Accelerated Development Procedure for Molecularly Imprinted Polymers Using Membrane Filterplates

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A novel technique for the synthesis and testing of large numbers of molecularly imprinted polymers is described requiring much less time than the commonly used miniMIP approach. Instead of vials, the polymers are synthesized on the surface of microfiltration membranes in multiwell filterplates. The thin polymeric films enable accelerated template removal. The MIP development procedure is thereby shortened to two days. Performance of the system was demonstrated by creating a combinatorial library of MIPs selective for cimetidine, an antiulcer drug. The polymer composition has been optimized. An experimental design combined with a multivariate analysis (i.e., response surface modeling) was used to minimize the number of experiments in the optimization process. The highest imprinting factor was obtained using a MAA/EDMA/template molar ratio of 3.5:19.5:1.

Introduction

Molecularly imprinted polymers (MIPs) are receiving more and more attention not only from researchers but also from practicing analytical chemists. Some imprinted polymers are already on the market, sold as solid-phase extraction sorbents and applied as sample preparation media for chromatography. New start-up companies have been founded targeting specific needs of practicing analytical chemists by providing them with custom-made MIPs of predefined selectivities. This type of activity needs fast polymer development and optimization procedures. Although the development of MIPs is a lot faster than that of their biological counterparts, that is, the production of antibodies, still the techniques are time-consuming, requiring weeks. Customers often cannot wait so long to solve their analytical problem.

The earliest established procedure, that is, bulk polymerization for the preparation and testing of molecularly imprinted polymers is rather tedious and time-consuming. It requires free-radical cross-linking polymerization of the monomers in the presence of the template in glass tubes using either thermal initiation or UV irradiation, and the results are hard monolithic blocks of polymers. Thermal polymerization takes about one day, while UV polymerization lasts only a few hours. The polymers are then crushed in a mortar or in a ball-mill, and the appropriate particle size range is separated by wet-sieving. This process can take a day for each polymer depending on the amount synthesized. The following step is template removal from the polymer matrix to free the specific binding sites. This must be carried out very thoroughly because "template bleeding" from the polymer can cause false results when low amounts of template are to be detected. The usual way to clean the polymers is Soxhlet extraction requiring 24−72 h. Other methods, such as supercritical fluid extraction, microwave- or ultrasonic-assisted extraction, and accelerated solvent extraction are also in use and can be more efficient than Soxhlet extraction.[1,2] After they are dried, the resulting polymers are tested either by elution or frontal chromatography or in equilibrium batch rebinding experiments. These procedures also add at least one or two days to the preparation process.

Optimization of the MIPs requires the variation of relatively large numbers of parameters like the type and concentration of functional monomer and cross-linker, their relative amount to the template, and the type of polymerization solvent. Temperature can also be an important variable, determining the timing of phase separation and the extent of the template−monomer associations. It is obvious that the traditional polymerization, processing, and testing method is not applicable when optimized compositions are to be obtained in a short time. Therefore new approaches appeared in the literature based on combinatorial design and high-throughput synthesis and testing. The two pioneering papers of Lanza et al. and Takeuchi et al. appearing at almost the same time, introduced miniMIPs,[3,4] the scaled down version of monolithic MIPs. These are approximately

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40−100 mg polymer discs of few millimeters thickness that are synthesized in autosampler vials simultaneously, with varying compositions, and tested in situ for template release, and after complete template removal, for template rebinding. The procedure can be easily automated using liquid handling robotic arms.[5] Template concentrations are determined by sequential injection into a HPLC-UV system, which substantially slows down the procedure. To obviate the need for HPLC analyses, microtiter plates and fluorescent microplate reader were introduced instead of the autosampler vials to accommodate miniMIPs and to detect template binding to the polymers simultaneously.[6] However this approach had limitations on the template (only fluorescent templates could be measured) and on the concentration of detectable bound template (above a certain amount of bound template saturation of the detector was observed). The idea was further improved by Dirion and her co-workers.[7] They synthesized miniMIPs in a 96-well microtiter plate and transferred them to a 96-well filterplate, where the template removal and the template rebinding step was taking place. Fractions containing the nonbound template were collected in another 96-well plate and transferred to a UV monochromator plate reader for parallel quantification. This improved method was suitable to synthesize and evaluate a MIP library within one to two weeks.

The critical step in the above procedures is still template removal from the polymers. The optimization process is considerably slowed down by the extensive washing procedures. MiniMIPs are washed consecutively with small portions of washing solvents, each wash step requiring considerable time because of the slow diffusion kinetics of the template. According to our experience, template removal can require up to 20−30 wash steps, each taking at least 30−60 min. Therefore to clean one set of miniMIPs can cost up to a week of work.

