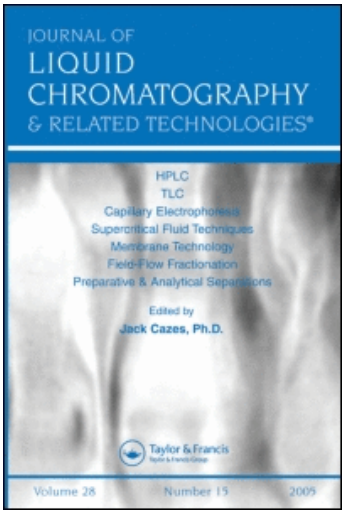

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Synthesis of Molecularly Imprinted Copolymer and its Application as a SPE Sorbent for Preconcentration of Metoprolol and Vitamin B_6 from Water

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Abstract: Molecularly imprinted polymer – copolymer of methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EGDMA) with imprints of metoprolol molecules was synthesized. To optimize conditions of its synthesis, the following parameters were studied: influence of molar ratio of the monomer (MAA) to the crosslinker (EGDMA), chemical nature of porogen, and its volume ratio to monomers mixture on the porous structure of the final copolymer.

In order to study the sorption properties of this polymeric material, the recoveries and breakthrough volume of metoprolol and vitamin B_6 were determined. Their values are compared with those obtained for the MAA-EGDMA reference copolymer.

Keywords: Ethyleneglycol dimethacrylate, Methacrylic acid, Metoprolol, Molecular imprinting

INTRODUCTION

Molecular imprinting is the process, which leads to preparation of a polymer with imprints of the template molecule in its structure. In an illustrative way, it is possible to say, that molecularly imprinted polymers

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(MIPs) have "holes" of shapes and sizes similar to those of the template molecule. These places in the polymer structure are characterized by high affinity to molecules of structures similar to those of templates. Similarity between the template molecules and their imprints in the MIPs structure is caused by suitable location of functional groups, which are compatible with proper functional groups in the template molecules, on the polymer surface. In this way, complementary interactions between the template molecules and the surface of polymer matrix occur. Imprints of template molecules in the polymer structure are formed during polymerization.[1–5]

The imprinting process (covalent or non–covalent) consists of three following stages:[6,7]

1. formation of prepolymerization complex: monomer(s) template
2. polymerization of monomer(s) and crosslinker(s)
3. washing of the obtained polymer off the template molecules.

The most important stage of imprinting process is formation of the complex between monomer(s) and molecule of template. This stage decides about success of the whole process. Interactions between monomers and template can be of covalent character, but covalent bonds between functional groups of monomers and template have to be reversible. Ester, acetal, ketal, and imine (Schiff's base) bonds are the most common reversible covalent bonds exploited in covalent imprinting.[8] The advantage of covalent imprinting consists of information in the polymer structure sites of very high affinity for template molecules because covalent bonds are stronger than non-covalent interactions. The main disadvantage of this method is necessity of using waterless, expensive reagents, which prevent hydrolysis of reversible bonds during polymerization.

Prepolymerization complex can be also formed on the basis of non–covalent interactions between monomers and template molecules. The most common non–covalent interactions used in the imprinting process are hydrogen bond, ion pair, dipole interactions, and Van der Waals forces. The disadvantage of non–covalent imprinting is associated with instability of the prepolymerization complex. Stability of the prepolymerization complex is affected by temperature of polymerization, types and concentration of solvents, etc. On the other hand, specific, high purity, and expensive reagents are not necessary in this method.

A polymerization mixture has the following components:

- template, which is the most important component of polymerization. Its molecules determine properties of other components, especially monomer(s). These molecules can not contain functional groups, which are able to take part in polymerization or which inhibit free

radical polymerization. The template must be stable under polymerization conditions.[9]
- monomer(s) with suitable functional groups. Molar ratio of the monomer to the template determined in an empirical way is also important.[9,10]
- crosslinker, which is responsible for morphology of the obtained polymer and its mechanical resistance.[10]
- solvent (porogen), which performs two main functions: dissolve all components of the polymerization mixture and is responsible for formation of the polymer porous structure.[10,11]
- initiator of polymerization directly responsible for the synthesis of molecularly imprinted polymer, according to the free radical mechanism.

