents were of HPLC quality.

Preparation of the Glass Substrate. Imprinted polymer films were polymerized on standard microscope cover glass plates (BDH, $\Phi = 13$ mm, borosilicate thickness no. 0). Before being imprinted with acrylic polymers, the glass plates were modified with acrylic functions with trimethoxysilyl propyl methacrylate (Aldrich, 2% in dry toluene, O.N., with shaking).[31] The glass plates were rinsed in toluene and acetone and dried in ambient temperature for at least 2 h.

Polymerization Cell. The need to maintain an inert atmosphere with slight overpressure during the polymerization lead to the construction of a simple polymerization apparatus. The polymerization cell was built from stainless steel, fitted with a quartz window and nitrogen inlet. An overpressure of 0.1 atm of N_2 was maintained throughout the polymerization process. The polymerization cell was placed on a rotating stage (60 rpm) and was illuminated by a mercury lamp (Oriel Q series model 6000) fitted with a band-pass filter (Schott, 300−400 nm) and an IR filter (Schott). A 45° mirror (Oriel) directed the light perpendicular to the cell.

Preparation of Imprinted Acrylic Polymer Membrane. A typical polymerization mixture was comprised of MAA (200 μL, 2.4 mmol), EGDMA (400 μL, 2.1 mmol) or TRIM (400 μL, 1.1 mmol), 200 μL of chloroform, and 5 mg (0.03 mmol) of azobis(isobutyronitrile). The template molecule, propranolol (5 mg, 0.02 mmol) was finally added for the imprinted films, or omitted, for the reference films. A thin film of the monomer solution was cast on the modified glass plates (30 μL of monomer mixture, Headway 101, 4000 rpm, and 20 s). Immediately after the casting, batches of 5−9 plates were placed horizontally in the polymerization cell. Polymerization was carried out for 10 min. The resulting polymerized films were transparent, smooth, and uniform. The films were 1-μm thick (the thickness of the films was measured by the reflectance spectrum using Filmetrics F-20). After the polymerization, the coated glass plates were Soxhlet extracted with acidic methanol (10% acetic acid) for at least 3 h to remove the template molecule and unpolymerized material.

Preparation of Imprinted Sol−Gel Films. The preparation of the sol−gel polymer consisted of two stages—preliminary hydrolysis of the monomers to the sol phase, followed by coating and drying.[21] The polymerization mixture typically

(18) Hedborg, E.; Winquist, F.; Lundstrom, I.; Andersson, L. I.; Mosbach, K. *Sensors Actuators A* **1993**, *37−38*, 796−799.
(19) Malitesta, C.; Losito, I.; Zambonon, P. G. *Anal. Chem.* **1999**, *71*, 1366−1370.
(20) Piletsky, S. A.; Pilestskaya, E. V.; Panasyuk, T. L.; El'skaya, A.; Levi, R.; Karube, R.; Wulff, G. *Macromolecules* **1998**, *31*, 2137−2140.
(21) Makote, R.; Collinson, M. M. *J. Chem. Soc., Chem. Commun.* **1998**, 425−426.
(22) Gao, L.; Shi, Y.; Slaterbeck, A. F.; Seliskar, C. J.; Heineman, W. R. *Proc. SPIE* **1998**, *3258*, 66−73.
(23) Andersson, L. I. *Anal. Chem.* **1996**, *68*, 111−117.
(24) Schwaitz, L.; Andersson, L. I.; Nilsson, S. *Anal. Chem.* **1997**, *68*, 1179−1183.
(25) Fischer, L.; Muller, R.; Ekberg, B.; Mosbach, K. *J. Am. Chem. Soc.* **1991**, *113*, 9358−9360.
(26) Suedee, R.; Srichana, T.; Saelim, J.; Thitirat, T. *Analyst* **1999**, *124*, 1003−1009.
(27) Haginaka, J.; Sakai, Y.; Narimatsu, S. *Anal. Sci.* **1998**, *14*, 823−826.
(28) Martin, P.; Wilson, I. D.; Morgan, D. E.; Jones, G. R.; Jones, K. *Anal. Commun.* **1997**, *34*, 45−47.
(29) Haupt, K.; Nowortya, K.; Kunter, W. *Anal. Commun.* **1999**, *36*, 391−393.
(30) Schubert, U.; Husing, N.; Lorentz, A. *Chem. Mater.* **1995**, *7*, 2010−2027.
(31) Glad, M.; Norrlow, O.; Sellergren, B.; Siegbahn, N.; Mosbach, K. *J. Chromatog.* **1985**, *347*, 11−23.