Another argument against the above approaches was raised by Perez-Moral et al.[8] indicating that the miniMIP format does not allow for evaluation of the test polymers in different conditions, although the various polymer compositions may exhibit their best binding properties under disparate conditions (e.g., the materials cannot be used in SPE conditions).

Our idea was to create thin polymer layers/films for the MIP development process, where template release is enhanced by the drastically decreased diffusional path length and washing can be done in a flow-through mode.

Thin molecularly imprinted polymer films have already been successfully prepared on the surface of porous microfiltration membranes for affinity separation purposes. Different approaches exist to prepare these composite membranes with selective transport properties. Wang et al. photografted a layer of imprinted polymer onto the surface of a polyacrylonitrile membrane modified with photosensitive dithiocarbamate groups.[9] A more general method has been developed by Piletsky et al. A photoinitiator was coated on the surface of a microporous membrane which, after UV irradiation, created free radicals. These free radicals in turn served as starters for graft copolymerization of the functional monomers in the presence of a template. This method has been tested with different membrane materials and both in aqueous and organic solvents.[10,11] MIP nanoparticles have also been used to modify the surface of a methylmethacrylate-*co*-acrylic acid copolymer membrane prepared by the phase inversion method.[12] Zhu et al. had presented a simple approach to prepare surface-modified MIP-composite membranes by simply casting the prepolymerization mixture onto a Nylon microfiltration membrane and polymerizing it by thermal initiation.[13]

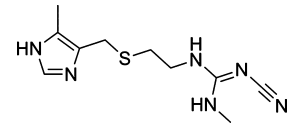

Figure 1. Structure of cimetidine.

In this paper, we describe a novel system for the fast, high-throughput synthesis and testing of large numbers of molecularly imprinted polymers. The system is based on filtration microplates that are available in 24, 96, and 384 well formats and in a wide range of filter materials. These are commonly used in HPLC sample preparation, combinatorial chemistry, drug screening, and ELISA. For the purpose of MIP screening, we have modified the filters with thin layers of MIPs following Zhu's method, while preserving the filterability of the membranes. This approach allowed accelerated template removal because the obtained thin polymeric layers could be washed much faster because of the shortened diffusion length. Template removal could be carried out in a flow-through mode instead of numerous and time-consuming consecutive steps of batch washing. Recognition properties of the polymers could also be tested in situ. Another advantage of the MIP supported membranes is the possibility to test them directly under SPE conditions. The feasibility of the system has been proved by preparing and testing a combinatorial library of polymers against cimetidine, an antiulcer drug (Figure 1). To further speed up the development procedure the library was created by an experimental design combined with a multivariate analysis approach, first applied to the optimization of selective MIPs by Navarro-Villoslada et al.[14]

Experimental Section

Materials. Functional monomers methacrylic acid (MAA) and hydroxyethyl methacrylate (HEMA), cross-linking monomer ethylene glycol dimethacrylate (EDMA), free radical initiators 2,2′-azobisisobutyronitrile (AIBN) and benzoin ethyl ether (BEE), cimetidine, adiponitrile, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO). All solvents used were HPLC grade; acetonitrile (MeCN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany).

Glass fiber GF/C membrane was from Whatman (Kent, England), the Teflon filter was obtained from MSI (Westboro, MA) and cellulose filter paper was from Millipore (Billerica, MA).

The Swin-Lok filter holder, 25 mm diameter made with polypropylene, was the product of Whatman.

Twenty-four well 10 mL UNIPLATE microplate, 24 well 10 mL UNIFILTER Microplate with melt-blown polypro-

pylene membrane, and 24 well 10 mL UNIFILTER Microplate with glass fiber GF/C membrane were also from Whatman.

Water was purified with a Millipore Synergy UV system (Millipore). The monomers were purified before use by distillation or using an inhibitor remover column from Sigma-Aldrich. AIBN and BEE were used without further purification.

Cling film was obtained from Cole-Parmer (Vernon Hills, IL) and checked for UVC transparency (85% transmittance at 254 nm) with a spectrophotometer.

Equipment. The diaphragm vacuum pump was from Ilmvac (Ilmenau, Germany), and the vacuum manifold was from Biotage (Uppsala, Sweden).

The 40 W UVC (254 nm) germicidal lamp was a generous gift of László Korondán (LightTech Ltd., Dunakeszi, Hungary).