In preparation of MIPs different methods are used. Besides the most popular bulk polymerization, suspension, precipitation, emulsion, and multi step swelling polymerizations are attempted to be applied.[11–13] The most important is the fact that is possible to synthesize MIPs of high affinity for many organic compounds. High affinity of MIPs makes it possible to analyze very complicated samples, especially of biological origin, in which analytes occur in trace amounts.[12]

The paper presents the synthesis of polymers imprinted with the metoprolol molecules using the bulk polymerization. Choice of proper monomer and influence of different solvents on the structure of molecularly imprinted polymer are studied.

The obtained MAA-EGDMA copolymer was used for preconcentration of metoprolol and vitamin B_6 from the water samples. Efficiency of preconcentration was compared with that on the MAA-EGDMA copolymer without imprints.

EXPERIMENTAL

Materials

2,2′–Azobisisobutyronitrile (AIBN), and methacrylic acid purity $\geq$98% came from Fluka AG (Buchs Switzerland). 1,4–Dioxane purity (GC) $\geq$ 99.8%, acetonitrile purity (GC) $\geq$ 99.8%, dichloromethane purity (GC) $\geq$ 99.9%, ethyleneglycol dimethacrylate purity (GC) $\geq$ 98%, and hexane purity (GC) $\geq$ 99.8% were from Merck (Darmstad Germany). Acetone, toluene, dichloromethane, sodium hydroxide, hydrochloric acid (35–38%) reagent grade, and bromothymol blue were from POCh (Gliwice, Poland). Metoprolol was obtained from the commercially available drug produced by ICN Polfa (Rzeszów, Poland) by extraction

with dichloromethane. B_6 vitamin was a commercially available injection fluid produced by PLIVA (Kraków, Poland).

In SPE and HPLC analyses Milli-Q water (Millipore, Bedford, USA) with conductivity of $18.2\,m\Omega \cdot cm^3$ and methanol of LiChrosolv quality from Merck were used.

Optimalization of Polymerization Procedure

Methacrylic acid (MAA) and ethyleneglycol dimethacrylate (EGDMA) were copolymerized in the presence of the following porogens: toluene, 1,4–dioxane, chloroform, methanol, and acetonitrile. The total mass of both components was 5 g, while the volume of porogen was increased from 1 to 10 mL. 2,2′–azobisisobutyronitrile (AIBN) was used as the initiator in the amount of 0.02 g.

The polymerization process was carried out in a test tube at 60°C. In order to avoid evaporation of solvent – porogen, test tubes were plugged up. In every plug two glass capillaries were located, preventing the increase of the pressure inside the test tubes during the process.

The obtained copolymers were ground. Next, they were sieved through the sieve with a diameter of screen opening Ø 0.063 mm. The particles with diameters smaller than 0.063 mm were decanted in acetone, in order to separate particles with too small diameters. The particles of diameters in the range from 5 to 25 μm were isolated, and extracted in the Soxhlet apparatus using acetone and methanol (4–5 hours). Finally, they were dried in a vacuum dryer.

Synthesis of Polymer with the Imprints of Metoprolol and Reference Polymer

The polymer imprinted with metoprolol molecules was obtained in exactly the same way as the reference polymer. Both polymers were obtained in bulk polymerization. The previous studies showed that homogeneous mixtures were obtained when the molar ratio of MAA to EGDMA was 1:4, thus this ratio was maintained in the syntheses presented here. The total mass of MAA (monomer) and EGDMA (crosslinker) was always 5 g. The volume of the added porogen was always constant and it was equal to 5 mL.

The synthesis procedure was as follows:

1. 0.49 g (6 mmol) methacrylic acid and 4.51 g (23 mmol) ethyleneglycol dimethacrylate were placed into the test tubes.
2. The contents of test tubes were mixed by shaking very thoroughly.