consisted of TMOS (3 mL, 20.3 mmol), PTMOS (0.37 mL, 0.98 mmol), MTMOS (0.3 mL, 2.1 mmol), ethoxyethanol (3 mL), H_2O (1 mL), and 0.1 N HCl (1 mL). This mixture was stirred for 2 h at RT, after which an aliquot of 2 mL was taken, and 10 mg (3.8 mmol) of propranolol was dissolved in 100 mL of ethoxyethanol and 50 μL of 0.1 N HCl was added. This solution was used for the imprinted sol−gel films, while the remaining solution was used for the nonimprinted reference films. Glass plates were coated by spin coating (30 μL, 4000 rpm, 20 s) and were allowed to polymerize and dry overnight. After the polymerization, the coated glass plates were Soxhlet extracted with acidic methanol.

Radioligand Binding Assay. Radioligand binding assay was performed according to the following procedure: polymer-coated glass plates were incubated in a solution of ^{3}H-propranolol (typically, 2 nM) and 10 μM of propranolol in PB (10 mM, pH = 7.6) or in an organic solvent, at 25 °C for durations of 0.5−24 h. The plates were rinsed thoroughly with buffer (or with the organic solvent), placed in scintillation liquid, and read in the β-counter. Reference polymers (same chemical composition, no imprinting) underwent the same procedure. Batches of four different plates were measured for each data point.

Indirect Fluorescent Assay. Coated glass plates were immersed in 2 mL of 10 μM propranolol in 10 mM PB, pH = 7.6, at 25 °C. After 24 h the plates were taken out of the solution, rinsed with the buffer, and immersed in 3 mL of acidic PB (10 mM, pH = 4.1) for 2 h. After the incubation period, the fluorescence of the solution was read at λ_{em} = 352 nm (SLM 500 fluorimeter, λ_{ex} = 288 nm). The concentration of the liberated propranolol was calculated using a calibration graph.

Results and Discussion

The formation of thin films of acrylic polymers is not simple. Factors that govern the polymerization process and resulting polymer characteristics, like pressure and solvent concentrations, are more difficult to control when dealing with thin films. The role of the solvent on the imprinting process has been recognized,[32] but this problem was addressed only in bulk polymers. It was found that chloroform was a good solvent for the polymerization process. Surprisingly, sufficient solvent remained in the thin liquid film of monomer mixture to maintain the polymerization process. Another factor that controls the imprinting properties of the polymer is the cross-linking degree. Most bulk imprinted polymers include a high degree of cross-linking, generally 80−100%. This immense degree of cross-linking is needed also to maintain the three-dimensional network structure in pressurized HPLC columns. Polymerization of thin films with such high cross-linking in the polymerization cell resulted in opaque, brittle films that flaked immediately. The more applicable mixture included only 34% cross-linking, and the polymerization resulted in clear stable films that were polymerized in 10 min. The polymerization cell was designed to ensure that during the polymerization an overpressure of 0.1 atm was maintained and that the whole batch of glass plates was exposed to the same average light intensity. The resulting films were smooth and transparent and were about1-μm thick. Prefunctionalization of the glass plates with the bifunctional reagent trimethoxysilyl propyl-methactylate constituted a monolayer that acts as an adhesive between the glass substrate and the film. It is envisioned that, during the polymerization process, the growing polymer chains near the glass surface cross-link with the methacrylate groups, thus adhering the film chemically to the surface.