The HPLC analyses of the templates were made with a Perkin-Elmer Series 200 HPLC, equipped with a pump, autosampler, and UV detector.

Cimetidine was separated on a Waters Nova-Pak C18, 3.9 × 75 mm, 4 μm reversed-phase column. The mobile phase was a mixture of 0.1% formic acid (85%) and MeCN (15%). The flow rate was 0.8 mL/min; a 20 μL sample was injected, and the UV wavelength was set to 220 nm.

Polymer coated and uncoated filter membranes were characterized by scanning electron microscopy after sputter coating with Au/Pd using a JEOL JSM-5500LV instrument.

The experimental design was generated and all the statistical analysis treatments were accomplished by the software Design Expert 7.1 (Stat-Ease, MN).

Measurement of the Nonspecific Adsorption on Single Membranes. Each membrane was cut into pieces of about 50 mg each and put inside different vials. The template solution (1 mM cimetidine in MeCN) was added to each vial in the ratio of 20 μL per milligram of membrane. The vials were sealed and left to equilibrate at 25 °C for 24 h. Afterward the amount of free cimetidine in the supernatant was quantified by HPLC.

Preparation of Single-Membrane MIP. The membrane was cut into a circle of 26 mm diameter and washed with 10 mL of MeOH by fitting it into a Swin-Lok membrane holder and flowing through the solvent. The membrane was then removed and soaked with the monomer solution. It was placed into a closed container and polymerized with UV light for 40 min, while a slight overpressure of argon was applied to prevent oxygen entering the container. The degree of modification, M was evaluated from the difference in weight of the membrane before and after the polymerization, that is, the polymer weight, as $M = w_{\text{polymer}}/A$, where w_{polymer} is the weight of the polymer deposited onto the membrane in milligrams and A is the surface of the membrane in square centimeters. The filterability of the resulting membrane was verified by placing it inside a membrane holder and flowing MeOH through it or, eventually, applying low vacuum, if the membrane was not filterable by gravity.

UV Polymerization of MIPs in Filterplate Membranes. The membranes of the filterplate were washed with MeOH and dried before use. The mother solutions containing the template (0.1 mmol cimetidine), the functional monomer (MAA 0.4 mmol), the cross-linker (EDMA 2 mmol), the UV initiator (BEE 0.012 mmol), and the porogen (adiponitrile 585 μL) were prepared in glass vials in advance. Nonimprinted polymers (NIPs) were also prepared by omitting the template. The prepolymerization solutions were purged with argon for 2 min, and then 40 μL of each solution was placed over a membrane of the filter microplate with an Eppendorf pipet, taking care to completely wet it. The plate was then placed over a vacuum manifold and covered with a layer of UVC transparent cling film (Figure 2). A small flux of argon was maintained during the whole procedure to ensure an oxygen-free environment. After 10 min of argon flushing, the whole system was placed under a UVC germicidal lamp and irradiated for 45 min. The temperature of the filter microplate was monitored with a thermocouple thermometer and kept at 35 ± 2 °C by using a fan during the whole process. For 40 μL of mother solution, 17.5 ± 0.5 mg of polymer was formed in each membrane.

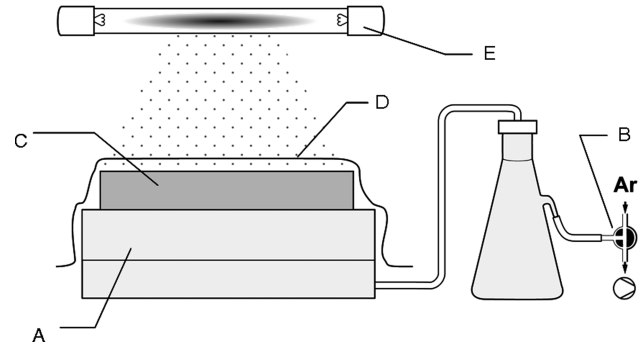

Figure 2. System setup for UV polymerization of the MIPs supported in microfiltration plates: (A) vacuum manifold, (B) argon and vacuum sources, (C) filterplate, (D) cling film, and (E) UVC lamp.

Template Removal from the Filterplate Membrane MIPs. After polymerization, the membrane-supported polymers were washed 10 times with 0.5 mL of warm (45 °C) MeOH containing 1% formic acid, five times with 0.5 mL of water, and five times with 0.5 mL of MeOH. Each washing step was done by placing the washing solution over the membrane for two minutes, and then slight vacuum was applied to remove it. To test the efficiency of the washing steps, each fraction was collected in a 24 well plate placed under the filterplate in the vacuum manifold and further analyzed by HPLC to quantify the template.

