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Restricted access molecularly imprinted polymers obtained by bovine serum albumin and/or hydrophilic monomers' external layers: a comparison related to physical and chemical properties

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Abstract

Molecular imprinting polymers (MIPs) can be modified with external layers in order to obtain restricted access molecularly imprinted polymers (RAMIPs) able to exclude macromolecules and retain low weight compounds. These modifications have been frequently achieved using hydrophilic monomers, chemically bound on the MIP surface. Recently, our group proposed a new biocompatible RAMIP based on the formation of a bovine serum albumin coating on the surface of MIP particles. This material has been used to extract drugs directly from untreated human plasma samples, but its physicochemical evaluation had not been carried out yet, mainly in comparison with RAMIPs obtained by hydrophilic monomers. Thus, we proposed in this paper a comparative study involving the surface composition, microscopic aspect, selectivity, binding kinetics, adsorption and macromolecule elimination ability of these different materials. We concluded that the synthesis procedure influences the size and shape of particles and that hydrophilic co-monomer addition as well as coating with BSA do not alter the chemical recognition ability of the material. The difference between imprinted and non-imprinted polymers' adsorption was evident (suggesting that imprinted polymers have a better capacity to bind the template than the non-imprinted ones). The Langmuir model presents the best fit to describe the materials' adsorption profile. The polymer covered with hydrophilic monomers presented the best adsorption for the template in an aqueous media, probably due to the hydrophilic layer on its surface. We also concluded that an association of the hydrophilic monomers with the bovine serum albumin coating is important to obtain materials with higher capacity of macromolecule exclusion.

Keywords: molecularly imprinted polymers, restricted access materials, restricted access molecularly imprinted polymers, MIP, RAMIP, polymer characterization.

Introduction

The low molecular weight compounds' analysis, in biological samples, requires simple, reliable and quick analytical methods. However, the complexity of the biological matrix makes it necessary to have a sample pretreatment in order to remove the concomitants (often at high concentrations) as well as to concentrate the analytes [1-3].

For this reason, studies about selective sorbents that are able to purify complex samples have been growing continuously [4], where two sorbents have been gaining space: i) molecularly imprinted polymers - MIPs, which are synthetic polymers capable to selectively bind to the target analytes [5] and ii) restrict access materials – RAMs, which are able to retain low molecular weight analytes and remove macromolecules such as proteins and polypeptides [6]. Despite their high selectivity to a molecule or a class, MIPs can retain macromolecules on their surfaces. Therefore, biological fluids, such as plasma or serum, can only be extracted by MIPs after eliminating the macromolecules by a previous sample preparation step. As far as RAM, they are very efficient in removing macromolecules, but they have no molecular recognition and, therefore, they are poor in selectivity [7-8]. Thus, in order to join the advantages of both MIPs and RAM, some researchers have proposed the combination of these technologies. Their aim was to obtain a polymer for the selective extraction of analytes from complex samples, with molecular recognition enhanced in aqueous medium and, at the same time, capable to eliminate macromolecules [9].

Haginaka and co-authors were the pioneers. They proposed the synthesis of a new kind of material called restricted access molecularly imprinted polymer (RAMIP) selective to (S)-naproxen [10-11], (S)-ibuprofen [10], and propranolol [12]. Their

proposal was to cover a conventional MIP with a layer of hydrophilic co-monomers. The obtained polymer presented good sensitivity and selectivity to extract the target analytes from biological samples and good capability to eliminate macromolecules due to the presence of polar groups on the particle surfaces. Similarly, Puoci and co-authors [13] as well as Hua and co-authors [14] obtained RAMIPs by using different hydrophilic co-monomers and had good results in terms of selectivity and the exclusion of macromolecules were obtained in both cases.

The most recent addressed strategy was developed by Moraes and co-authors [15]. They synthetized a MIP that was coated with bovine serum albumin (BSA) using glutaraldehyde as a cross-linker, resulting in a protein chemical shield around it (restricted access molecularly imprinted polymer covered by bovine serum albumin - RAMIP-BSA). In high pHs, this biocompatible material was able to selectively retain the template molecule. At the same time, ca. 99% of the protein from human plasma samples were eliminated, due to the electrostatic repulsion between these proteins and the BSA layer fixed on the polymer surface (both negatively charged).

Thereby, to better understand the behavior of the RAMIPs obtained by hydrophilic monomers and/or the BSA external layers, as well as the consequences of each superficial modification, we proposed a comparative study involving the characterization of different polymers selective to oxprenolol, with and without each one of the above-mentioned surface modifications.

Experimental

Chemicals and solutions

HPLC-grade acetonitrile and methanol were obtained from Vetec (Rio de Janeiro, Brazil). The work solutions and buffers were prepared by using deionized water (18.2 MΩcm) obtained from a Milli-Q water purification system (Millipore, Bedford, USA). For the syntheses of the MIPs, oxprenolol (OXP), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and 2,2'-azobisisobutyronitrile (AIBN) were used as the template, functional monomer, cross-linker and initiator, respectively (all from Sigma-Aldrich, Steinheim, Germany). HPLC-grade acetonitrile was used as the porogenic solvent. Glycerol dimethacrylate (GDMA) and hydroxy methyl methacrylate (HEMA) (both from Sigma-Aldrich) were used as hydrophilic co-monomers in the syntheses of the RAMIPs. Methanol and acetic acid (Merck, Darmstadt, Germany) were employed during the polymer washing steps. To perform the external coating of the materials with protein, bovine serum albumin (BSA) (Sigma-Aldrich), glutaraldehyde (Rioquímica, São José do Rio Preto, Brazil) and sodium borohydride (Sigma-Aldrich) were used. Monobasic and dibasic potassium phosphates (Synth Diadema, Brazil) were used to prepare a phosphate buffer pH 7.0, 0.01 mol L^{-1}.