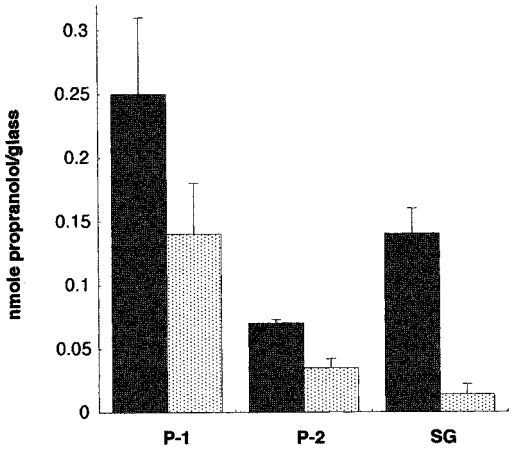

Figure 2. Steady-state binding of propranolol to molecularly imprinted thin films of acrylic (P-1 and P-2) and sol−gel (SG). Solid bars: imprinted films. Dotted bars: nonimprinted films. Glass plates were incubated for 24 h at 25 °C, in 10 μM of propranolol in phosphate buffer pH = 7.6 containing 2 nM ^{3}H propranolol (n = 4).

Molecular imprinting of propranolol in sol−gel was achieved using a mixture of alcoxysilane functional monomers to produce a hybrid organic−inorganic matrix. The thickness of the films was 700 nm.

Propranolol Binding. *Steady-State Binding.* The molecular recognition of the imprinted films was studied by radioligand binding and by fluorescence. Radioligand binding of ^{3}H-propranolol to imprinted polymer particles was first shown by Andersson.[23] The binding assay performed by us was simple and direct: the amount of bound propranolol was read directly from the coated plate after incubation periods of up to 24 h. Nonspecific adsorption was evaluated using the nonimprinted polymer films that contained the exact same composition of monomers, but lacked the presence of propranolol in the imprinting stage.

Two imprinted acrylic polymer films and the sol−gel imprinted films were subjected to the radioligand binding experiment (Figure 2): P-1, which contained EGDMA as the cross-linker, and P-2, which contained TRIM as the cross-linker. The P-1 imprinted films bound ca. 1.7 times more propranolol than the reference nonimprinted films (0.25 ± 0.06 and 0.14 ± 0.04 nmol/glass plate, respectively). The P-2 imprinted films bound twice as much propranolol than the reference film (0.07 ± 0.03 and 0.035 ± 0.007 nmol/glass plate, respectively). This result is in accordance with similar binding experiments[23] carried out in bulk polymers. The similarity in the binding properties of film and bulk polymers (from the literature) indicate that the process of film forming and lowered degree of cross-linking did not impair the binding properties of the imprinted polymer.

The high uptake of the acrylic polymer toward propranolol was accompanied by a high degree of nonspecific binding, probably to the nonspecific sites. The polymer matrix includes a high molar ratio of methacrylic acid, the monomer that is responsible for the

(32) Yoshizako, K.; Hosoya, K.; Iwakoshi, Y.; Tanaka, N. *Anal. Chem.* **1998**, *70*, 386-389.

hydrogen bonds, and this may be the reason for the relatively high nonspecific binding.