3. Porogen consisting of 4 mL of toluene and 1 mL of methanol was added into every single test tube, and mixed.
4. 0.29 g (0.85 mmol) tartrate of metoprolol was added only into these test tubes, in which molecularly imprinted polymers were synthesized. The molar ratio of metoprolol cations to methacrylic acid in the initial polymerization mixture was 1:7, as 1 mole of tartrate metoprolol salt contains 2 moles of ammonium metoprolol cations. The contents of test tubes were mixed.
5. 0.02 g of 2,2′–azobisisobutyronitrile (AIBN) used as the initiator was added into every single test tube.
6. To homogenize solutions test tubes were placed on ultrasound bath for 5 minutes. In order to avoid increase of pressure inside the test tubes during the process, two glass capillaries were located in every plug of test tube.
7. Test tubes were maintained on the water bath at 60°C for 7 hours.
8. The obtained polymers were ground in a mill.
9. Particles of the diameters from 5 to 25 μm were isolated and extracted with acetone and methanol in a Soxhlet apparatus in order to remove template molecules (4–5 hrs).
10. Finally, they were dried in a vacuum dryer.

Characterization

Porous structure of the obtained polymeric materials was characterized by nitrogen adsorption isotherms measured at −196°C using the adsorption analyzer ASAP 2405 (Micrometrics Inc., USA). Before measurements, the samples were outgassed at 150°C for 1 h. The specific surface area was calculated using the BET method, the total pore volume was calculated as the volume of liquid adsorbate at $P/P_0 = 0.99$, while the volume of micropores was calculated by the DR method.

Skeletal density of the copolymer particles was measured by pycnometry with methanol as a confining fluid.

To determine the number of carboxyl groups on the polymer surface the following procedure was used: 0.1 g of the studied polymer was placed into a conical flask containing 10 mL of the standard solution of NaOH (concentration 0.0099 mol/L). Next, 20 mL of the distilled water and 5 drops of 0.05% aqueous solution of bromothymol blue were added. The flask was maintained on the water bath at 70°C for 0.5 h. After cooling it to room temperature, the excess of NaOH was titrated using the standard solution of hydrochloric acid (concentration 0.0024 mol/L) in the presence of bromothymol blue. The stoichiometric end point of titration was indicated by the change of bromothymol blue colour from blue to yellow.

Chromatographic and Elemental Analyses of Metoprolol

The chromatographic analysis of metoprolol was performed using a Waters 2690 Alliance liquid chromatograph (Waters, USA). The apparatus was equipped with an automatic sampler, the UV detector Waters 2487, and a 250×4 mm I.D. LiChrosorb RP-18 (7 μm) column. As a mobile phase, water-methanol (20:80, v/v) at a flow rate of 1 mL min^{-1} was applied. Detection was performed at 254 nm. The elemental analysis was carried out on a Perkin Elmer CHN 2400 analyzer (Palo Alto, CA, USA).

SPE Experiments

SPE experiments were carried out using a chamber produced by J.T. Baker, type spe-12 G. Minicolumns were packed with 100 mg of MIP and reference sorbents. Experiments were carried out simultaneously in 10 minicolumns. Five of them contained MAA-EGDMA polymer with imprints of metoprolol, while the other 5 copolymers were without molecular imprints.

Recovery studies of metoprolol on both types of sorbents were determined. As adsorption of vitamin B_6 should be the same on both studied sorbents, it was included in recovery studies.

Stock standard solutions were prepared by weighing the compounds and dissolving them in methanol. A standard methanolic solution contained 1 mg/mL of each compound. Working aqueous solutions were prepared directly before use by diluting 0.5 mL of stock solution up to 500 mL with Millipore-Q water. The obtained solution contained 1 μg/mL of each compound.

Before sampling, each minicolumn was activated with 10 mL of methanol. Then, different volumes of working aqueous solution were sucked through the minicolumn with a flow velocity ~20 mL/min. After the sample solution was passed through the minicolumn, drying of the sorbent under vacuum was maintained for 15 min. Then the tested compounds were eluted with methanol.

