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Synthesis and characterization of molecularly imprinted polymer for controlled release of tramadol

Research Article

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Abstract: In this paper, we describe how to prepare a highly selective imprinted polymer by a bulk polymerization technique. We used tramadol as the template, (MAA) as functional monomers, and (EGDMA) as the cross-linker in chloroform as solvent. Results from Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Scanning Electron microscopy (SEM) show that this imprinted sorbent exhibits good recognition and high affinity for tramadol. Selectivity of molecularly imprinted polympers (MIP) was evaluated by comparing several substances with similar molecular structures to that of tramadol. Controlled release of tramadol from MIPs was investigated through *in vitro* dissolution tests and by measuring the absorbance at λ_{max} of 272 nm by (HPLC-UV). The dissolution media employed were hydrochloric acid pH 3.0 and phosphate buffers, pH 5.0 and 7.4, maintained at 37 and 25 ± 0.5°C. The results show the ability of MIP polymers to control tramadol release. In all cases, the release of MIPs was deferred for a longer time as compared to NMIP. At a pH of 7.4 and 25°C slower release of tramadol imprinted polymer occurred.

Keywords: *Molecularly • Imprinted polymer • Tramadol • Bulk polymerization • Drug release • Biological systems*

1. Introduction

Half a century ago, Dickey *et al*. [1] first reported molecular imprinting as an efficient method to introduce specific recognition sites into a polymer matrix. However, it is only in the last decade that the use of molecular imprinting as a useful tool has became well-established. An imprint is like a lock that is only compatible with the correct key, similarly to compatibility within biological systems, such as enzymes with substrates, antibodies with antigens, or hormones with receptors. The most widely adopted method to prepare MIPs is a non-covalent approach, which uses only non-covalent interactions between the template and the functional monomers, that provides flexibility with regard to selection of the functional monomers and possible template molecules [2]. In this process, functional and cross-linking monomers are co-polymerized in the presence of the template molecules [2-6]. Functional monomers initially form a complex with the imprint molecule followed by a process of polymerization, and then their functional groups are held in position by the highly cross-linked polymeric structure. The subsequent removal of the imprint molecule reveals the binding sites, which are complementary in size and shape to the template molecule Thus, molecularly imprinted polymers can often be used as selective separation media for the template [6].

The applicability of the MIPs has led to numerous reports such as sensors and biosensors [7,8], as stationary phases for affinity chromatography [9], for membrane separation [10], as adsorbent for solid phase extraction [11], enzyme like catalysts [12], enantioseparation [13,14], or in pharmaceutical applications [15].

Polymer systems that allow controlled-release of a drug are well-established. In most recent studies, MIPs with artificially fabricated receptor structures have been used to develop the design of drug delivery systems (DDS) [16-21]. Molecular imprinting technology can provide polymeric materials with the ability to recognize specific bioactive molecules with sorption and release behavior that can be made sensitive to properties of the surrounding medium. A potential advantage of imprinted polymer as DDS includes an increase of the residence

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time of the drug within a body by reducing a drug release rate. In cases where the drug has a narrow therapeutic index, MIP delivery vehicles may keep its concentration in the body below the level where adverse side effects become dominant.

Tramadol hydrochloride (1*R*,2*R*)-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl)-cyclohexanol (Fig. 1) is a white crystalline powder freely soluble in water and in methyl alcohol, and very slightly soluble in acetone It is an opioid analgesic which can rapidly, and almost completely, be absorbed after oral or parenteral administration. The presence of food does not significantly affect the rate or extent of absorption. It also has noradrenergic and serotonergic properties that may be contributing to its analgesic activity. Tramadol is used against moderate to severe pains. Tramadol hydrochloride is given by mouth, intravenously, or rectally as a suppository. It may also be given by infusion, as part of a patient-controlled analgesia system, intramuscularly, or with other analgesics such as paracetamol [22].

In this paper, we present first DDS based on molecularly imprinted polymers for controlled release of tramadol and we discuss the key factors controlling recognition and release by imprinted polymer matrices.

2. Experimental Procedure

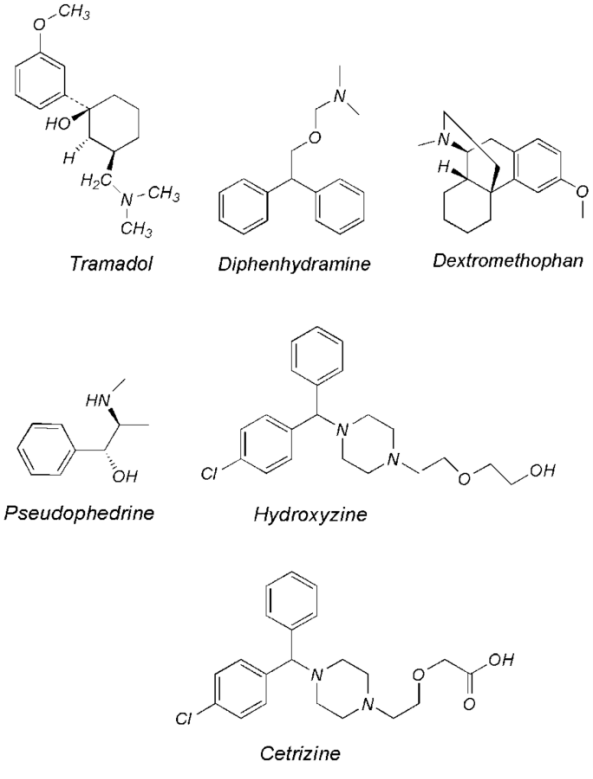


Figure 1. Chemical structures of investigated drugs.

