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Screening combinatorial libraries of molecularly imprinted polymer films casted on membranes in single-use membrane modules

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Abstract

A new and fast technique for screening combinatorial libraries of molecularly imprinted polymers is presented. The procedure is based on commercially available membrane modules which are rinsed with pre-polymerization imprinting mixtures. After the in situ polymerization and generation of MIP films on the PTFE membranes within the modules, the membranes are evaluated in terms of affinity towards the target molecule (template) *R*-(−)-phenylbutyric acid. Therefore, after template extraction from the freshly produced membranes a solution of this target molecule is flushed through the module. By analyzing the remaining analyte concentration in the permeate, the amount of analyte adsorbed on the membrane can be calculated and related to specific interactions with the molecular imprints. By this means, optimized recipes in terms of cross-linker to template ratios could be obtained in combination with the optimal porogen, when screening *p*-divinylbenzene or ethylene glycol dimethacrylate as cross-linker and porogens like acetonitrile, dimethylsulfoxide and methanol.

Keywords: Combinatorial libraries; Single-use membrane modules; Molecularly imprinted polymers

1. Introduction

Molecularly imprinted polymers (MIPs) are known to be applicable as highly specific receptors in affinity chromatography, sensor technology or catalysis [1–5]. However, finding the most efficient recipe for a MIP usually is a time and material consuming process. Due to the fact that at least five different components are used in an imprinting mixture, i.e., the template, functional monomer, cross-linker, porogen and initiator, the perfect composition is not easily achieved. Since it is not possible at this state to determine an optimal recipe based on molecular modeling, chemists are dependent on trial and error procedures involving a lot of variations of the components in terms of quality and quantity. A typical way of generating MIPs is the bulk polymerization of monomers in the presence of templates, followed by grinding, sieving and sedimenting the polymers. This procedure requires approximately 1 day and liters of organic solvents for producing a single polymer. In order to avoid this costly practice, scientists developed a technique of generating and screening combinatorial libraries of different MIPs [6,7]. The most important approach describes the use of an automated system, generating a variety of MIP coatings by dispensing different imprinting mixtures into glass vials [6]. After polymerization and extraction of the template, a solution of this target molecule is filled into the vials for adsorption measurements. By analyzing the remaining analyte concentration in the supernatant, the affinity of the different MIPs could be determined. However, the few publications showing such an approach were obviously not leading to a broad applicability, because of the fact that an apparatus had to be build or bought allowing the automated procedure. Thus, a simplified technique is needed which enables the user to perform a simple and fast screening of molecularly imprinted polymers. We have developed a technique based on ultrafiltration membrane modules (Fig. 1). Molecularly imprinted polymeric membranes are currently applied

Abbreviations: AcN, acetonitrile; AIBN, azobis(isobutyronitrile); CP, control polymer; DMSO, dimethylsulfoxid; DVB, *para*-divinylbenzene; EGDMA, ethylene glycol dimethacrylate; GC/MS, gas chromatography/mass spectrometry; MeOH, methanol; MIP, molecularly imprinted polymer; PP, polypropylene; PTFE, poly(tetrafluoroethylene); TRIM, trimethylolpropane trimethacrylate; 4-VPy, 4-vinylpyridine

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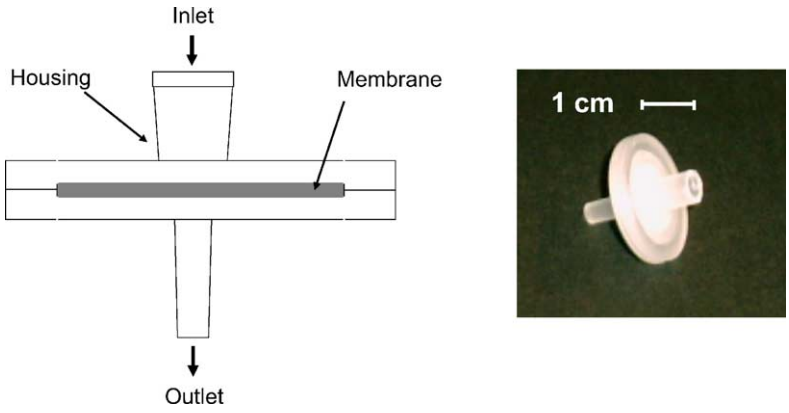

Fig. 1. Membrane module (original purpose: filtration). Left: sketch, right: photograph.

as sensors [8–12] and for separation [13–23], but not for screening procedures.