Stock solutions of OXP, metoprolol (MET), labetalol (LAB), propranolol (PROP) and nadolol (NAD) (all from Sigma-Aldrich, and at concentrations of 1.0 and 2.0 mg L^{-1}) were prepared in HPLC-grade methanol, placed in amber flasks and stored at −5.0°C for up to 30 days. HPLC-grade methanol and ammonium formate (Fluka, Seelze, Germany) were used to prepare the mobile phases for the LC-MS/MS analyses.

Syntheses of the polymers

Four imprinted polymers and their corresponding non-imprinted polymers (NIPs) were synthesized in this study. Table 1 summarizes each one of them, according to the presence or absence of each synthesis step. The detailed procedures are described below.

For the MIP synthesis, 1.0 mmol of OXP and 4.0 mmol of MAA were dissolved in 48.0 mL of acetonitrile in a 250 mL three-mouth glass flask. Then, 7.0 mmol of EGDMA and 25.0 mg of AIBN were added, and the mixture was purged with nitrogen for 20 min. The flask was connected to a condenser in order to avoid the solvent loss due to evaporation. The apparatus was immersed in a glycerin bath, agitating at 65^{o}C, for 24 h. A NIP was obtained as MIP , but in the absence of OXP (template).

The RAMIP was obtained employing the same quantities of OXP, MAA, EGDMA and AIBN previously used in the MIP synthesis. Initially, the reagents were dissolved in 24.0 mL of acetonitrile and the synthesis was carried out as it was for MIP, but for only 1 h. At this moment, a mixture of 7.5 mmol of HEMA, 0.5 mmol of GDMA (both hydrophilic monomers) and 24.0 mL of acetonitrile were purged with nitrogen for 20 min and added into the synthesis flask. The polymerization was carried out for 23 h more. A restricted access non-imprinted polymer (RANIP) was synthesized the same way as the RAMIP was, but in the absence of template molecule.

For each polymer, particles from 75.0 to 106.0 µm in size were selected using steel sieves. Approximately 2.0 g of each were washed in an ultrasonic bath with 10.0 mL of a 9:1 (v:v) methanol:acetic acid solution for 1 h. The washing procedure was repeated 10 times, and the washing solution was renewed for each repetition. After this, MIP, NIP, RAMIP and RANIP were dried at 70.0°C for 24 h.

Five hundred milligrams of each previously obtained polymer (MIP, NIP, RAMIP and RANIP) were placed in 5 mL polypropylene cartridges separately (one cartridge for each polymer). Then, 20 mL of 1% (w/v) albumin solution (prepared in 0.05 mol L^{-1} phosphate buffer, pH 6.0) were percolated through each cartridge at 1.0 mL min^{-1} flow rate. Subsequently, 25 mL of a 5% (w/v) glutaraldehyde aqueous solution were percolated through each one cartridge at 1.0 mL min^{-1} flow rate, and they were maintained in standby for 5 h. Finally, 10 mL of sodium borohydride 1% (w/v) aqueous solution were percolated through the cartridges at 1.0 mL min^{-1} flow rate. The coated polymers (MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA) were washed with water to remove residues of the reagents.

Polymers characterization

Initially, the materials were characterized by scanning electron microscopy (Zeiss LEO 440, Cambridge, England) equipped with an OXFORD detector, operating at 15 kV electron beam. The samples were coated with a 10-nm layer of gold, using a Coating System BAL-TEC MED 020 (BAL-TEC, Liechtenstein) and maintained in a desiccator before analysis.

The infrared (IR) spectra of the polymers were obtained using a Fourier transform infrared spectrometry (FT-IR) equipment (Shimadzu, Kyoto, Japan). Pellets were made, using 200 mg of KBr and 10 mg of MIP, NIP, RAMIP RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA or RANIP-BSA. The analyses were carried out at a spectral range from 500 to 4000 cm^{-1}, with a resolution of 4 cm^{-1} (20 scans).

The adsorption pH was studied adding 10.0 mg of each polymer in separated glass tubes containing OXP aqueous solutions at a concentration of 1.0 mg L^{-1}, in

different electrolytes (acetic acid aqueous solution 0.01 mol L^{-1}, pH 3.5; phosphate buffer solution 0.01 mol L^{-1}, pH 7.0; and sodium hydroxide aqueous solution 0.01 mol L^{-1}, pH 10.5). The tubes were shaken for 15 min and centrifuged at 1,000 × *g* for 10 min. The supernatant was collected and the remaining concentration of OXP was quantified by spectrophotometry. The mass, retained by the polymers (adsorption capacity - Qe), was calculated by subtraction. The same test was carried out individually for each polymer.

A kinetic study was carried out by adding 10.0 mg of each polymer (MIP, NIP, RAMIP, RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA or RANIP-BSA) to test glass tubes containing 1.0 mL of 100.0 mg L^{-1} OXP phosphate buffer solution (0.01 mol L^{-1}, pH 7.0). The tubes were shaken for 0, 15, 30, 45, 60, 75, 90 or 105 min at room temperature (approximately 25.0°C) and centrifuged at 1,000× *g*. The OXP, remaining in the supernatant (equilibrium concentration - Ce), was quantified by spectrophotometry. The Qe for each polymer was calculated by subtraction.