MiniMIP Preparation. Two mother solutions were prepared with compositions identical to the ones used in the filterplates (except that AIBN initiator was used instead of BEE); one containing the template (for the MIPs) and one without it (for the reference NIP). The solutions were purged with argon for 2 min, and 100 μL aliquots of the mother solutions were transferred to 1.5 mL glass vials and then sealed with PTFE/rubber silicon septa. Each vial was purged with argon to remove oxygen before the polymerization. The sealed vials were kept at 60 °C for 24 h to allow the polymerization to take place. The obtained white polymer discs, weighing about 45 mg each, were then extensively washed for template removal. To reach the same low bleeding as with the membrane supported MIPs, the mini-MIPs were washed 34 times: 22 times with acidic MeOH

Table 1. Nonspecific Binding (D) of Different Membrane Materials and Their Degree of Modification (M)

membrane type	D (L/kg)	M (mg/cm^2)
PTFE membrane	no binding	0.162
GF/C glass fiber membrane	no binding	7.6
polypropylene membrane	no binding	5.7
cellulose filter paper	5.3	
cellulose with polyethylene layer	5.0	

(10% formic acid), 6 times with water, and 6 times with methanol, requiring 3 days of work.

MIP Characterization. Both the membrane-supported polymers and the miniMIPs were characterized by equilibrium batch rebinding of the template molecule. A 1 mM solution of the template in MeCN was added to the dried and weighed polymers with a volume/mass ratio of 20 μL mg^{-1} and left to equilibrate after sealing for 16 h. The amount of nonbound template was quantified by HPLC using the appropriate method for the used template. The bound amount of template was calculated from the supernatant concentration by subtracting it from the initial concentration. Distribution coefficients were determined from the equilibrium concentration of the solid and liquid phase, respectively. ($D = q_{solid}/c_{liquid}$, where q is the bound concentration in millimoles per kilogram and c is the supernatant concentration in millimoles per liter.)

Results and Discussion

A high-throughput system for the accelerated development of molecularly imprinted polymers was developed based on composite MIP membrane filterplates. Previously, a molecularly imprinted polymer for cimetidine was synthesized in our laboratory using methacrylic acid functional monomer and ethyleneglycol dimethacrylate cross-linker in acetonitrile porogen.[15] The polymer showed high binding capacity for cimetidine because of the strong acid−base interactions of methacrylic acid and the template (the guanidine group in cimetidine is strongly alkaline, see Figure 1). We chose this MIP as a model polymer for setting up the system and to evaluate its performance.

Tests were made in advance to determine the best membrane support for the polymerization of the imprinted polymers. For our purposes, the ideal membrane should not bind any template by itself and should be thick enough to support a relatively high amount of imprinted polymer. Moreover, after the polymerization, the membrane should be still filterable. Further investigations were also carried out to find a proper porogen for the synthesis and to choose the appropriate filter format.

Membrane Materials. Various commercially available single membranes have been tested for nonspecific binding of cimetidine and the results are reported in Table 1. Unsurprisingly, the lowest values were found for Teflon, fiber glass, and polypropylene-based membranes.

The maximum amount of polymer that can be supported over the membrane (M) and still allows an acceptable flow rate is also reported in Table 1. Out of the three nonbinding membranes, the Teflon one was the thinnest; therefore the amount of polymer supported was very low. For our purposes, this amount was too small because the volume of the template solution in the batch rebinding experiment would have been too small to ensure the 20 μL/mg phase ratio.

Table 2. Comparison between Mini-MIPs Prepared in Adiponitrile or MeCN Porogen ($n = 3$)

porogen	D (L/kg)	imprinting factor
MeCN NIP	24.5 ± 2.9	2.35
MeCN MIP	57.5 ± 11.1	
adiponitrile NIP	22.0 ± 0.7	2.46
adiponitrile MIP	54.1 ± 6.7	

Porogen. A crucial point in molecular imprinting is the formation of the template-functional monomer complex in the prepolymerization mixture. A proper porogen should avoid splitting up that complex, should keep the polymerization components in solution, and should yield a good permeability (i.e., a proper pore structure) to the final polymer. Usual porogens for imprinted systems are, for example, toluene, acetonitrile, and dichloromethane. In the polymerization setup of supported membrane MIPs and of membrane filterplates, an argon overpressure is kept during polymerization. This can cause fast evaporation of the usual solvents, resulting in polymers with low porosity morphologies.[16] For this reason, we chose to use a low-volatility solvent, adiponitrile, which, to our best knowledge has not been used in MIP synthesis before. This porogen, having similar structure to acetonitrile, is able to solubilize the template−monomer mixture and does not interfere with H-bonding. A comparison using acetonitrile or adiponitrile as porogen in mini-MIP preparations shows similar binding properties and selectivity of the resulting polymers. The bound concentrations and the distribution coefficients together with the imprinting factors ($IF = D_{MIP}/D_{NIP}$) are shown in Table 2.