The concentration of tested compounds was measured by injecting 20 μL of eluate into the Waters 2690 Alliance liquid chromatograph. For calibration, the same volume of stock solution was also injected into the chromatograph. The recovery (in %) of the tested compound was calculated by comparing peak areas.

As a mobile phase, water-methanol (35:65; v/v) at a flow rate of 1 mL/min was applied. Detection was performed at 254 nm.

RESULTS AND DISCUSSION

Chemical Structure of Metoprolol and Choice of Monomers for the Polymerization Process

Metoprolol is a popular drug from a group of β-blockers. Metoprolol blocks only β_1 receptors, that are located in the muscles of heart, blood vessels, and brain. This selective β-blocker diminishes the influence of epinephrine and norepinerphrine on β_1 receptors. In order to choose suitable reagents for polymerization (monomers and crosslinkers) it is necessary to take chemical structure of metoprolol into consideration.

The molecule of metoprolol contains one chiral atom and occurs in two enantiometric forms (Figure 1). The S isomer gives more effective therapeutic results, but methoprolol as a drug is rather produced as the recemate.[14]

In its chemical structure four functional groups, capable of interacting with the functional groups of monomers and crosslinkers, are presented. There are: 2^o amino, hydroxyl, and two ether groups. Two oxygen atoms, belonging to ether group, can generate hydrogen bonds with hydrogen atoms, which are connected with strong electronegative atoms. Hydrogen atoms from the hydroxyl group of metoprolol can form hydrogen bonds with strong electronegative atoms, like oxygen or nitrogen. 2^o Amino group of metoprolol can generate ionic bonds with the acidic reagent. Methacrylic acid was chosen as a monomer capable of forming compatible interactions with metoprolol (Figure 2).

Due to occurrence of four different possible interactions between the molecules of metoprolol and methacrylic acid, a variety of prepolymerization complexes should be expected.[15] In Figure 2, two cases are presented. The simplest prepolymerization complex (I), in which only one interaction (ionic bond) between the template and the monomer occurs and the most complicated prepolymerization complex (II), in which four different interactions between metoprolol and methacrylic acid are possible.

Due to formation of different forms of prepolymerization complexes, which are generated during polymerization of monomer and crosslinker, adsorption centers could show different affinity for template molecules. Homogeneity of adsorption centers in a produced molecularly imprinted

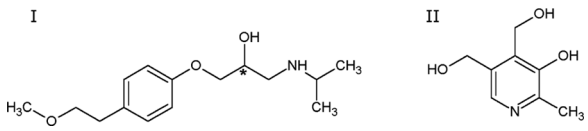

Figure 1. Chemical structures of metoprolol (I) and vitamin B_6 (II).

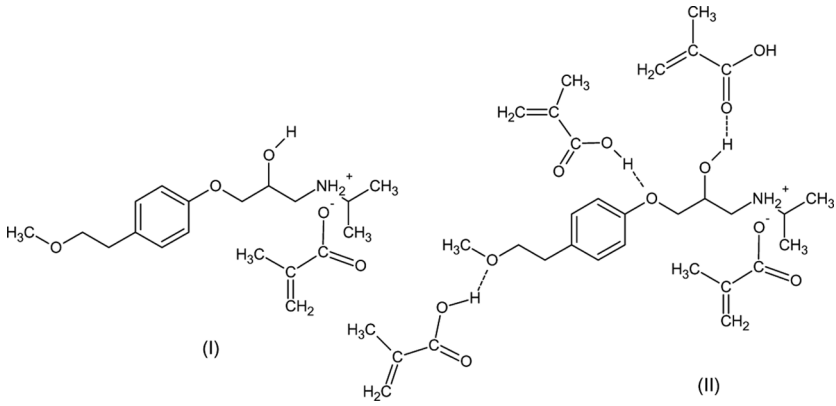

Figure 2. Chemical structure of prepolymerization complex.

polymer is very important in the case of their analytical applications. Figure 3 presents formation of adsorption centers of the strongest affinity for metoprolol.