Adsorption isotherms were constructed in order to evaluate the extraction capacities of all obtained materials. Each material was studied individually. OXP standard solutions (25.0, 50.0, 75.0, 100.0, 200.0, 300.0, 500.0, 1000.0 and 1500.0 mg L^{-1}) were prepared in a phosphate buffer solution (0.01 mol L^{-1}, pH 7.0). One milliliter of each solution was transferred to glass tubes containing 10.0 mg of each polymer. The tubes were shaken for 60.0 min at room temperature (approximately 25°C), then each sample was centrifuged at 1,000× *g* for 10.0 min. The Ce for each polymer was determined by spectrophotometry and the Qe was calculated by subtraction. The data were modeled according to the Freundlich and Langmuir models, with the accepted adequacy standard being the linear correlation coefficient (r). The Langmuir isotherm

was given by: Ce/Qe = Ce/M + 1/(KM), where Qe was obtained in mg g^{-1} and Ce in mg L^{-1}. K and M were the OXP maximum adsorption capacity and binding constant, respectively [16-17].

To perform the selectivity tests, a LC-MS/MS method, for the detection and quantification of LAB, NAD, MET, OXP and PROP, was initially developed. A LC-MS 8030 instrument from Shimadzu® (Kyoto, Japan), equipped with a Shim-Pack XR-ODS C18 (100 x 3 mm, 2.2 µm) chromatographic column and a triple-quadrupole mass analyzer were used for this. The positive electrospray ionization mode was selected with the SRM (selected reaction monitoring) transitions and optimal collision energies optimized for each analyte (Table 2). The identification criterion was the simultaneous presence of the three fragments of each molecule (Table 2), the ratio between these fragments when compared to beta-blockers standards analyses and the fragments' relative abundance. The quantitative analyses were carried out using the TIC (total ion chromatogram) of the three SRM transitions of each molecule. The oven, interface and heat block temperatures were set to 40.0, 250.0 and 400.0°C, respectively. The nebulizing and drying gas flow rates were 1.5 and 15.0 mL min^{-1}, respectively. A gradient elution starting with 5:95% methanol:ammonium formate buffer solution 0.01 mol L^{-1} pH 3.5 was used. The methanol proportion was linearly increased, first to 20% reaching at the mark of 0.5 min, then to 43% until reaching at 1.5 min, to 58% until 3.5 min, to 98% untill5.5 min, then to 10% until 6.5 min and finally turning back to 5% until 7.0 min. The flow rate was 0.5 mL min^{-1}. The volume of the sample loop was 100.0 µL and the data files were acquired using LabSolutions® software. A calibration curve of LAB, NAD, MET, OXP and PROP was constructed at concentrations of 0.5; 0.7; 1.0; 1.5; 2.0 and 3.0 mg L^{-1}.

For the selectivity test, 10 mg of each polymer (MIP, NIP, RAMIP, RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA AND RANIP-BSA were individually placed in test tubes containing 1.0 mL of LAB, NAD, MET, OXP and PROP phosphate buffer solution (0.01 mol L^{-1}, pH 7.0) at a concentration of 500 mg L^{-1} each. The tubes were shaken for 60 min at room temperature (approximately 25°C) and centrifuged at 1000x *g*. Subsequently, the supernatant was removed and the polymers were placed in contact with 1 mL methanol. Once again, the tubes were shaken for 10 min, centrifuged at 1000x *g* and the supernatant was collected. An aliquot of this supernatant was diluted about 100 times and 25 µL of this solution was injected into LC-MS/MS to determine the beta-blockers' concentrations. The data were acquired by LabSolutions ® software. The Qe was calculated by subtraction. The selectivity constants were calculated as the Qe for OXP per the Qe of other beta-blockers (LAB, NAD, MET, PROP). Values greater than one (1) indicate that more OXP (template) was retained by the studied materials, compared to others, while values less than 1 (one) indicate a lower retention of the template.

To evaluate the macromolecules' elimination capacity, first, 25 µL of 44 mg mL^{-1} BSA standard in phosphate buffer 0.01 mol L^{-1} pH 7.0 (approx. the same concentration found in human plasma) was injected in a HPLC system without analytical column and with phosphate buffer 0.01 mol L^{-1} pH 7.0 as mobile phase at 1 mL min^{-1} and UV detector operating at 254 nm. Subsequently, 70 mg of each polymer were individually packed into HPLC pre-columns (10×4.6 mm i.d.). Each column was assembled in the analytical pathway and 25 µL of 44 mg mL^{-1} BSA standard in phosphate buffer 0.01 mol L^{-1} pH 7.0 were injected, following the same conditions described before. The percentage of protein exclusion was defined as the ratio

between the peak areas obtained in the system with and without the columns, multiplied by 100.

Results and discussion

The syntheses of the MIP and RAMIP, selective to OXP, were based on non-covalent interactions between functional monomer and template. The synthesis procedures were carried out by the precipitation method, when a large volume of solvent is used [18].

Two chemical reactions are involved in the BSA covering process (for MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA): i) reaction 1 occurs between the amine groups from the BSA and the aldehyde groups from glutaraldehyde (cross-linker); and ii) reaction 2 occurs between the free aldehyde groups of BSA-glutaraldehyde complex and the amino groups of another molecule of BSA, forming BSA polymeric network around the materials [15]. The reaction between glutaraldehyde and albumin results in imines, which are very labile functions. This fact justifies the use of sodium borohydride solution 1% (w/v), which reduces the imines to amines (more stable compounds) [15].

Scanning electron micrographs (Fig. 1) revealed that the morphological structures of the MIP, RAMIP, NIP, RAMIP, MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA presented macrospores formed by microsphere agglomerates, as frequently obtained by the precipitation method [19]. In MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA, the microspheres were more regular and velvety, probably due to the presence of the BSA layer on the surface of the polymers.