2.1. Chemicals and reagents

Methacrylic acid (MAA) from *Merck* (Germany) was distilled in vacuum prior to its usage in order to remove the stabilizers. Ethylene glycol dimethacrylate (EGDMA), 2,2-azobis isobutyronitrile (AIBN) from *Sigma–Aldrich* (Germany) were of reagent grade and were used without any further purification. Tramadol HCl, Dextromethorphan HBr, Pseudoephedrine HCl, diphenhydramine HCl, hydroxyzine HCl and cetirizine HCl were obtained from The Ministry of Health and Medical Education (Tehran, Iran) with the degree of purity of all drugs above 95%. Tramadol stock solutions were prepared in water as standard solution (1000 µg L^{-1}) and stored at 4°C. Intermediate standard solution of 50 µg L^{-1} was prepared by dilution of stock solutions with water. Working standard solutions of different concentrations were prepared daily by diluting the intermediate standard solution with mobile phase solution. Phosphate buffer, with desired pH value was prepared in de-ionized water. All solvents were HPLC grade and supplied by *Merck* (Germany).

2.2. Instruments

The HPLC system consisted of a Waters 515 pump, a 486Waters UV/vis detector, *a model 7725i Rehodyne* injector with a 25 µL sample loop and a micro-Bondapak *C18 column* of 4.6 mm×150 mm HPLC column. HPLC data was acquired and processed using a PC and Millennium 2010 Chromatogram Manager software (Version 2.1 Waters). Water bath (*memmert WNB14*) was used to carry out the polymerization. Sonic bath (*EURONDA 4D*) with power of 350 W and frequency of 50 HZ was used to disperse the mixture. Scanning electron microscope (*Philips XLC*) was used to study morphology of polymer particles. The pH of solutions was adjusted using *model 630 digital Metrohm* pH meter equipped with a combined glass-calomel electrode. FT-IR spectra of ground polymers were recorded (*Bruker model EQUINOX 55*). The thermal analysis of polymer was carried out on model *PL-STA-1500,* thermo gravimetric analysis (TGA) was carried out on a *Perkin Elmer TGS-2* instrument at the maximum heating rate of 20°C min^{-1} in oxygen atmosphere.

2.3. Procedures

2.3.1. MIP and NMIP preparation with bulk polymerization

Schematic representation of the imprinting and the removal of tramadol from the imprinted polymer are shown in Fig. 2. For the preparation of tramadol imprinted polymer, the template (64 mg, 0.24 mmol) was dissolved in chloroform in a 25 mL thick walled glass tube. A functional monomer MAA (0.129 mL, 1.5 mmol), cross-linker EGDMA (2.6 mL, 14 mmol), and initiator

Table 1. Compositions of the polymer's extraction.

MIP	MAA (mmol)	Tramadol (mmol)	EGDMA (mmol)	AIBN (mmol)	Extraction (%) [mean ± S.D] [a]
MIP_1	2	1	60	0.34	24 (±1.5)
MIP_2	4	1	60	0.34	55 (±2.0)
MIP_3	6	1	60	0.34	85 (±3.8)
MIP_4	8	1	60	0.34	69 (±2.4)
$NMIP_1$	2	0	60	0.34	20 (±1.0)
$NMIP_2$	4	0	60	0.34	41 (±1.9)
$NMIP_3$	6	0	60	0.34	15 (±2.1)
$NMIP_4$	8	0	60	0.34	62 (±2.2)

[a] Average of three determinations.

AIBN (24 mg, 0.146 mmol) were then added to the above solution. The mixture was degassed under vacuum in a sonicating water bath while being purged with nitrogen for 5 min. While maintaining flow of nitrogen, the reaction flask was removed from the sonicating bath, sealed and placed inside a water bath at 60°C to allow initiation of the reaction. Under these conditions, the reaction was continued for 20 h. After drying in air overnight, white polymer, P (MAA-co-EGDMA) with a rigid structure was obtained. It was ground into fine particles using mortar and pestle. The polymer particles were washed with 10% (*v/v*) AcOH/MeOH three times and with distilled water twice. A complete removal of the template was followed by HPLC-UV. After washing the product three times, spectra of tramadol were not observable. In order to verify that the retention of the template was due to molecular recognition and not due to a non-specific binding, a control non-imprinted polymer (NMIP) was prepared according to the same procedure, but excluding the target molecule, tramadol. Compositions of the polymer's extraction are compared in Table 1. The size of the particles used after crushing and sieving was between 30 and 45 µm.