Influence of Molar Ratio of MAA to EGDMA

In these studies, constants volume of porogen was used. Independently of the type of porogen, a homogeneous solution was formed when its volume ratio to the monomers mixture was 1:1. This ratio was maintained in all syntheses carried out in the presence of toluene, 1,4-dioxane, chloroform, methanol, and acetonitrile as porogens.

The results obtained for MAA-EGDMA copolymer synthesized in the presence of toluene are presented in Table 1. From these data, one can see that with the increase of molar ratio of the monomer to the crosslinker from 1:1 to 1:4, specific surface areas of the obtained copolymers also increased. Further increase of this ratio does not cause increasing of the specific surface area. Similar tendency is observed for the copolymer pore volume. Simultaneously, the increase of molar ratio of EGDMA to MAA is accompanied by the increase of microporous volume. Large differences in values of average pore diameters and the most probable pore diameters confirm that all synthesized copolymers have two dispersive structures. Mesoporous structure with rather small contribution of miroporess caused that copolymer synthesized from MAA and EGDMA in the molar ratio of 1:4 to be chosen as the most suitable for further studies.

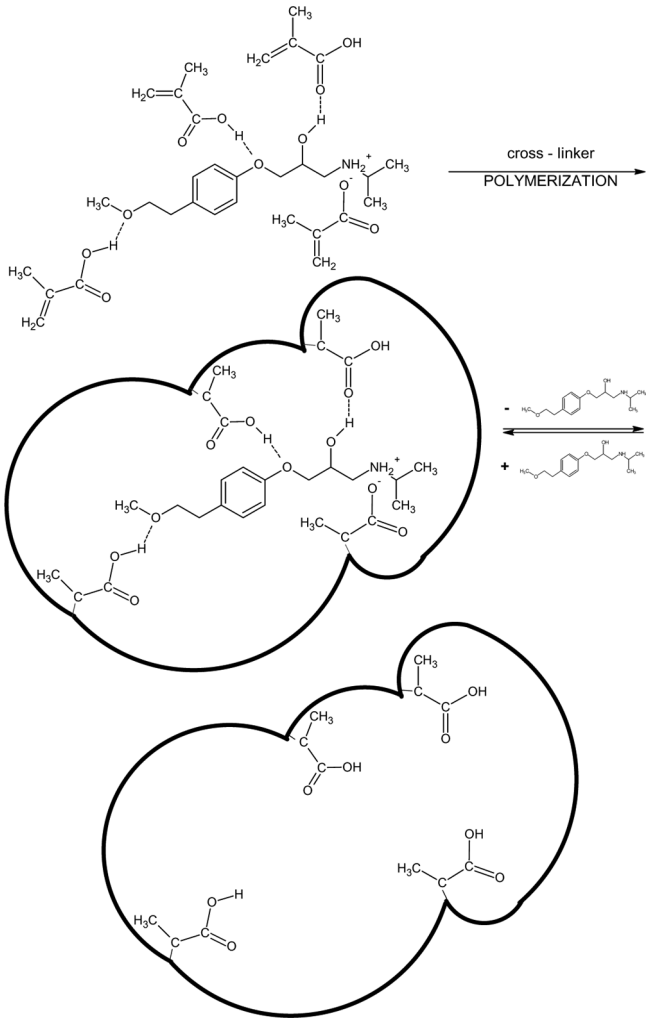

Figure 3. Formation of adsorption centers of the strongest affinity for metoprolol.

Influence of Type of Porogen Used

Influence of type of porogens used was studied for copolymers synthesized from MAA to EGDMA in the molar ratio 1:4. In Figure 4, the relationship between type of porogen used in the polymerization process and specific surface areas of the obtained copolymers is presented. The solvents are put in order of their increasing dipole moments. From these data, one can see that the polymer synthesized in the presence of toluene has the smallest surface area, while the one in methanol has the largest one.

