Study of Selectivity of Molecularly Imprinted Polymers Prepared Under Different Conditions

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Abstract

Molecularly imprinted polymers were prepared and tested in different ways. 1-Methyl-2-piperidinoethylester of 4-decyloxyphenylcarbamic acid was used as the template for imprints formation. Acrylamide, 4-vinylpyridine, and methacrylic acid as monomers and methanol and acetonitrile as a porogene were used. Non-imprinted polymers were prepared for each imprinted polymer by the same procedure. Polymers were employed as sorbents for solid-phase extraction. In this work the influence of polymerization mixture composition on polymer properties, such as capacity and selectivity, was investigated. The influence of alkoxy-chain length and the position on benzene ring on the selectivity of polymers was also investigated.

Introduction

Molecularly imprinted polymers (MIPs) are tailor-made materials with a predefined selectivity for a given analyte or closely related compounds for which they were synthesized. These materials are obtained by polymerizing functional and cross-linking monomers around a template molecule, leading to a highly cross-linked three-dimensional network polymer. The monomers are chosen according to their ability to interact with the functional groups of the template molecule. Once polymerization has taken place, template molecule is extracted and binding sites with shape, size, and functionalities complementary to the target analyte are established. The resulting imprinted polymers are stable, robust, and resistant to a wide range of pH, solvents, and temperature (1).

In recent years, MIPs have shown to be useful materials as selective sorbents for a given target analyte and, in some cases, related substances in solid-phase extraction (SPE) procedures, namely MISPE. In contrast to classical sorbents used for clean-up procedures (reversed-phase or ion-exchange sorbents), MIPs provide high selectivity to SPE, allowing analytes to be eluted from cartridges almost free of co-extracted compounds (1). MISPE can be used for preconcentration of the target analyte and also for removing of other compounds from the sample matrix. In recent years, MISPE has been applied to the extraction of several compounds from different matrices, such as environmental samples (river water, ground water, wastewater, sea water, and soil extracts) (2–5), biofluids (urine, serum, plasma, blood) (6–8), tissue samples (9,10), food samples (11,12), and plants (8,12,13).

Three important factors that affect the formation of the pre-polymerization complex are: the type of functional monomer employed for complexation with the template; the relative ratio of functional monomer to template able to determine the degree of complexation of the template, and finally, the solvent (or porogen). The latter will regulate important features in the pre-polymerization complex such as hydrogen bond formation and the formation of pores of differing sizes and volumes. The functions of the porogen are to dissolve all of the components of the polymer and to allow the arrangement of the template-functional monomer pre-polymerization complex (14).

In this study, the influence of functional monomer and porogen, respectively, on the sorptive properties was investigated. The structurally related compounds (analytes with alkoxy-chain with in different position or different length) were utilized to study the selectivity of MIPs. All MIPs were prepared by non-covalent approach by bulk polymerization and used as the sorbent for SPE.

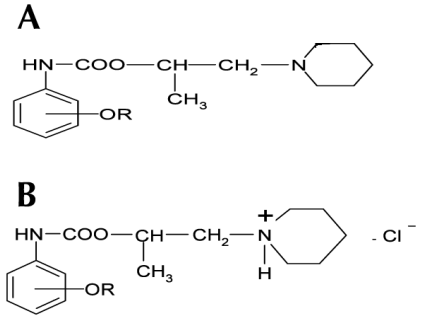

Figure 1. Structure of compounds used in research: (A) free base and (B) hydrochloride. Template molecule: R= - $C_{10}H_{21}$ in 4- position (4-DPCA). Other analytes used in research: R= - $C_{10}H_{21}$ in 2- position (2-DPCA).

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Experimental

Materials

1-Methyl-2-piperidinoethylesters of alkoxyphenylcarbamic acid were synthesized by the Pharmaceutical Faculty in Bratislava (Figure 1) (15). Acetonitrile, methanol, toluene, methacrylic acid, and diethylamine were purchased from Merck (Darmstadt, Germany). 4-Vinylpyridine was obtained from Aldrich (St. Louis, MO). Acrylamide and azobisizobutylonitrile were obtained from Fluka (Buchs, Switzerland), and acetic acid was purchased from Lachema (Brno, Czech Republic).

HPLC analysis

An HP 1100 system (Hewlett-Packard, Boeblingen, Germany), consisting of a pump with a degasser, a diode-array detector (DAD), a 50 µL injector, and a HP ChemStation were used. Analyses were carried out on the analytical column Separon SGX C_{18} (125 × 4 mm, 7 µm) (Watrex, Prague, Czech Republic) at laboratory temperature. Mobile phase consisted of methanol, acetonitrile, acetic acid, and diethylamine (80:20:0.1:0.1) at a flow rate of 0.5 mL/min. Isocratic elution was used. DAD worked in the range of 190–400 nm, and the chromatograms were acquired at a wavelength of 240 nm.