Steady-state binding experiments of propranolol to imprinted sol−gel films were carried out in a similar way. After an incubation period of 24 h in 10 μM of propranolol, the imprinted sol−gel films bound 0.14 ± 0.02 nmol, while the nonimprinted sol−gel films bound only 0.014 ± 0.008 nmol of propranolol. The ratio of specific to nonspecific binding is 10, which is considerably higher than the ratio found for the acrylic polymers. The binding capacity of the sol−gel system toward propranolol is lower than the capacity of the acrylic system in ca. 50%, but this lower capacity is compensated by high specific binding and also by better reproducibility of the polymer films. The more specific binding of propranolol by the sol−gel material is probably due to the different natures of the noncovalent bonds that exist between the template and the matrix. Two major binding contributions probably come from hydrophobic and $\pi-\pi$ interactions between the PTMOS and MTMOS monomers and propranolol. The contribution of hydrogen bonds to the recognition is lower, with respect to the considerable contribution of the acrylic moieties. It is therefore envisioned that, in the sol−gel system, the imprinting induces a more defined cavity that attracts the functional monomers, and only a small amount of functional monomers are scattered randomly on the surface. This functional cavity may be responsible for the specific binding, while the silanol groups that remain on the surface could be responsible for the nonspecific binding. Another difference between the sol−gel and the acrylic system is the ratio between the functional monomers and the backbone. If we consider MAA as the functional monomer for the acrylic system and EGDMA for the backbone, then the molar ratio between function and backbone is 1.1, while in the sol−gel system the molar ratio between the functional monomers, PTMOS and MTMOS to the backbone monomer, TMOS is 0.21. This may lead to the suggestion that increasing the EGDMA amount in the acrylic polymer will increase the specificity, but then one must take into account that increasing the cross-linker concentration changes dramatically the physical properties of the polymer film, while a careful interplay between the alcoxysilane monomers concentration does not have that marked influence on the sol−gel physical properties. In addition, the effect of hydrophobic and $\pi-\pi$ interaction is evident because the TMOS monomer is actually a functional monomer, responsible for the hydrogen bonds, but the specificity and selectivity are affected more by the PTMOS and MTMOS monomers.

An alternative indirect fluorescent assay for propranolol binding was also investigated. The assay was carried out indirectly. After an incubation period, the polymer-coated glass plates were immersed in acidic buffer (pH = 4.4) to break the hydrogen bonds. The fluorescence of the propranolol liberated into the solution was measured at $\lambda = 355$ nm. After an incubation period of 24 h, the acrylic-imprinted film, P-1, bound 0.46 ± 0.3 nmol of propranolol/glass plate whereas the nonimprinted film bound 0.27 ± 0.14 nmol of propranolol/glass plate. This ratio of specific to nonspecific binding, 1.7, is similar to the ratio found in the radioligand binding assay. The sol−gel imprinted polymers were subjected to the same assay procedure. After 4.5 h of incubation (which was shown to be a sufficient period of time for saturation), the imprinted sol−gel bound 0.343 ± 0.055 nmol of propranolol/plate whereas the nonimprinted polymer bound 0.071 ± 0.028 nmol/plate. The difference in the amount of bound propranolol measured by radioligand binding and fluorescent assay probably arises from the fact that the fluorescent assay is measured directly from the glass plate whereas the fluorescent assay is indirect and involves a stage of extraction of the bound propranolol into an acidic solution.

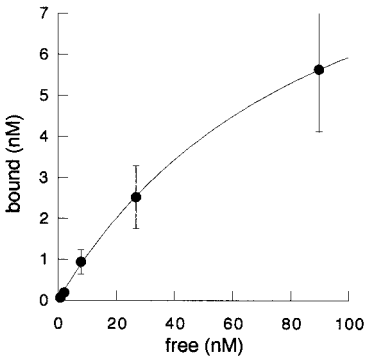

Figure 3. Binding of propranolol to SG imprinted polymer. Imprinted SG coated glass plates were incubated for 24 h at 25 °C, in 10μM of propranolol in phosphate buffer pH = 7.6 containing 2 nM ^{3}H propranolol (n = 4). The dissociation constant, K_d, was calculated by the mathematical simulation of the total binding curve: $B = ((B_{max} + T + K_d)/2) - \sqrt{((B_{max}+T+K_d)^2/2)-B_{max}T}$, where B = amount of bound propranolol and T = total propranolol concentration.