In general, in our new approach, the pre-polymerization mixture of template, functional monomer, cross-linker, initiator and porogen is rinsed through the membrane, and, after removing the excess of the solution, the polymerization is executed within an oven. In the following, the templates are extracted and the affinity of each membrane towards the target molecule is investigated by pumping a defined amount of the solution of the target molecule through the membrane. The adsorbed amount is determined by measuring the remaining amounts of the target molecule in the permeate solution. Finally, the adsorption on the MIP membranes is related to the control polymer (CP) membrane in order to exclude non-specific adsorption effects. It is demonstrated that this fast technique can be applied for finding optimized cross-linker to template ratios in different porogens by screening MIPs of different compositions.

2. Chemicals and methods

2.1. Chemicals

R-(−)-Phenylbutyric acid, DVB, EGDMA, TRIM and methacrylic acid were from Sigma–Aldrich (Taufkirchen, Germany). All solvents were from Carl Roth (Karlsruhe, Germany). The membrane modules (Minisart SRP 25, pore size 0.45 μm, PTFE membrane in PP housing) were from Sartorius (Göttingen, Germany).

2.2. Casting MIP films on membranes

Forty-five different MIP recipes and 45 corresponding CPs were screened with respect to three different parameters: (1) cross-linker type; (2) cross-linker concentration; and (3) porogen type. Three cross-linkers were investigated: EGDMA, TRIM and DVB. Each cross-linker was applied with five different molar ratios with respect to the template. These molar ratios were: 4, 8, 12, 16, and 20 mol cross-linker to 1 mol template. The porogens used were acetonitrile (AcN), methanol (MeOH) and dimethylsulfoxide (DMSO). In all recipes 4-vinylpyridine (4-VPy) was used as the functional monomer in a molar ratio of 4 with respect to the template (Table 1). 1×10^{-5} mol of template (*R*-(−)-phenylbutyric acid) was used for every MIP recipe. Pre-polymerization solutions were mixed for 15 min at room temperature before rinsing through labeled, weighed, cleaned and hydrophilized PTFE membranes within single-use modules using 1 ml syringes (cleaning and hydrophilization were done with 3 l of methanol for all the modules in series using an automatic pump). Excess mixtures were removed by pumping air with 20 ml syringes for 10 times through the module, leaving a thin film on the PTFE membranes. The modules were then flushed with nitrogen for 3 min to remove the radical scavenger oxygen before being closed from both sides with a sealed canula at the outlet and a 1 ml syringe at the inlet. Polymerization was performed by heating the modules in an oven at 70 °C over night. After polymerization, the modules were washed with a 7:1 methanol–acetic acid solution to extract the template, rinsed with methanol to eliminate residual acetic acid, dried at 50 °C and weighed again. The masses of the generated polymer layers were calculated from the mass difference of the modules between the pre-casted and the casted states. Masses up to 0.5 g of polymer were achieved without reducing the permeability of the modules. The masses of MIPs

Table 1
The material and amounts used for casting 45 different MIPs on PTFE membranes within microfiltration modules

Function	Component	Amount (mol)
Cross-linker	EGDMA, TRIM or DVB	(4, 8, 12, 16 or 20) × 10^{-5}
Functional monomer	4-VPy	4 × 10^{-5}
Template	*R*-(−)-Phenylbutyric acid	10^{-5}
Porogen	AcN, MeOH or DMSO	3 × 10^{-4}
Initiator	AIBN	1.5 × 10^{-6}

generated on the membranes ranged from 20 to 200 mg. Control polymer membranes were generated and processed in the same way, but in the absence of any template.

2.3. Affinity evaluation

For analyzing samples derived from the affinity experiments GC/MS was applied due to the technique's high sensitivity to small concentration changes in the affinity testing. However, when methanol was used as a porogen, measurements were performed with gas chromatography combined with flame ionization detection instead of MS.

A clear evidence of MIP layer formation on membranes is the increase in module mass which was constant after three consecutive affinity tests. Another evidence is the pink-brown color observed in MIP casted modules that was removable by washing. This color is due to the formation of a charge-transfer complex between the template and 4-vinylpyridine. Obviously, the template was displaced from polymerized 4-vinylpyridine anchors within the imprint by washing. Only membranes coated with MIP films did show this color effect after polymerization.