Table I. Composition of Polymerization Mixtures

Polymer	Form of template molecule	Porogene	Monomer
MIP1	Base	Methanol	Acrylamide
MIP2	Hydrochloride	Methanol	Acrylamide
NIP1,2	–	Methanol	Acrylamide
MIP3	Base	Acetonitrile	Acrylamide
NIP3	–	Acetonitrile	Acrylamide
MIP4	Base	Toluene	Acrylamide
NIP4	–	Toluene	Acrylamide
MIP5	Hydrochloride	Methanol	4-vinylpyridine
NIP5	–	Methanol	4-vinylpyridine
MIP6	Base	Acetonitrile	4-vinylpyridine
NIP6	–	Acetonitrile	4-vinylpyridine
MIP7	Base	Toluene	4-vinylpyridine
NIP7	–	Toluene	4-vinylpyridine
MIP8	Hydrochloride	Methanol	Methacrylic acid
MIP9	Base	Methanol	Methacrylic acid
NIP8,9	–	Methanol	Methacrylic acid
MIP10	Base	Acetonitrile	Methacrylic acid
NIP10	–	Acetonitrile	Methacrylic acid
MIP11	Base	Toluene	Methacrylic acid
NIP11	–	Toluene	Methacrylic acid

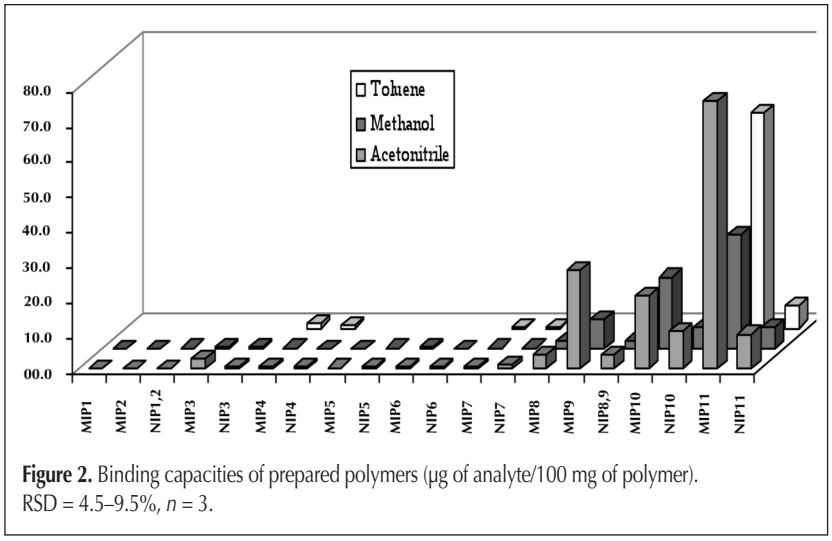

Figure 2. Binding capacities of prepared polymers (µg of analyte/100 mg of polymer). RSD = 4.5–9.5%, *n* = 3.

Polymer preparation

The molecularly imprinted polymer was prepared according to Zhang et al.'s method (16). For synthesis of MIPs, template (1-methyl-2-piperidinoethylester of 4-decyloxyphenylcarbamic acid – 4-DPCA) was applied as hydrochloride and base, respectively. The base was used mainly for polymers prepared in acetonitrile and toluene because of better solubility of base template in these solvents. The composition of polymerization mixtures is shown in Table I.

Evaluation of MIP

The cartridge capacity of each MIP and NIP was tested in methanol, acetonitrile, water, and toluene. Prior to applying the solution of derivative of 4-DPCA, the polymer was pre-equilibrated with 5 mL of methanol and then with 5 mL of solvent in which the capacity was studied. 4-DPCA solution (0.5 µg/mL) was applied onto the cartridge (effluent was collected in 1 mL fractions) until a release was detected. Each fraction was measured by high-performance liquid chromatography (HPLC). In the case of toluene, 5 mL of 4-DPCA solution dissolved in toluene (0.5 µg/mL in the case of MIP4, MIP7, NIP4, and NIP7) was applied. In the case of MIP11 and NIP11, 20 mL of 4-DPCA (5 µg/mL) was applied onto the cartridges. Then the cartridges were dried, and the adsorbed analyte was desorbed by methanol–acetic acid mixture (95:5). Effluent was dried, re-dissolved in methanol, and measured by HPLC. For polymers' capacity, the template was used in the same form (free base or hydrochloride) as it was used during polymerization.

The selectivity of MIP3, MIP 9, MIP10, and MIP11 for 1-methyl-2-piperidinoethylester of 4-methoxyphenylcarbamic acid (4-MPCA) and 2-decyloxyphenylcarbamic acid (2-DPCA) was tested. The selectivity of MIP3 and MIP11 in acetonitrile and MIP9 and MIP10 in methanol was determined. The procedure and the solution concentration were the same as described for 4-DPCA.

Results and Discussion

1-Methyl-2-piperidinoethylesters of phenylcarbamic acids are potential anaesthetics (15). Basic chromatographic parameters are described by Rencová (17).

Capacity of polymers

As it was described earlier, the capacities of MIPs were evaluated in different solvents. The

same procedure was performed on MIPs and NIPs, respectively, and the resultant values of polymer capacities for template molecules are shown in Figure 2. The recoveries of tested analytes adsorbed on MIPs and NIPs were in the range of 98–100%.