Table 1. Influence of molar ratio of the MAA to EGDMA on porous structure of the obtained copolymers

	Molar ratio MAA : EGDMA				
Porous structure	1:1	1:2	1:3	1:4	1:5
Specific surface are (m^2/g)	25.1	183.7	267.3	351.0	338.4
Pore volume (cm^3/g)	0.063	0.449	0.583	0.665	0.617
Volume of micropores (cm^3/g)	0.0	0.001	0.006	0.014	0.014
Average pore diameter (Å)	100	100	90	75	75
The most probable pore diameter (Å)	550	500	300	415	250

Influence of Porogen Concentration

Despite the fact that toluene forms a polymer with the smallest specific surface area, for further studies this solvent was chosen as a porogen component. This choice was imposed by the nature of interactions in the prepolymerization complex of metoprolol and methacrylic acid in the initial polymerization mixture. In a predicted complex, three hydrogen bonds and one ionic pair can be formed. As stabilization of hydrogen bonds can be ensured by a non polar solvent we decided to use toluene as one porogen component. As the second polar solvent, methanol was used. This solvent can stabilize ionic bonds between the

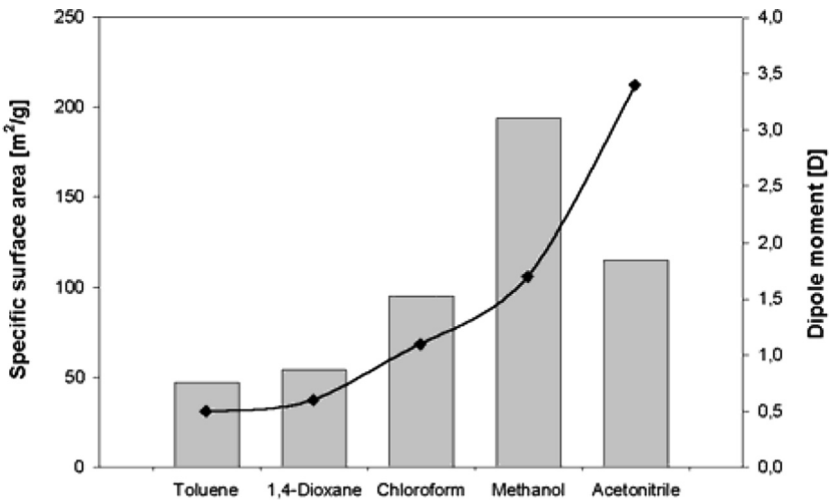

Figure 4. Influence of type of porogen used on copolymers specific surface area in relation to solvents dipole moments.

Table 2. Influence of volume ratio of diluents mixture (toluene + methanol) to the mass of monomers (MAA + EGDMA) on porous structure of the obtained copolymers

	Volume ratio of diluents mixture to the mass of MAA + EGDMA						
Porous structure	0.25:1	0.5:1	0.75:1	1:1	1.5:1	2:1	2.5:1
Specific surface are (m^2/g)	80.9	142.5	262.2	289.2	321.8	367.7	351.0
Pore volume (cm^3/g)	0.165	0.388	0.412	0.720	0.505	0.582	0.529
Volume of micropores (cm^3/g)	0.0	0.0	0.0	0.01	0.01	0.01	0.02
Average pore diameter (Å)	80	180	65	100	60	75	60
The most probable pore diameter (Å)	100	230	320	400	20/320	20/400	20/350

ammonium cation of metoprolol and the anion of methacrylic acid. Concentration of methanol in the porogen mixture was constant. It was equal to 20%. Good solubility of metoprolol in methanol was another reason for its addition. Pure toluene is a not a good enough solvent for a template.