Filterplate. The 24 well plate configuration was chosen in our system to allow the support of a comfortable amount of polymer over a membrane. Two different filterplates were tested: one with polypropylene membrane (10−12 μm melt blown polypropylene, no thickness specified) and the other with fiber glass membrane (GF/C, 1.2 μm, thickness 260 μm). Since fresh batches of polypropylene-type filterplates were found to be thinner than earlier ones, we switched to the glass fiber membrane filterplates that had a specified constant thickness reported in the vendor specifications.

SEM images taken of the glass fiber membrane filter before and after polymerization are shown in Figure 3a and b. The deposition of the MIP film cannot be directly inferred from an increased fiber diameter by comparing these pictures because the glass fibers have largely varying diameter in the membrane. However, the thin, web-like deposition of the molecularly imprinted polymer between the fibers is clearly seen in Figure 3b, and possibly it also covers the fibers too. Filterability of the membranes before and after polymerization was quantitated by measuring the time required for 5 mL of MeOH to pass through a membrane keeping a constant vacuum pressure of 1 in. Hg. The space velocity (SV), defined as the flow rate/membrane's volume was calculated. Before polymerization, the glass fiber membrane showed a SV of 16.4 s^{-1}. After polymerization, the NIP and MIP

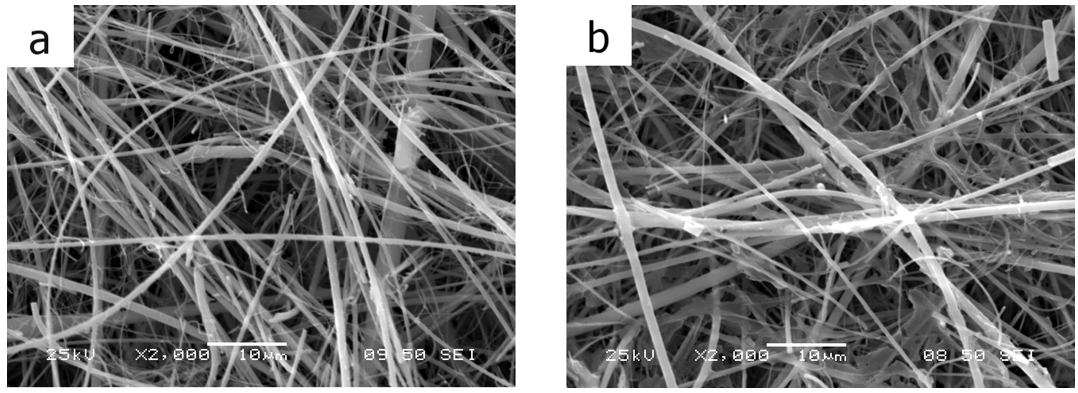

Figure 3. Scanning electron microscopic image of the glass fiber membrane of a filterplate (a) before and (b) after the polymerization of the MIP film. The bars in the pictures denote 10 μm.

membranes had much lower, but similar SV values of 2.0 and 2.3 s^{-1}, respectively. These values still indicate a good filterability.

Set-up of the UV Polymerization System. A UV initiated polymerization system was assembled to prepare cimetidine MIPs in the filterplates (Figure 2). The filterplate with the polymerization mixture in the wells was mounted onto a vacuum manifold. Different ways have been tested to keep an oxygen free atmosphere during polymerization. First argon was bubbled directly through the membranes from underneath during UV irradiation. This resulted in a nonfilterable membrane, probably, because of the accumulation of the mother solution on the upper membrane surface, causing the formation of a thick layer of polymer. Therefore another method was used to exclude oxygen during the MIP synthesis. One well was sacrificed by removing one membrane, creating a low resistance path, thereby allowing the argon to flow from the lower part of the device to the upper part through this hole. Since argon is heavier than air it displaces the latter one. The top of the filterplate was closed with a layer of cling film allowing the passage of the UV rays. To have a proper flow of argon through the device, the film was pierced with a needle to allow the exit of the gas.