The infrared spectra of the polymers (Fig. 2) did not present significant chemical differences in terms of presence or absence of specific chemical groups. The use of the hydrophilic monomers (GDMA and HEMA) in the RAMIP and RANIP syntheses resulted in a hydrophilic layer on each polymer, but with chemical composition similar to the MIP and NIP. For the MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA, we believe that thin BSA layers were formed on the polymer surfaces, and their chemical nature was not adequately detected. Probably most of the radiation penetrated through it, reaching the polymeric cores. Moreover, a possible evidence for the BSA layer existence can be the transmittances decreased for all the polymers after their covering with the BSA. All the spectra presented a strong and broad bands between 3600 cm^{-1} and 3300 cm^{-1} approximately, that indicates the presence of associated OH, possibly due to the polymeric association; bands between 2960 cm^{-1} and 2850 cm^{-1}, indicating the presence of aliphatic CH; bands between 1760 cm^{-1} and 1710 cm^{-1}, indicating the presence of C=O of esters, likely from the polymeric association (functional monomer and cross-linker); bands at about 1635 cm^{-1}, indicating the presence of vinyl groups (also association between functional monomer and cross-linker) and bands at about 1160 cm^{-1} indicating the presence of C-O from ester groups [20].

Adsorption can be defined as a process in which a component present in a solution adheres to a solid surface. The intensity of adsorption effect depends on the adsorption temperature, adsorbent nature, adsorbate concentration, pH solute solubility, contact time, agitation, among others [21]. The adsorption phenomena can be classified as physical or chemical adsorptions. In the physical adsorption, the process occurs reversibly and quickly, and the adsorbate binds to the adsorbent

surface. By the way, chemical adsorption or chemisorption involves the interaction between the adsorbent and adsorbate, with energy almost as high as the chemical bonds formation [21-22].

All the polymers retained the OXP from the solutions at pH 7.0 more efficiently. It was also observed that the adsorption equilibrium was reached in 60.0 min, according to the adsorption kinetic studies (Fig. 3). Thus, pH 7.0 and adsorption time of 60 min were chosen as optimum for carrying out the adsorption studies.

At constant temperature, the adsorbed amount increases with the adsorbate concentration increasing and the relationship between the adsorbed amount and the remaining concentration is known as adsorption isotherm [23]. Adsorption isotherms were constructed for MIP, NIP, RAMIP, RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA at different concentrations of OXP. As shown in Fig. 4, the adsorption presented a linear relationship with the OXP concentration until equilibrium was reached (approximately 100.0 and 200.0 mg L^{-1}, respectively for the imprinted and non-imprinted polymers). Based on the molecular recognition, imprinted polymers showed higher adsorption capacities, probably because there were selective interactions between these materials and OXP, whereas only nonspecific interactions prevailed between non-imprinted polymers and OXP [4,24-25]. However, these adsorption differences are more evident in materials that were coated with hydrophilic comonomers (RAMIP, RANIP, RAMIP-BSA and RANIP-BSA). This fact corroborates with the theory that affirms that hydrophilic comonomers (like HEMA and GDMA) contribute to a better molecular recognition in aqueous media. In fact, water molecules can interact with the surface instead of the selective binding sites. In this way, the solvent interferes less in the template/binding site interactions [26-27].

The Freundlich and Langmuir models were tested for all the obtained isotherm data. Table 3 shows the correlation coefficients (r) for both models, as well as the maximum adsorption capacities and the equilibrium parameters for the Langmuir model. It is possible to see that the best fit was obtained with the Langmuir model for the imprinted and non-imprinted polymers (higher correlation coefficients). The maximum adsorption capacities showed that all polymers had an OXP satisfactory retention. The Langmuir model indicated that the molecules had a uniform distribution in the binding sites around the polymer and that each binding site was able to receive only one molecule. Furthermore, according to the model, the analytes were retained in a monolayer on the MIP surface, and the energy involved in this process was the same for all binding sites surrounding the polymer [27-30].

In the selectivity studies (Fig. 5), the RAMIP presented the best beta-blockers' adsorption, which suggest that molecular recognition in aqueous media is improved with the addition of hydrophilic comonomers. The NAD adsorption was negatively influenced by BSA coating and it was little retained by MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA. Thus, we believe that the NAD binds to NIP and RANIP surface by non-specific interactions and, when the surface is blocked by the BSA layer, the NAD adsorption is impaired. The NAD interaction with the binding sites of the OXP imprinted polymers is difficult because NAD presents 3 chiral centers, and may take 8 different conformations. Fig. 6 shows the studied beta-blockers' chemical structure.

From the selectivity constants (Ks) showed in Table 4, it is possible to observe that more OXP was retained by the studied polymers than the other beta-blockers, except when compared to PROP. A possible explanation is that PROP has a very similar

chemical structure compared to OXP, as well as due to its lower molecular weight, which can facilitate the adaptation to the OXP imprinted binding site.

Macromolecule elimination tests were carried out with all the polymers (Table 5), in order to understand the influence of the hydrophilic comonomers or BSA layers in the exclusion mechanisms. By these results, it is possible to see that the hydrophilic comonomers addition (HEMA and GDMA) or the BSA coating alone are ineffective for a satisfactory macromolecule elimination. Probably because, in pH 7.0, the negative charge's density, derived from comonomers addition, as well as from the BSA coating is not enough to generate the negative charge amount needed to repel all proteins present in the samples. However, the combination of these processes produces a material (RAMIP-BSA) able to eliminate almost 100% of the macromolecules from the sample. Thus, this sorbent is suitable for direct biological sample extractions of specific analytes from plasma and serum, for example. As a possible explanation, in pH 7.0, the BSA (isoelectric point about 4.7 [31]) and hydrophilic monomers (hydroxyl group pka < 7.0) from the RAMIP-BSA surface are negatively charged and thus repell the proteins from the sample [15].