Association Constant of Propranolol to the Sol−Gel Matrix. The association constant of propranolol to the imprinted matrix was determined by a numerical solution of the binding formula.[33] Figure 3 displays the saturation binding curve of propranolol to the "receptor"—the imprinted matrix. The dissociation constant derived was $K_d = 80 \pm 6$ nM. This value indicates high affinity between the template molecule and the artificial sol−gel receptor. In a study on propranolol binding to a bulk imprinted acrylic polymer by Andersson,[23] it was found that the polymer had two populations of binding sites, one with high affinity, $K_d = 40 \pm 22$ nM, and one with lower affinity, $K_d = 23 \pm 8$ μM. The value found in our study indicated a homogeneous site population, with only one high-affinity association constant. The number of binding sites that was calculated from this analysis was found to be 1.03 ± 0.04 nmol of sites/glass plate. Because for a 10 μM propranolol solution it was found that the imprinted SG polymer binds 0.14 nmol of propranolol, the surplus of sites remains vacant. This may result from the long diffusion times into the bulk of the polymer film, and it is expected that a higher percentage of the sites will be occupied in thinner or more porous films.

Selectivity in Binding—Competition Studies with Other β-Blocker Derivatives. Binding of radiolabeled propranolol in the presence of varying concentrations of competing ligands was analyzed under conditions

(33) Boeynaems, J. M.; Dumont, J. E. *Outlines of Receptor Theory*; Elsevier: Amsterdam, 1980; pp 1−16.

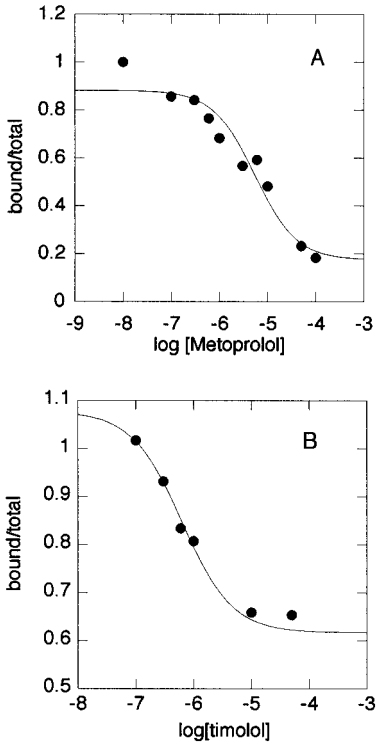

Figure 4. Displacement of ^{3}H-propranolol binding to imprinted SG by (A) metoprolol and (B) timolol in 10 mM phosphate buffer pH = 7.6. Bound/total is the ratio of bound ^{3}H-propranolol in the presence of the displacing ligand to the amount of bound propranolol in the absence of the displacing ligand. Curve was calculated using mathematical simulation of one binding site competition: (bound/total) = $A + ((B - A)/1 + 10^{(\log[\text{inhibitor}] - \log IC_{50})})$, where A = bound/total ligand ratio that induces maximal inhibition and B = bound/total ligand ratio that induces no inhibition (n = 4).

similar to immunoassay competitive tests. The IC_{50} values—the concentration of the competing ligand that is required to displace 50% of the specifically bound ligand—indicate the selectivity of the matrix toward foreign ligands. IC_{50} values for the β-blockers metoprolol and timolol were found to be 5.5 μM and 630 nM, respectively. Figure 4a displays the displacement plot of propranolol by the β-blocker metoprolol and Figure 4b for timolol. The different selectivity profiles that were obtained are the result of the difference between the hydrophobic and polar interaction of the β-blocker and the cavity in water. Individual drugs differ by the ether substituents—α-naphthyl for propranolol, β-phenoxyethyl phenol for metoprolol, and morpholine−thiadiazole heterocycle for timolol. This selectivity profile shows the effect of hydrophobic interaction on the imprinting yield: the hydrophobic and $\pi-\pi$ interaction between the naphthalene group of propranolol and the PTMOS and MTMOS monomers probably contribute in a major part to the recognition process because the other functional groups (hydroxyl, amine, and alkyl groups) are also present in the two β-blocker derivatives. These IC_{50} values can be compared to the values found by Andersson[23] for the selectivity of the acrylic polymer imprinted for propranolol: 64 μM and 250 μM for metoprolol and timolol, respectively. The selectivity in sol−gel imprinted polymers has been shown to be high, in cases where the imprinting was performed on the surface of silica particles.[34,31]