Our attention was focused on preparing the MAA-EGDMA polymer of developed porous structure (Table 2). The increase of volume ratio of diluents mixture (toluene + methanol) in the range from 0.25:1 to 1.5:1 causes the increase of specific surface area from 80 to 320 m^2/g. During further increase of this ratio the specific surface area remains at a constant level. For the volume ratio larger than 1:1, two dispersive porous structures are formed. The copolymer synthesized in the presence of the ratio equal to 1:1 has the most regular structure. As the smallest volume of solvent is advantageous in preparation of molecularly imprinted polymers, in other syntheses the volume ratio of diluents to monomers mixture equal to 1:1 was used.

Comparison of the Polymer with the Imprints of Metoprolol and the Reference Copolymer

In Tables 3 and 4 the properties of the MAA-EGDMA copolymer are compared with those of the imprinted metoprolol molecules. Both copolymers were synthesized under the same conditions. From these data one can see that their properties are very similar. Concentration of carboxyl groups does not indicate any differences. Their porous structure, e.g., specific surface area and pore diameter are almost the same. Only pore volume is larger in the case of the imprinted polymer.

Table 3. Comparison of the porous structures of the studied copolymers with and without imprints of metoprolol

	MAA-EGDMA copolymer	
Porous structure	Imprinted	Reference
Specific surface are (m^2/g)	289.2	287.0
Pore volume (cm^3/g)	0.720	0.670
Volume of micropores (cm^3/g)	0.010	0.006
Average pore diameter (Å)	100	95
The most probable pore diameter (Å)	400	410

Their swelling properties are also different. Copolymers imprinted with metoprolol molecules swells greatly in all the studied solvents. Its swellability coefficient in dichloromethane is especially high. The lowest differences in the volumes are observed after their swelling by acetonitrile.

Sorption Properties

In these studies, besides metoprolol vitamin B_6 is used. Vitamin B_6 (pyridoxine) possesses similar functional groups to those of metoprolol but its chemical structure is quite different (Figure 1). Both compounds can appear in physiological fluids.[16]

Recovery studies require complete separation of the peaks of the standard compounds and large peak areas to minimize errors.[17,18] The best separation between metoprolol and vitamin B_6 was obtained in the mobile phase water–methanol (35:65; v/v) at a wavelength of 254 nm. The results from elemental and HPLC analyses confirmed that metoprolol used in SPE experiments was pure.

Table 4. Properties of the MAA-EGDMA copolymers with and without imprints of metoprolol

	MAA-EGDMA copolymer	
Properties	Imprinted	Reference
Swellability coefficient (%)		
Methanol	33.5	21.5
Dichloromethane	38.9	13.0
Acetonitrile	18.7	14.8
Concentration of COOH groups (mmol/g)	.600	0.606
Skeletal density (g/cm^3)	.11	1.16

Table 5. Comparison of recoveries of metoprolol and vitamin B_6 on the studied sorbents for 100 mL samples of fortified water

	Recovery (%)	
Compound	Imprinted	Reference
Metoprolol	58.6	2.8
Vitamin B_6	0.7	2.8

In Table 5 the recoveries of the studied compounds from 100 mL of aqueous solution obtained on the imprinted and reference copolymers are presented. These data show that recovery of metoprolol on the imprinted polymer is much higher than that on the reference sorbent. Recoveries of vitamin B_6, which can not be adsorbed specifically, are comparable on both studied sorbents.

Recovery of these compounds as a function of the sample volume for both studied sorbents, are presented in Figure 5. For volumes smaller than 25 mL recovery of metoprolol on the imprinted polymers is 100%. Its recovery decreased from 58.6 to 2.0% with the increase of sample solution volume from 100 to 500 mL. Recovery of metoprolol on reference