Conclusion

The synthesized polymers were efficient for beta-blockers' adsorption. Through physical characterization, it was observed that the way in which the materials synthesis is conducted influences the size and shape of the particles, and that hydrophilic comonomers' addition, as well as BSA coating, do not alter their chemical recognition. Adsorption studies showed that: i) adsorption equilibrium is reached in 60 minutes, ii) BSA coating does not alter its adsorption profile, iii) there is an evident

difference between imprinted and non-imprinted polymers' adsorption, iv) the best fits to describe the adsorption profile of the materials was the Langmuir model and RAMIP was the best polymer to adsorb beta-blockers in an aqueous media. It was also possible to conclude that both hydrophilic monomers and BSA coating presence on the polymer surface are very important to obtain materials able to completely eliminate the macromolecules, during an extraction procedure. Thus, we believe that the RAMIP-BSA is the best polymer model to be used for the solid phase extraction of these analytes from untreated biological samples.

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Table 1: Compositions of each polymer in terms of molecular imprinting, presence of hydrophilic monomers and presence of BSA layer.

Polymer	Presence of template during the synthesis	Presence of hydrophilic monomers	Presence of BSA layer
MIP	Yes	No	No
NIP	No	No	No
RAMIP	Yes	Yes	No
RANIP	No	Yes	No
MIP-BSA	Yes	No	Yes
NIP-BSA	No	No	Yes
RAMIP-BSA	Yes	Yes	Yes
RANIP-BSA	No	Yes	Yes

Table 2: Analytes and their precursors, fragments and collision energies optimized for detection using LC-MS/MS in SRM mode.

Analyte	Precursor (*m/z*)	Fragments (*m/z*)	Collision Energy (kV)
LAB	329.2	91.1	-35
		162.1	-25
		294.1	-20
NAD	310.1	254.2	-20
		74.1	-25
		201.1	-25
MET	268.2	116.5	-25
		98.1	-25
		133.2	-25
OXP	266.2	72.1	-25
		224.9	-15
		116.1	-20
PROP	260.2	116.1	-20
		98.1	-20
		183.2	-20

Table 3: Langmuir and Freundlich linear coefficients, maximum adsorption capacities and equilibrium parameter values for MIP, NIP, RAMIP, RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA.

Polymer	Linear Coeficient (r) Langmuir	Linear Coeficient (r) Freundlich	Maximum adsorption capacities (mg of OXP per g of polymer)
MIP	0.99	0.91	81.30
NIP	0.99	0.90	75.19
RAMIP	0.99	0.93	82.64
RANIP	0.99	0.92	67.11
MIP-BSA	0.99	0.93	90.09
NIP-BSA	0.97	0.92	87.72
RAMIP-BSA	0.99	0.93	92.59
RANIP-BSA	0.96	0.92	79.37

Table 4: Polymers' selective constants for LAB, NAD, MET and PROP.

Polymer	Ks LAB	Ks NAD	Ks MET	Ks PROP
MIP	1.01	2.07	1.03	0.91
NIP	0.97	5.54	1.16	0.82
RAMIP	1.03	1.21	1.08	0.89
RANIP	0.90	5.56	1.21	0.73
MIP-BSA	1.01	5.69	1.17	0.84
NIP-BSA	1.24	Not determined	1.95	0.89
RAMIP-BSA	1.16	3.50	1.73	0.97
RANIP-BSA	0.78	Not determined	1.75	0.68

Table 5: BSA elimination percentages for MIP, NIP, RAMIP, RANIIP, MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA.

Polymer	BSA elimination (%)
MIP	14.7
NIP	18.1
RAMIP	87.8
RANIP	84.5
MIP-BSA	87.3
NIP-BSA	89.4
RAMIP-BSA	98.7
RANIP-BSA	98.3

Figure captions

Fig. 1. Scanning electron micrographs of the MIP, MIP-BSA, NIP, NIP-BSA, RAMIP, RAMIP-BSA, RANIP and RANIP-BSA magnified 50,000x.

Fig. **2.** Infrared spectra of (A) MIP and MIP-BSA, (B) NIP and NIP-BSA, (C) RAMIP and RAMIP-BSA and (D) RANIP and RANIP-BSA.

Fig. 3. Adsorption kinetics for the imprinted and non-imprinted polymers.

Fig. 4. Adsorption isotherms of OXP for (1) MIP and NIP, (2) RAMIP an RANIP, (3) MIP-BSA and NIP-BSA and (4) RAMIP-BSA and RANIP-BSA.

Fig. 5. Retention graph of LAB, NAD, MET, OXP and PROP by MIP, NIP, RAMIP, RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA.

Fig. 6. Molecular structures of beta-blockers used to perform selectivity tests.