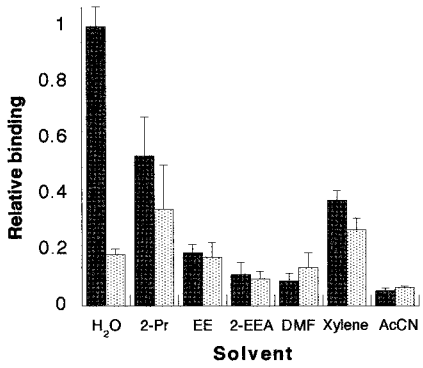

Figure 5. Binding of propranolol to imprinted (solid bars) and reference (dotted bars) SG in several solvents. The binding was normalized to the amount bound in aqueous solution. Plates were incubated in 10 μM propranolol with 2 nM ^{3}H-propranolol for 24 h at 25 °C. H_2O, 10 mM phosphate buffer pH = 7.6; 2-Pr, 2-propanol; EE, ethoxyethanol; 2-EEA, 2-ethoxyethyl acetate; DMF, dimethylformamide; AcCN, acetonitrile (n = 4).

Ligand Binding Analysis in Organic Solvents with Sol−Gel Imprinted Films. The interaction of propranolol with the imprinted SG matrix was investigated in a variety of organic solvents, both protic and a-protic. Figure 5 exhibits the steady-state binding of propranolol to a series of polar organic solvents in comparison to that in an aqueous medium. The binding of propranolol was noticeably high in water and in 2-propanol and very low in all of the other organic solvents that were investigated (ethoxy ethanol, 2-ethoxy ethyl acetate, acetonitrile, DMF, and xylene). This could result from the preferred solubility of propranolol in the organic solvents than an in aqueous medium. This assumption may be justified in part by the partition coefficient of propranolol in the octanol/water system. The value of the partition coefficient is log p(octanol/pH 7.4) = 1.2,[35] which indicates that propranolol is more soluble in organic solvents than in water. In addition, the nature of the hydrophobic interaction between the phenyl residue of the sol−gel matrix and the α-naphthyl of the propranolol influence the solvent effect. The hydrophobic interaction is stronger in water and weaker in organic solvents, which may explain the high binding determined in water and in the polar protic 2-propanol, relative to the organic solvents. An alternative explanation could arise from the entropic origin—as the propranolol molecule enters the specific cavity, it displaces water molecules that are bound to the matrix, thus increasing the entropy. The organic solvents are weakly bound to the matrix and the molecules are bigger, so the entropy change is small. Both considerations, enthalpic and entropic, lead to the increase in the association between propranolol and the matrix.

Effect of pH on Binding. The dependence of ligand binding on pH was investigated over the pH range of 4.5−9.6 (Figure 6). The binding of propranolol to the imprinted SG polymer increased with the pH, reaching an optimum in pH lower than the pK_a of propranolol, 9.5.[35] The amount of nonspecific binding, as apparent from the binding to the nonimprinted sol−gel films,

(34) Iqbal, S. S.; Lulka, M. F.; Chambers, J. P.; Thompson, R. G.; Valdes, J. J. *Mater. Sci. Eng. C* **2000**, *7*, 77−81.