For affinity analysis 15 ml of a 1×10^{-3} mol/l solution of *R*-(−)-phenylbutyric acid in the selected porogen was pumped through each module automatically within 1.5 min. The concentrations of the obtained permeates were compared to the concentration of the permeate obtained from a non-casted module to eliminate the effect of non-specific adsorption on the membrane support material (PTFE) and the module housing (PP). The affinities for MIP and CP were calculated per gram of polymer by subtracting the amount of target molecule in the permeate from the amount of target molecule in the original solution. The relative affinity of each MIP recipe was determined by dividing the affinity of this MIP by the affinity of the corresponding CP. Calculation of concentrations from peak areas of chromatograms were done using a calibration curve obtained from measuring the concentrations of 1×10^{-2}, 5×10^{-3}, 1×10^{-3}, 5×10^{-4}, 1×10^{-4} mol/l *R*-(−)-phenylbutyric acid.

3. Results and discussion

In this new technique, thin layers of MIPs were casted on the surface of microfiltration PTFE membranes. *R*-(−)-Phenylbutyric acid had been chosen as template and a combinatorial library of 45 MIPs and 45 CPs differing in cross-linker content and porogen were prepared and studied. After optimizing the technique, it was possible to generate and evaluate the 90 different polymers in 2 days which is approximately 45 times faster than generating MIPs via bulk polymerization. A very small amount of template was needed for every MIP (<0.01 g). Fig. 2 presents relative affinities of MIPs cross-linked with DVB in AcN. The maximum relative affinity of 4.7 was obtained at a molar cross-linker to template ratio of 8:1. Higher concentrations of DVB (12, 16 and 20) showed lower relative affinities. DVB is capable to interact with the template by π–π interaction and contributes to the strength of the imprinting effect. A low imprinting effect at high concentrations of DVB may be due to its rigidity. DVB is a relatively rigid cross-linker compared to EGDMA or TRIM which makes it harder to adapt the template orientation in the recognition cavities inside the polymer matrix. Rebinding of target molecules may be more difficult due to the lack of flexibility.

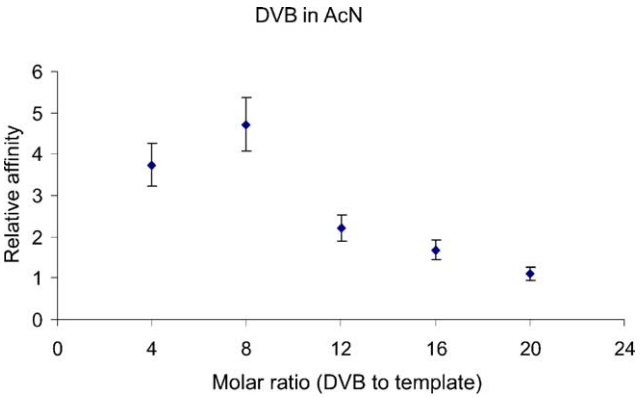

Fig. 2. Relative affinity of MIPs generated with different cross-linker to template ratios. Using *p*-divinylbenzene (DVB) as cross-linker, *R*-(−)-phenylbutyric acid as template and acetonitrile (AcN) as porogen.

Relative affinities of MIPs cross-linked with DVB in methanol show a very weak imprinting effect at all cross-linker concentrations (Fig. 3). A maximum imprinting effect of 1.7 was obtained at a molar cross-linker to template ratio of 4:1. Relative affinities of DVB–MIPs generated in DMSO (Fig. 4) were higher than in methanol but also lower than in AcN. This is probably due to the fact that the polar DMSO disturbs the self-assembly of the template and the functional monomer more than AcN, but less than methanol with its relatively high protonic and even stronger polar character. Maximum relative affinity obtained was 2.5 at a molar cross-linker to template ratio of 4:1. High concentrations of DVB showed again to be disadvantageous.