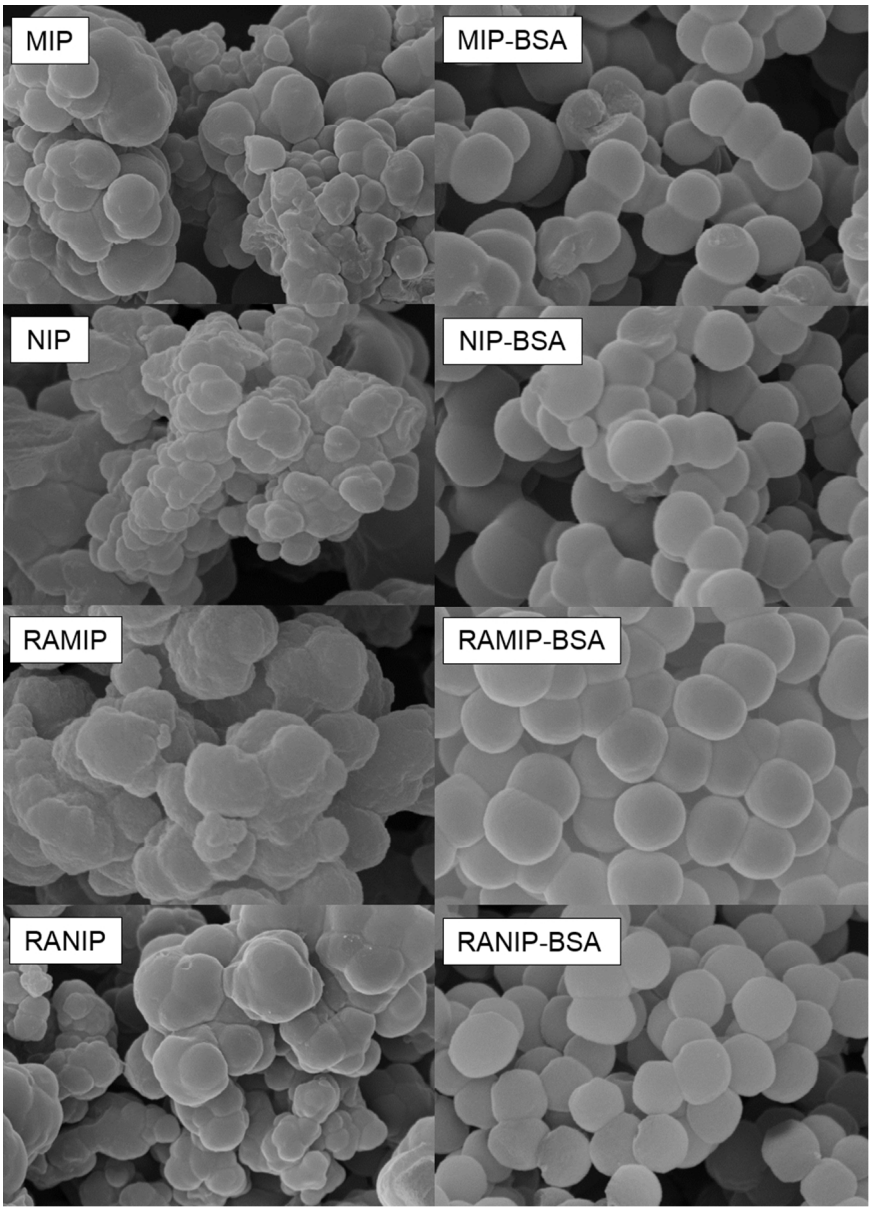

Fig. 1. Scanning electron micrographs of the MIP, MIP-BSA, NIP, NIP-BSA, RAMIP, RAMIP-BSA, RANIP and RANIP-BSA at magnification of 50,000x.
159x221mm (150 x 150 DPI)

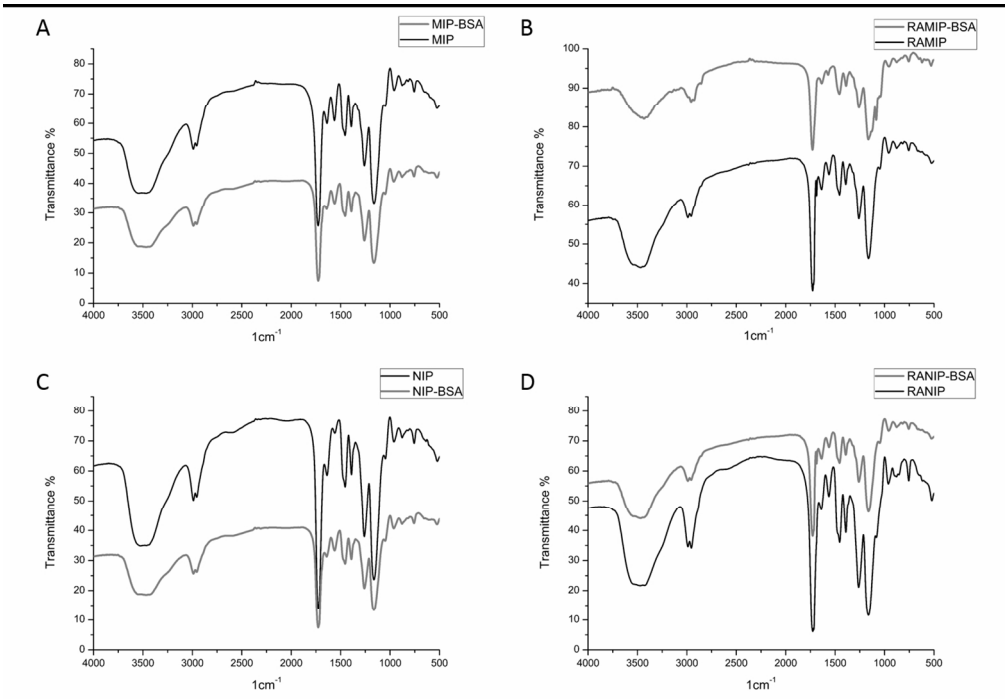

Fig. 2. Infrared spectra of (A) MIP and MIP-BSA, (B) NIP and NIP-BSA, (C) RAMIP and RAMIP-BSA and (D) RANIP and RANIP-BSA.
254x179mm (150 x 150 DPI)