(35) *Clarke's Isolation and Identification of Drugs*; Moffat, A. C., Ed.; The Pharmaceutical Press: London, 1986; pp 936−937.

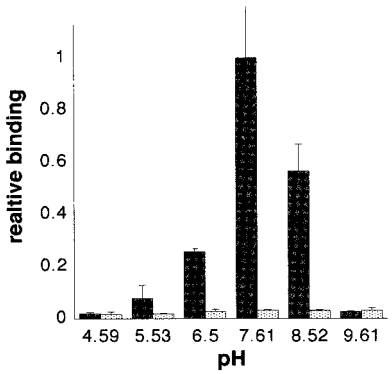

Figure 6. Binding of propranolol to imprinted (solid bars) and reference (dotted bars) SG as the function of pH. Binding was normalized to the binding at pH = 7.6. Plates were incubated in 10 μM propranolol with 2 nM ^{3}H-propranolol for 24 h at 25 °C in 10 mM phosphate buffer in the appropriate pH (n = 4).

increased only slightly and is probably due to nonspecific hydrogen bonds between the amino group of the propranolol and the silanol groups of the matrix. The optimal binding pH is probably a combination of electrostatic attraction between the positively charged propranolol and the negatively charged matrix followed by penetration of propranolol to the cavity and the formation of hydrophobic interactions between the cavity and propranolol. For comparison, in a study on the pH dependence of propranolol binding to bulk acrylic imprinted polymers[23] a similar pH-dependence profile was observed with an optimum at pH = 8.0, but was accompanied by high nonspecific binding, ca. 50% at pH 7.5, relative to the 30-fold specific binding found in this experiment. The shift in the pH binding optimum relative to the pK of propranolol is probably due to the matrix effect. The pKi of acid-catalyzed sol−gel is between 2 and 3;[36] thus, the interplay between the pK of the target molecule and of the matrix results in preferential binding at pH = 7.6. The fact that the nonspecific binding is independent of the pH indicates that the nonspecific binding is not a result of hydrophobic interaction. If this was the case, the nonspecific binding would have been expected to rise with increased pH.

Kinetics of Binding. The general kinetic profile of propranolol binding to P-1 and to SG imprinted polymers was investigated (Figure 7). It can be seen that the acrylic polymer P-1 possesses a slow uptake profile, reaching saturation only after ≈10 h. The nonspecific binding increases with time as well. In contrast, the sol−gel imprinted polymer shows a faster uptake profile, with significant specific binding after only 5 min, and saturation in <1 h. In addition, the nonspecific binding is negligible, even after long periods of incubation. A possible explanation is the higher porosity of SG relative to that of P-1. The porosity of the matrix has a pronounced effect on the diffusion times of the target molecule into the polymer. The porosity of the acrylic polymer is controlled mainly by the porogenic solvent. In bulk preparation the solvent is entrapped in the cross-linked polymer and creates the pores. However, in the thin film, most of the solvent evaporates, thus creating a less porous matrix. In the sol−gel matrix the

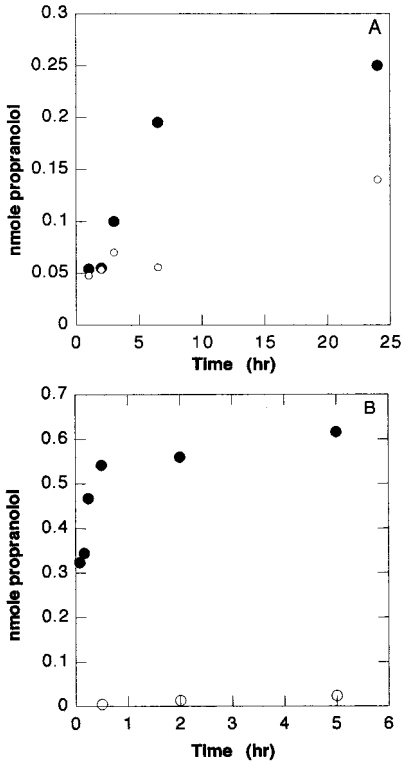

Figure 7. Kinetic binding profile of propranolol binding to the following: (A) P-1 coated glass plates: solid circles, imprinted P-1; open circles, reference P-1. (B) SG coated glass plates: solid circles, imprinted SG; open circles, reference SG (n = 4).