Optimization of the Template Removal and the Equilibrium Rebinding Test. In high-throughput methods, apart from making and testing the samples in parallel, it is important to keep the process time at minimum. For this reason we optimized the two critical points in the MIP synthesis and evaluation: the washing and the rebinding step.

Optimization of the template removal was done using freshly made cimetidine MIPs, supported over glass microfiber membrane filterplates. Four parallel imprinted polymers were made, and each well was washed with 10 mL of MeOH to remove the porogen and the unreacted monomers. Afterward warm acidic methanol (1% formic acid in MeOH) was applied in 0.5 mL fractions for 2 min each and collected for further analysis. Furthermore, 5 washes were carried out with 0.5 mL of water to remove the acid and 5 more washes with MeOH to remove water. Figure 4 shows the template concentration in the 10 acidic methanol wash fractions.

It can be seen that, after five to six washes, the template concentration of the wash solution does not change any more.

To test the efficiency of the template removal, the polymers were dried, and MeCN was added to each well in

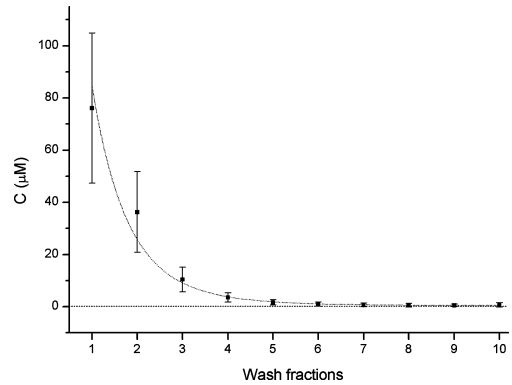

Figure 4. Template concentration in the consecutive wash fractions (1% formic acid in MeOH) during template removal. (n = 4).

the same phase ratio as in the equilibrium batch rebinding experiments, that is, 20 μL/mg polymer. The membranes were allowed to equilibrate with the solvent for 20 h to check the bleeding. The template concentration in the supernatant was below 1 μM in all cases. This amount can be considered acceptable for further evaluation in the batch rebinding experiment using 1 mM template solution.

It can be concluded that the whole wash procedure is unparalleled in terms of time requirement because it takes only about 30 min as opposed to 3−5 days.

Because of the fast wash times and the relatively small quantity of solvents needed, a 10 step wash procedure with acidic methanol was used further on.

Optimization of the rebinding time in the evaluation of the polymers was done by measuring the free cimetidine concentration at different time intervals during the batch rebinding experiment. Each data point was obtained from different wells. A 1 mM cimetidine solution was pipetted over the supported polymers into each well, and the filter plate was sealed. The supernatant was removed after 4, 6, 8, 16, 24, and 32 h. Figure 5 shows the free cimetidine concentration at each equilibration time. The equilibrium is reached after 16 h.

After the batch rebinding experiment, the polymers were first washed with 0.5 mL of MeCN to remove unbound cimetidine and then again with subsequent portions of acidic MeOH according to the protocol. The template removal can be followed in Figure 6.

It can be concluded that approximately 6−7 washings are sufficient to regenerate the polymers.

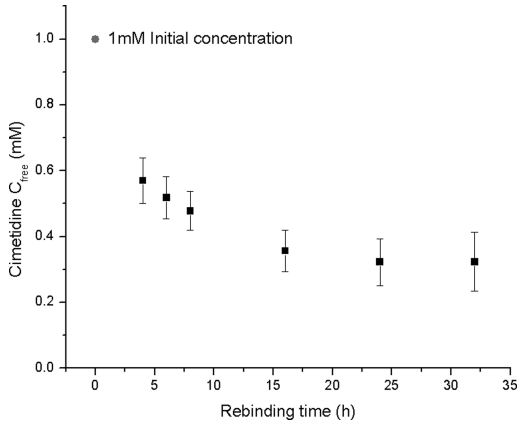

Figure 5. Template concentration in the supernatant after different equilibration times with1 mM cimetidine in the batch rebinding experiment ($n = 4$).