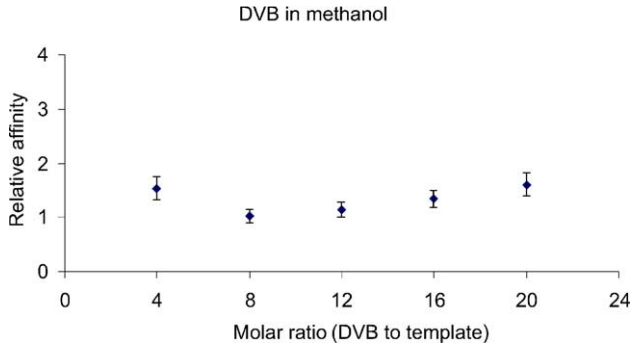

Fig. 3. Relative affinity of MIPs generated with different cross-linker to template ratios. Using *p*-divinylbenzene (DVB) as cross-linker, *R*-(−)-phenylbutyric acid as template and methanol as porogen.

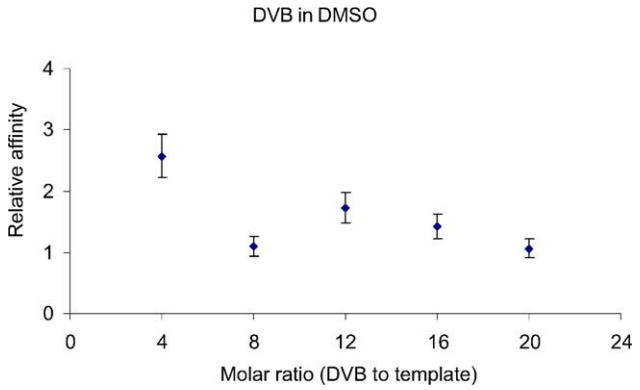

Fig. 4. Relative affinity of MIPs generated with different cross-linker to template ratios. Using *p*-divinylbenzene (DVB) as cross-linker, *R*-(−)-phenylbutyric acid as template and dimethylsulfoxide (DMSO) as porogen.

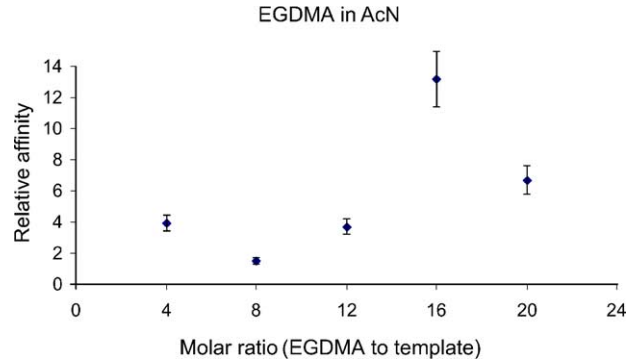

Fig. 5. Relative affinity of MIPs generated with different cross-linker to template ratios. Using ethylene glycol dimethacrylate (EGDMA) as cross-linker, *R*-(−)-phenylbutyric acid as template and acetonitrile (AcN) as porogen.

Interestingly, MIPs prepared in acetonitrile with EGDMA as cross-linker showed a significantly high affinity at a molar cross-linker to template ratio of 16:1 (Fig. 5). When using methanol as porogen, the EGDMA–MIPs exhibited very low specific affinities of similar values (Fig. 6), comparable with

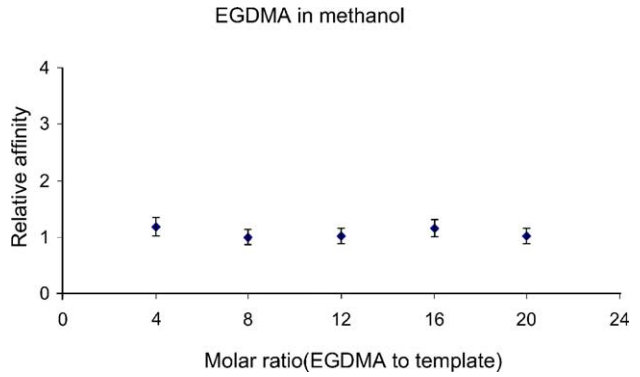

Fig. 6. Relative affinity of MIPs generated with different cross-linker to template ratios. Using ethylene glycol dimethacrylate (EGDMA) as cross-linker, *R*-(−)-phenylbutyric acid as template and methanol as porogen.