porosity is controlled by several factors[37] such as the method of preparation, addition of surfactants, monomer composition,[38] and more.[39] In the polymer SG preparation, the solvent, H_2O−ethoxyethanol, is not only less volatile than $CHCl_3$, the solvent for P-1, but it is also in higher content in the monomer mixture (ca. 60% v/v in SG, relative to ca. 25% v/v in P-1).

Conclusions

The successful molecular imprinting of propranolol in thin films of two chemically different polymer matrixes has been achieved. The more explored acrylic matrix has been shown to be less favorable for molecular imprinting in thin films due to the nontrivial preparation method that reduces the reproducibility and due to the high nonspecific binding. The amount of specific binding in this matrix is probably satisfactory for chromatography and other flow systems, which rely on dynamic binding, but certainly not for sensor application. The sol−gel system has been shown to be superior in terms of preparation process, faster diffusion times, and reduced nonspecific binding, It is likely that the role of a hybrid organic−inorganic sol−gel matrix will increase in sensor application as indicated by recent reports.

Acknowledgment. This research was supported in part by the infrastructure (Tashtiot) project of the Israel

(36) Iler, R. K. *The Colloid Chemistry of Silica and Silicates*; Cornell University Press: Ithaca, NY, 1955.

(37) Brinker, C. J.; Scherer. G. W. *Sol−Gel Science*; Academic Press: New York, 1990.

(38) Rottman, C.; Grader, G. S.; De Hazan, Y.; Avnir, D. *Langmuir* **1996**, *12*, 5505−5508.

(39) Elferink, W. J.; Nair, B. N.; de Vos, R. M.; Keizer, K.; Verweij, H. *J. Colloid Interface Sci.* **1996**, *180*, 127−136.

Ministry of Science, Culture & Sport. We thank Sharon Fireman-Shoresh for experimental assistance.

Note Added in Proof

Chiral Sol−Gel Film Imprinting. Since propranolol is a chiral molecule, an intriguing question has been whether the imprinted cavity retains the chirality of the molecular shape. Preliminary imprinting experiments of the sol−gel films with the pure enantiomers (and not with the racemate, as used above) indeed revealed capability of the film to discriminate between the two enantiomers. Sol−gel films that were imprinted with (S)-propranolol were incubated with ^{3}H−(S)-propranolol with ca. 4000-fold excess of either (S) or (R) enantiomers. The (S)-imprinted sol−gel films were able to concentrate the (S)-radioactive ligand from the mixture of ^{3}H−(S) and nonradioactive (R) enantiomer to yield a signal 14 times larger than that found for films incubated in the mixture of ^{3}H−(S) and nonradioactive (S) enantiomer (0.18 and 0.013 pmol of radioactive ligand, respectively). This indicates that the (S)-imprinted film binds the ^{3}H−(S) enantiomer preferably from the ^{3}H−(S) and (R) enantiomers mixture due to chiral recognition of the former. It is noteworthy that despite being in very large excess, the (R) enantiomer is unable to displace the preferred, adsorbed ^{3}H−(S) enantiomer. In comparison, in the use of the ^{3}H−(S) and nonlabeled (S) enantiomers mixture, both compounds bind, as should be the case, nondiscriminatingly, and the radioactive reading reflects the ratio of radioactive label to nonlabeled (S) in the solution. Two other blank tests, one on (R)-imprinted films and the other on nonimprinted films, showed with the above two types of enantiomer mixtures no (S) specificity.

CM000983Z