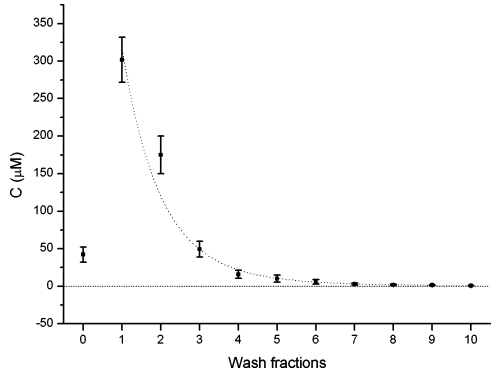

Figure 6. Template concentration in the consecutive wash fractions (0, 0.5 mL MeCN; 1−10, 0.5 mL 1% formic acid in MeOH) during template removal after the batch rebinding experiment ($n = 4$).

The total template recovery was also calculated from the cumulative amount of template found in the wash fractions and the theoretical bound amount obtained from the rebinding step. Thereby recoveries between 110% and 130% were obtained for each well. The excess recovered template can be explained by the incomplete removal of the rebinding solution.

Preparation of a Polymer Library for Cimetidine. The performance of the high-throughput polymerization system was tested by preparing a library of MIPs for cimetidine. The purpose of the study was to find an optimal polymer formulation that leads to high binding affinity for the cimetidine in the MIP, and at the same time, low binding affinity in a control polymer. An experimental design combined with a multivariate analysis approach was used to minimize the number of polymers to be synthesized in the optimization process and to take into account possible interactions of the parameters. In addition to methacrylic acid, another hydrophilic functional monomer, hydroxyethyl methacrylate (HEMA), was also tested as a parameter that could lend water-compatibility to the polymer. The three parameters chosen for the experimental design were the amount of the functional monomers (MAA and HEMA) and the amount of cross-linker (EDMA). Table 3 shows the settings of the parameters in the combinatorial library.

The amount of template and initiator was considered constant and was set to 1 μmol and 1% of the total amount of monomers, respectively. The volume ratio of the porogen (adiponitrile) regarding the total volume of the prepolymerization mixture was also kept constant at 0.57. Thereby, 14 different experiments and a central point were calculated according to a face-centered central composite design (i.e., $2^k + 2k + 1$, k = number of parameters). Three replicates of some experiments, as well as four replicates of the central point, were carried out. The experiments are listed in Table 4. A library was prepared for NIPs with identical polymer compositions but omitting the template.

Table 3. Setting of the Factors in the Combinatorial Library

	setting		
factor	low	center	high
MAA (μmol)	3	4.5	6
HEMA (μmol)	0	1.5	3
EDMA (μmol)	15	22.5	30

Table 4. Molar Ratio of Monomers and Template Used in the Cimetidine MIP Library

run	MAA	HEMA	EDMA	cimetidine
1	3	0	15	1
2	3	0	30	1
3	3	3	30	1
4	3	3	15	1
5	3	0	30	1
6	3	1.5	22.5	1
7	4.5	1.5	22.5	1
8	4.5	1.5	22.5	1
9	4.5	1.5	30	1
10	4.5	0	22.5	1
11	4.5	1.5	22.5	1
12	4.5	3	22.5	1
13	4.5	1.5	15	1
14	4.5	1.5	22.5	1
15	6	0	30	1
16	3	0	30	1
17	6	3	30	1
18	6	3	15	1
19	6	3	15	1
20	6	3	15	1
21	6	0	15	1
22	6	1.5	22.5	1
23	3	0	30	1

The polymer libraries were tested in batch rebinding experiment using 10^{-3} M cimetidine solution in acetonitrile as described in the Experimental Section. Distribution coefficients were calculated for the individual polymers and entered as response to model the polymer libraries by a quadratic model.

The response surfaces generated for this experimental design have been used to verify the parameters that have significant influence on the distribution coefficient of cimetidine, as well as to calculate the optimum values of the significant parameters to enhance the selectivity on the MIP. Figure 7 shows the response surfaces that describe the variation of the distribution coefficient for the MIP and NIP libraries. Both models showed a correlation coefficient higher than 0.94 with a coefficient of variation of 6.1% and 5.3% for the MIP and NIP regression model, respectively.