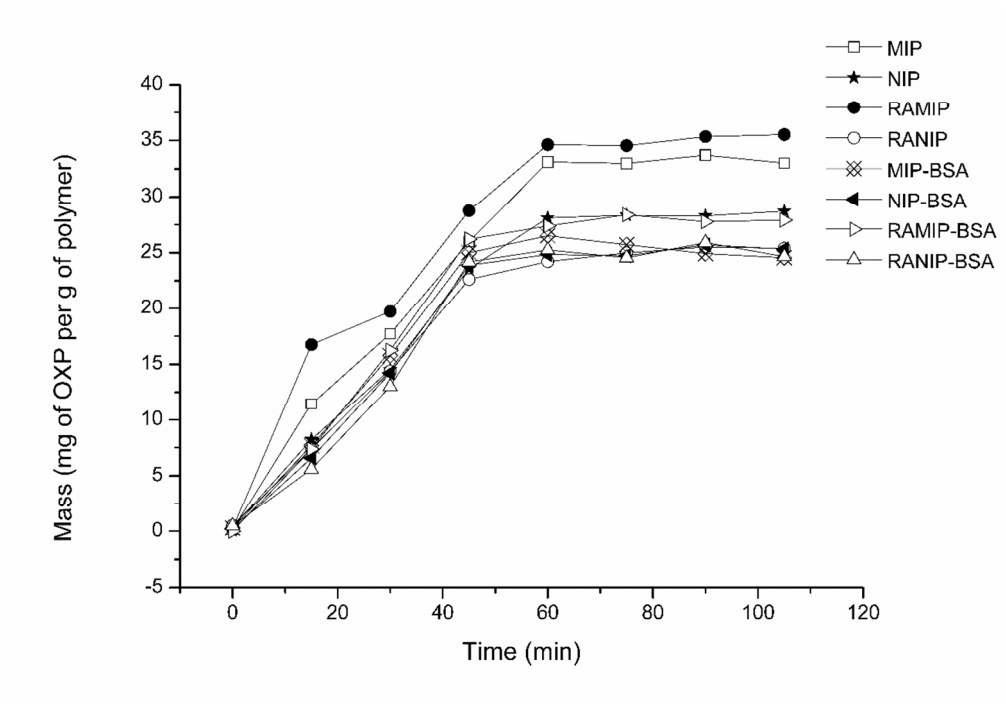

Fig. 3. Absorption kinetics for the imprinted and non-imprinted polymers.
149x105mm (220 x 220 DPI)

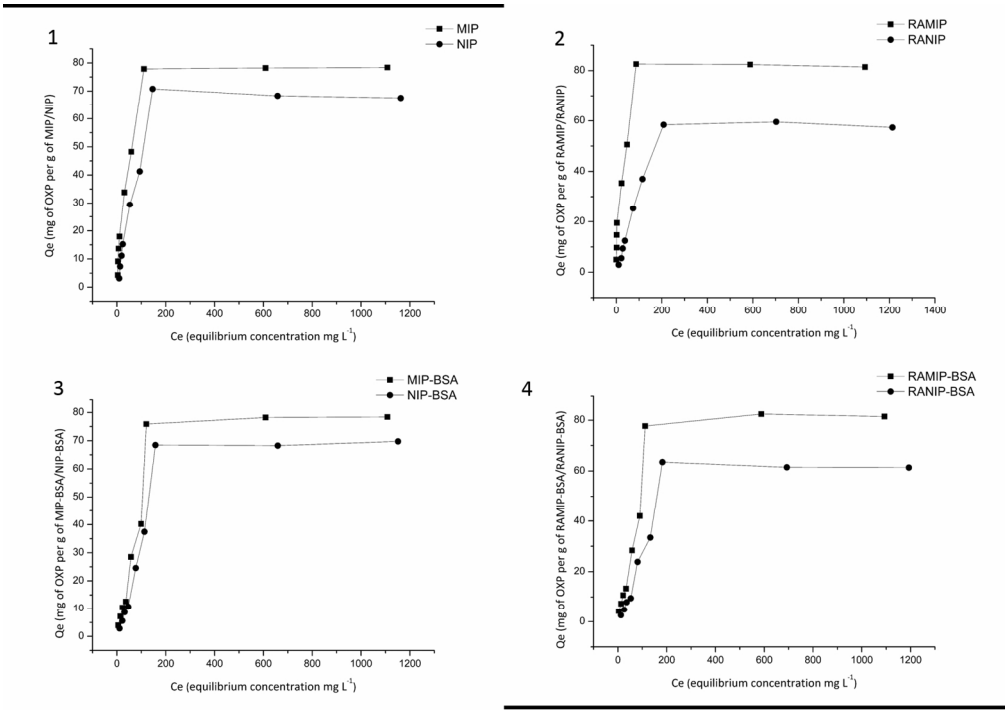

Fig. 4. Adsorption isotherms of OXP for (1) MIP and NIP, (2) RAMIP an RANIP, (3) MIP-BSA and NIP-BSA and (4) RAMIP-BSA and RANIP-BSA.
285x200mm (150 x 150 DPI)