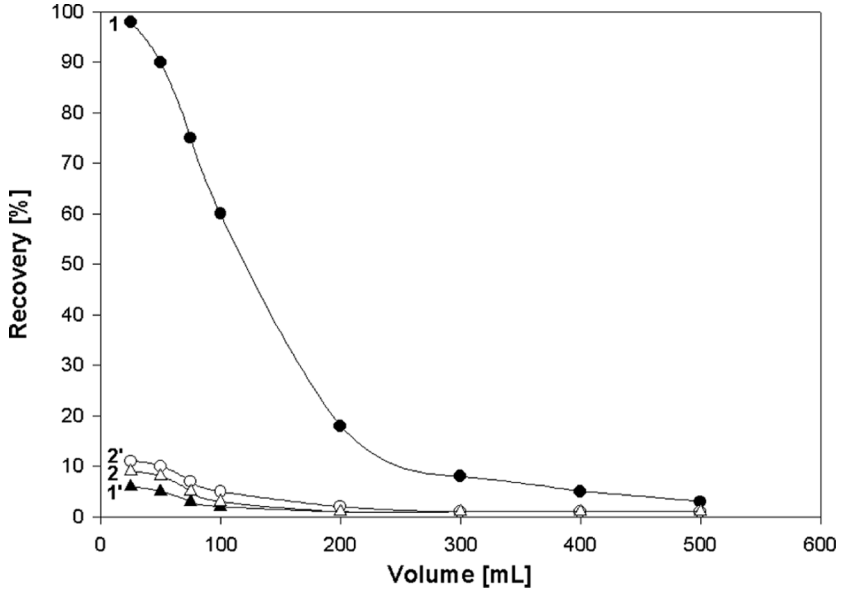

Figure 5. Recoveries of metoprolol (1 and 1′) and vitamin B_6(2 and 2′) as a function of sample volume for the imprinted and reference polymer, respectively.

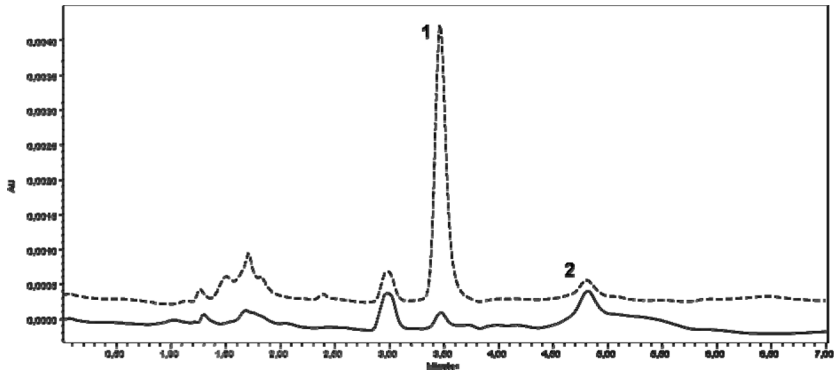

Figure 6. Chromatogram of eluates obtained for 100 mL preconcentarted samples on imprinted (dotted line) and reference (continuous line) polymers Peaks: 1 = metoprolol; 2 = vitamin B_6.

sorbent is small. Its value changes from 5.8% for 25 mL to 2.8% for 100 mL and 0.1% for 500 mL volume of preconcentrated sample.

Differences in efficiency in extraction and concentration of metoprolol and vitamin B_6 from water are shown on the chromatogram of eluates obtained for 100 mL preconcentarted samples (Figure 6). On the chromatogram for the molecularly imprinted polymers the peak of metoprolol is much higher than that of vitamin B_6, while on that for the reference polymer their peaks are small and have comparable surface areas. The results of the blank test for 5 samples of imprinted polymers show that ~4.8% of metoprolol appear in eluate.

CONCLUSIONS

The choice of suitable monomer and crosslinker for preparation of molecularly imprinted polymers with the imprints of metoprolol molecules is discussed. A non-covalent prepolymerization complex characterized by a strong affinity for metoprolol is formed when MAA and EGDMA are used for copolymerization.

The reference MAA-EGDMA copolymer synthesized in the presence of a mixture of toluene and methanol is also synthesized. Both copolymers are used in preconcentration of metoprolol and vitamin B_6. Recoveries and breakthrough volumes confirm higher efficiency of sorption of MAA-EGDMA copolymer imprinted with metoprolol molecules.

The results show that the imprinting process was successful. The obtained copolymer can find applications in SPE of physiological fluids.

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