The predicted models showed that the amount of HEMA is not a significant parameter. It did not increase the distribution coefficient (i.e., selective binding) in the MIPs nor did it decrease the nonselective binding in the NIPs. This could be a result of its neutral character that avoids

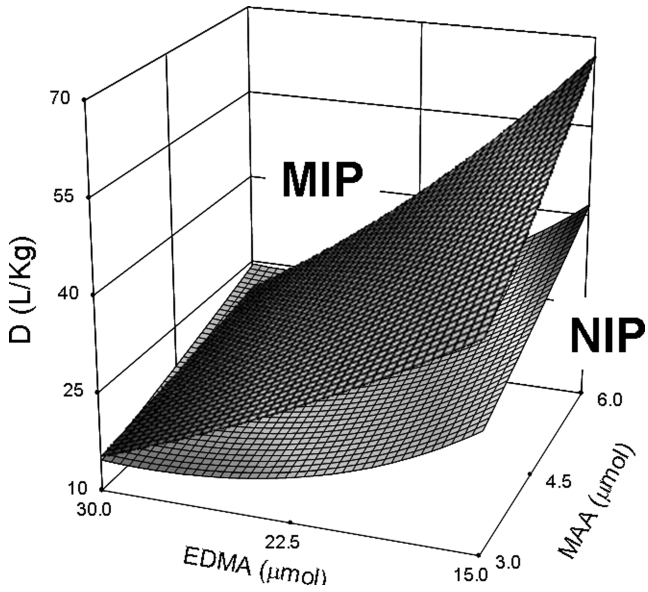

Figure 7. Estimated response surfaces from the central composite design of the MIP and NIP libraries.

interacting with the template and, therefore, not affecting the distribution coefficient of the polymers.

On the other hand, MAA and EDMA are significant parameters in the response for both polymers (MIP and NIP), showing an increase in the distribution coefficient of cimetidine when the amount of MAA increases and the amount of EDMA decreases. However, the distribution coefficient for the MIP increases more rapidly than for the NIP; therefore, selective binding on the MIP is observed in these conditions. The significant effect of the amount of MAA on the distribution coefficient could be explained considering the interaction with the template as well as the presence of nonselective binding when the amount of MAA is too high.

The increase of the distribution coefficient for both polymers when the amount of EDMA decreases could be related with the chemical environment, the site preservation, and the morphology (i.e., rigidity) of the polymer. Lower amounts of EDMA would impart less rigidity to the polymers, but with these compositions, the MIPs showed higher selectivity and loading capacity than the NIPs. Regardless the amount of MAA, when the amount of EDMA increases, which implies higher cross-linking density and rigidity, the models predict low distribution coefficients for cimetidine on the MIP and on the NIP. This behavior could be attributed to a lack of accessibility to the selective binding sites.

A numerical optimization combined with a desirability function[17] was applied to calculate the optimum values of MAA and EDMA amounts to enhance selective binding on the MIPs. This multicriterion approach was developed for the simultaneous optimization of multiple responses, and it is simple and easy to apply and allows the user to make subjective judgments on the importance of each response. The desirability function is based on the search of a global optimum taking into account a compromise between possible conflicting situations, such as the achievement of the highest selective binding on the MIP with the lowest nonselective binding on the NIP that affects the optimum values of each parameter. The goal of the optimization was to maximize the distribution coefficient in the MIP model and minimize the distribution coefficient in the NIP model, at the same time. The optimum conditions generated by the optimization were 3.5 μmol of MAA and 19.5 μmol of EDMA. Interestingly, the optimum composition is almost the typical 1:4:20 (template/functional monomer/cross-linker) prepolymerization ratio described in the literature for MIP syntheses.[18] The distribution coefficient predicted by the MIP model and NIP model under this optimum composition was 36.6 L/kg and 18.8 L/kg, respectively; resulting in an imprinting factor of 1.9. This predicted imprinting factor is close to the best imprinting factor obtained from the experiments of the experimental design. The predicted distribution coefficients are slightly lower than those obtained with miniMIPs (Table 2). This might be caused by the different polymerization conditions (UV vs thermal polymerization with different initiators).

Summary and Conclusions

In this paper, we presented a novel high-throughput system for the fast synthesis and screening of large numbers of molecularly imprinted polymers. Polymers were prepared as thin layers on microporous fiber glass membranes of 24-well filterplates. Polymerization was carried out using UV initiation under a gentle flow of argon. This design allows for extremely rapid and efficient template removal because the washing solution is semicontinuously filtered through the membranes and because the diffusion path length of the template is very short because of the small, micron dimensions of the polymer. Cimetidine, a model template was used throughout the experiments to establish the design of the apparatus and to optimize the critical, most time-consuming, steps, that is, the template removal, the batch rebinding test, and the regeneration of the polymers. Finally a combinatorial library of cimetidine MIPs was prepared and tested in equilibrium batch rebinding experiment. The whole procedure required less than two days. Optimization of the polymer composition was done by experimental design techniques. The optimum composition was found to be very close to the typical 1:4:20 (template/functional monomer/cross-linker) ratio described in the MIP literature.

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References and Notes

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