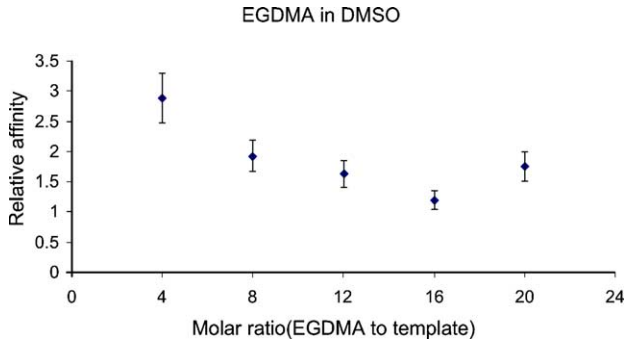

Fig. 7. Relative affinity of MIPs generated with different cross-linker to template ratios. Using ethylene glycol dimethacrylate (EGDMA) as cross-linker, *R*-(−)-phenylbutyric acid as template and dimethylsulfoxide (DMSO) as porogen.

the results obtained with DVB–MIPs in methanol. The affinities of EGDMA–MIPs prepared in DMSO show the highest imprinting effect again at a molar cross-linker to template ratio of 4:1 with a relative affinity of 2.89 (Fig. 7). The imprinting effect obtained with the EGDMA–DMSO combination appeared to be similar to those of the DVB–DMSO membranes. DMSO as a polar porogen may interact with functionalities of template and functional monomer (carboxylic group, hydroxyl group, pyridine) and affects the complexation. The absence of aromatic groups in EGDMA capable of π–π interaction may also account for the less efficient imprinting effect.

Finally, one exemplary set of results is given for the TRIM–MIPs generated with methanol (Fig. 8). In contrast to the effects of DVB- or EDMA–MIPs produced with methanol, TRIM–MIPs show significantly high affinities at molar cross-linker to template ratios of 4:1 and 8:1. When changing to other solvents, TRIM–MIPs exhibited their highest affinities at molar cross-linker to template ratios of 12:1 (DMSO) and 20:1 (AcN).

Reproducibility experiments showed that the standard deviation of the affinities towards the target molecule of five

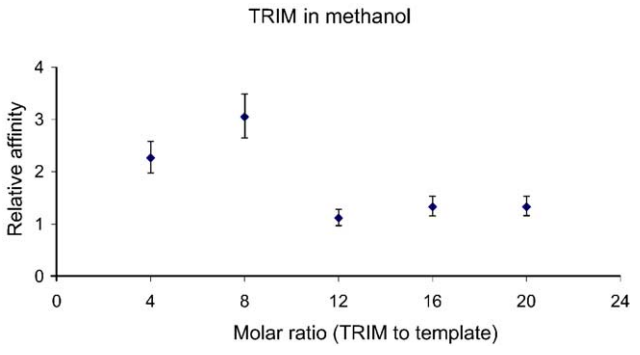

Fig. 8. Relative affinity of MIPs generated with different cross-linker to template ratios. Using trimethylolpropane trimethacrylate (TRIM) as cross-linker, *R*-(−)-phenylbutyric acid as template and methanol as porogen.

different MIPs prepared with the same recipe from a 1×10^{-3} mol/l solution of the target molecule in the porogen (acetonitrile) was 14%.

Furthermore, bleeding of templates from MIPs during rebinding experiments could be eliminated by simple and intensive washing of the polymer coated PTFE membranes during template extraction. In the published approaches of screening combinatorial libraries in vials, bleeding of template may occur, causing lack of sensitivity of the affinity measurement. Probably, the washing of the MIP can not be as extensive as in the technique presented here, and also the MIP layers inside the vials seem to be not thin enough to allow complete template extraction. Another advantage of this newly presented casting technique over the techniques of screening combinatorial libraries of MIPs described in literature is the shorter time of MIP generation and the smaller amount of material needed. Further experiments focussing the scale-up potentials of this technique are in progress.

4. Conclusion

A fast, easy and efficient procedure for screening combinatorial libraries of molecularly imprinted polymers has been developed. This technique of casting thin layers of MIPs on membranes in microfiltration modules for later screening of MIPs saves time, money and material compared to procedures published in literature. It can be applied by any MIP researcher for fast qualitative screening with acceptable reproducibility. Quantitative screening and higher reproducibility would be possible after optimizing the thickness of the casted MIP layer and using a more sensitive analytical tool (based on fluorescence or radio-labeling) for a better quantification of the exact amount of target molecule adsorbed on the membrane material.

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