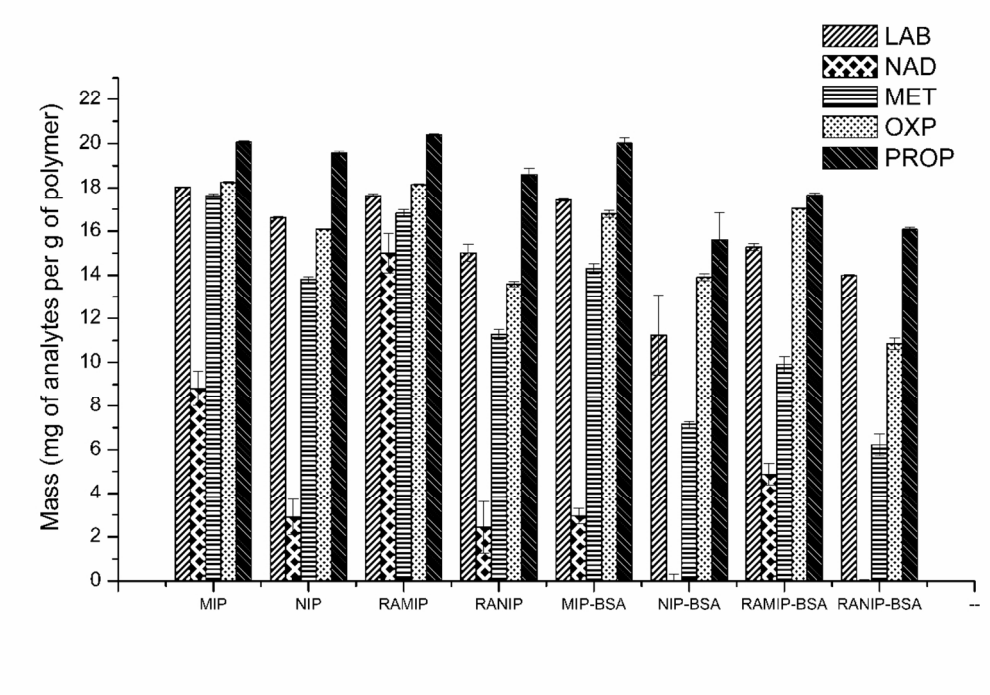

Fig. 5. Retention graph of LAB, NAD, MET, OXP and PROP by MIP, NIP, RAMIP, RANIP, MIP-BSA, NIP-BSA, RAMIP-BSA and RANIP-BSA.
149x105mm (220 x 220 DPI)

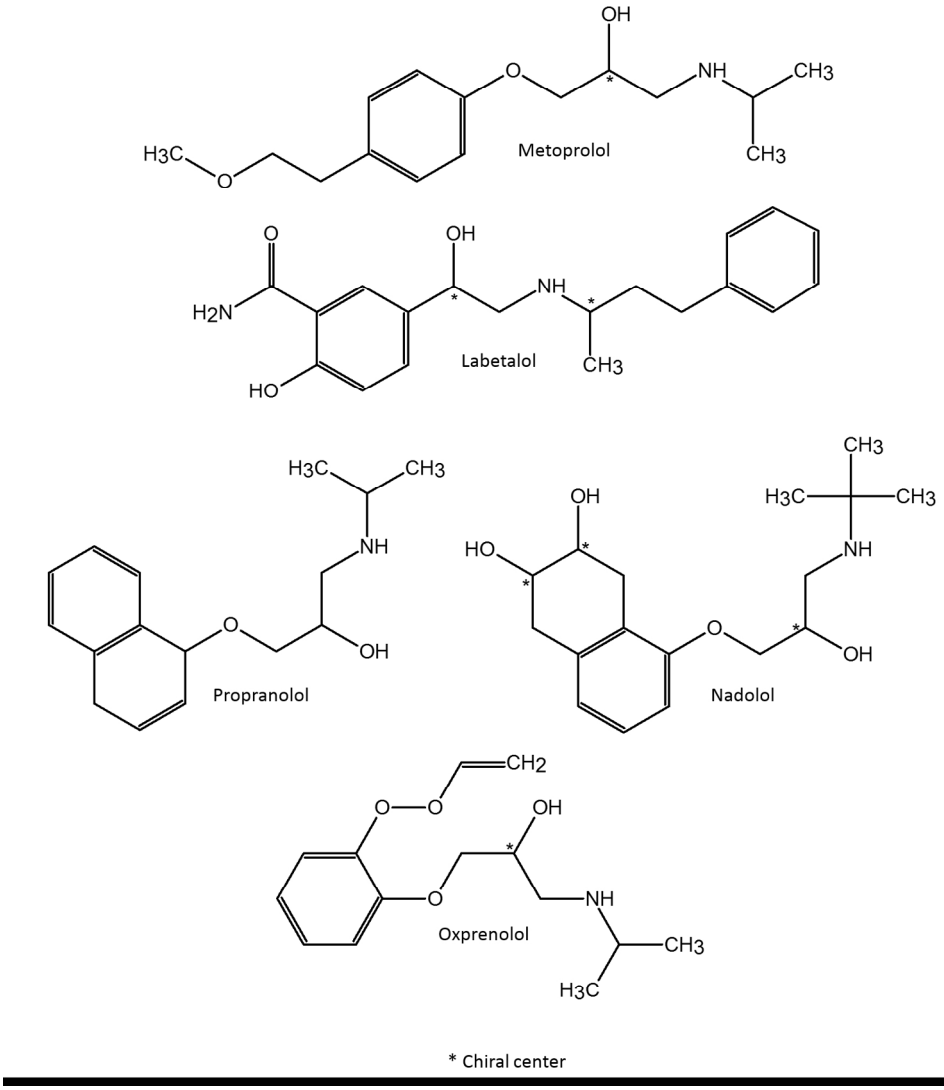

Fig. 6. Molecular structure of beta-blockers used to perform selectivity tests.
313x343mm (150 x 150 DPI)