Polymers prepared with hydrochloride form as a template were tested for both forms (hydrochloride and base, respectively), and the values of capacities of each polymer (MIP1, 5, 8) were similar for both forms of template.

As it was mentioned in the introduction, the type of functional monomer and porogen plays an important role during the formation of pre-polymerization complex. It is obvious from Figure 2 that the specific capacity of polymers prepared with 4-vinylpyridine as a functional monomer is very low. Therefore, 4-vinylpyridine is not a suitable monomer for this type of template.

Table II. Binding Capacities of Prepared Polymers*

Polymer	Capacity (µg of analyte/100 mg of polymer)		
	Acetonitrile	Methanol	Toluene
MIP1 (AA-MeOH)	0.1	0	–
MIP2	0	0	–
NIP1,2	0.1	0.1	–
MIP3 (AA-ACN)	2.5	0.5	–
NIP3	0.4	0.4	–
MIP4 (AA-Tol)	0.7	0.1	1.2
NIP4	0.5	0	0.9
MIP5 (4VP-MeOH)	0.2	0.1	–
NIP5	0.3	0	–
MIP6 (4VP-ACN)	0.5	0.2	–
NIP6	0.6	0	–
MIP7 (4VP-Tol)	0.7	0.1	0.4
NIP7	0.8	0.1	0.4
MIP8 (MAA-MeOH)	3.8	2.0	–
MIP9	28.0	8.0	–
NIP8,9	4.0	1.9	–
MIP10 (MAA-ACN)	20.4	20.2	–
NIP10	10.7	5.9	–
MIP11 (MAA-Tol)	76.0	32.0	61.1
NIP11	9.7	6.2	6.6

* RSD = 4.5–13.5%, n = 3.

Table III. Binding Capacities of Prepared Polymers*

	Capacity (µg of analyte/100 mg of polymer)		
	Ester of 4DPCA	Ester of 4-MPCA	Ester of 2-DPCA
MIP3 (AA-ACN)	2.5	1.3	1.9
NIP3	0.4	0.3	0.4
MIP9 (MAA-MeOH)	8.0	9.2	4.8
NIP8,9	1.9	1.9	1.8
MIP10 (MAA-ACN)	20.2	18.1	10.2
NIP10	5.9	3.2	6.2
MIP11 (MAA-Tol)	76.0	75.1	76.2
NIP11	9.7	10.2	8,0

* RSD = 4.5–13.5%, n = 3.

Methacrylic acid seems to be the most convenient monomer for our template. All MIPs, with the exception of MIP8, prove the ability to bind the template molecule specifically. Different capacities of MIP8 in comparison to MIP9 demonstrate the influence of the template form on the ability to form the pre-polymerization complex. The MIP9 was prepared by using the base form, and the MIP8 was prepared with hydrochloride form.

When we compare the MIPs prepared with acrylamide, only MIP3 is able to bind the template specifically. It indicates the influence of porogene on the formation of pre-polymerization complex. Acetonitrile is a less polar solvent in comparison to methanol. In this case, less polar solvents optimize the interactions between monomer and template. More polar solvents such as methanol and water cancel the hydrogen bonding between monomer and template (18). The difference between acetonitrile and methanol is also in the way of creation of the hydrogen bonds – donor and acceptor.

The solvent used in the process of capacity determination is also very important. The nature of solvent employed for this step influences the relative swelling of the polymer. Swelling of the polymer causes changes in the binding site cavities. Solvent is also responsible to solvation process. It means that the size and shape of template molecule depends on the solvent. Combination of these factors affects the change of capacity of MIP.

The highest values of binding capacities were obtained using water (Table II) for sample loading for all MIPs and also for NIPs. The whole amount of loaded template (100 µg) was sorbed onto the sorbents. In aquaeous environments, hydrogen-bonding and electrostatic interactions could be disrupted, and hydrophobic interactions, which are non-specific, could govern analyte retention (19). This allows use of water samples for extraction, of course, after sample loading. The cartridge should be dried and washed with selective organic solvent able to disrupt the non-specific interaction of analyte with polymer.

Capacity of MIPs for structurally related compounds

The procedure of determination of capacity of MIP3, MIP9, MIP10, and MIP11 for structurally related compounds was the same as for template. The influence of length of alkoxy-chain on benzene ring was tested using by 4-MPCA. The influence of position of alkoxy-chain was investigated by measuring of MIPs' capacity for 2-DPCA. The polymer capacities of MIPs and relevant NIPs are shown in Table III.

As it is obvious from Table III, the capacity of MIP3 is lower for analyte with shorter alkoxy-chain (4-MPCA) than for template. Therefore, the length of alkoxy-chain impacts the capacity of analyte, and MIP3 can recognize template molecule from structurally related compounds.

The influence of alkoxy chain position on benzene ring was also tested. The difference between capacity values of the template (4-DPCA) and the analyte with alkoxy chain in ortho-position (2-DPCA) is not very significant. But in the case of MIP9 and MIP10, the capacity of MIPs is two times higher for template than for the analyte with alkoxy-chain in ortho-position. It demonstrates that MIP9 and MIP10 have different selectivities for compounds with decyloxy-group in other positions on the benzene ring.

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