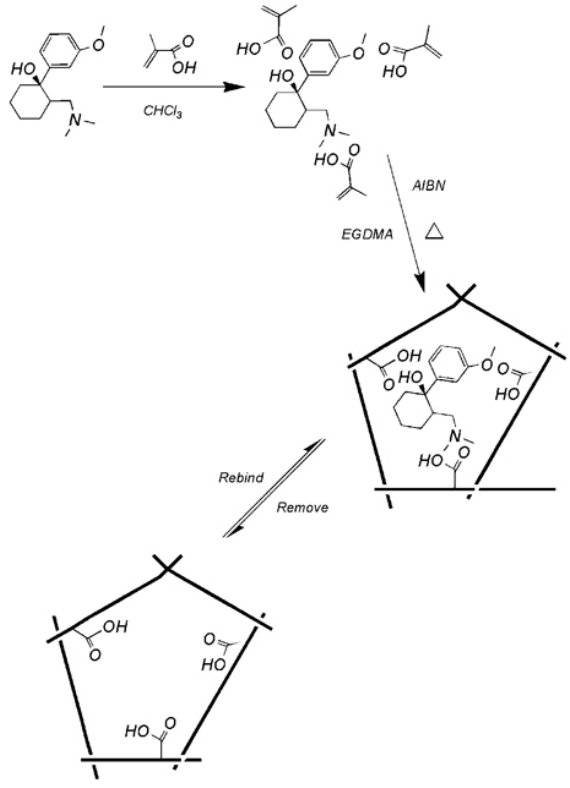


Figure 2. Schematic representation of the MIP synthesis for tramadol.

2.3.2. Chromatographic conditions

The HPLC was carried out at room temperature. A degassed mixture of acetonitrile: phosphate buffer (0.01 mol L^{-1}, pH 3.0) (30:70) at a flow rate of 1.0 mL min^{-1} was selected as a mobile phase [23]. All of the analyses were carried out at a wavelength of 272 nm and the results were recorded by Millennium chromatography software.

2.3.3. Batch rebinding experiment

Batch adsorption experiments were used to evaluate binding affinity of the imprinted polymer as reported before [2,24]. A general procedure for the extraction of tramadol by the MIP was as follows: tramadol standard solution was prepared in water and the pH was adjusted to 7.5. Then, 50 mg of imprinted polymer was placed in

a 10 mL flask containing tramadol solutions of various concentrations (5-100 µg L^{-1}). Each suspension was magnetically stirred for 1h and then passed through a paper filter at a flow rate 100 mL min^{-1} by applied vacuum. Free concentration of tramadol after the adsorption was recorded by HPLC-UV at 272 nm. Three replicate extractions and measurements were performed for each aqueous solution. The adsorbed tramadol was desorbed from the MIP by treatment with 5 mL of 10 % (v/v) AcOH/MeOH. The extraction percentage of tramadol was calculated from Eq. 1:

$$\text{Extraction\%} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where Ci and Cf are the concentrations of tramadol before and after extraction in the solution. In order to compare specific and non-specific interactions with tramadol, a control procedure was performed using NMIP particles.

2.3.4. Drug loading by soaking procedure

Tramadol loading was carried out by immersing the known weight of polymers (50 mg) in tramadol solution (10 mL of 50 µgL^{-1}) at different pH. Each suspension was magnetically stirred for 30 min and then passed through a paper filter at a flow rate 100 mL min^{-1} by applied vacuum. Loaded polymers were removed from the tramadol solution and dried in vacuum overnight at 40°C.

2.3.5. In vitro drug release studies

The release studies were performed using the dissolution method [25]. Two parallel experiments for MIP_3 and $NMIP_3$ matrices were performed. All tests were conducted in 10 mL of dissolution medium maintained at 37 ± 0.5°C under 50 rpm stirring. Dissolution media employed were hydrochloric acid, 3.0 pH and phosphate buffers, 5.0 pH and 7.4 pH. 2 mL of each samples solution mLwas withdrawn at regular intervals and returned to the vial immediately after their tramadol concentration was measured by HPLC-UV at 272 nm. The cumulative amount of tramadol was calculated and plotted as a function of time. Experiments were repeated three times.

3. Results and Discussion

3.1. Characterization

The IR spectra of $NMIP_3$ and the unleached and leached MIP_3 displayed similar characteristic peaks, indicating similarity in the backbone structure of different polymers. The IR spectra of the unleached and leached imprinted poly (MAA co-EGDMA) are shown in Fig. 3. As a result of hydrogen binding with the –COOH group of MAA, the C=O and OH stretching, as well as the bending vibrations at 1710, 3457 and 1388 cm^{-1} in the unleached MIP_3 materials were shifted to 1722, 3473 and 1394 cm^{-1} in the corresponding, leached MIP_3. Furthermore, two other distinct differences between the IR spectra of the unleached and leached MIPs were noticable. In the leached polymer, there was one sharp band with low relative intensity at 1522 cm^{-1} and one band with high relative intensity at 2965 cm^{-1}. Corresponding peaks in unleached MIP_3 appeared at 1538 and 2940 cm^{-1}. Other absorption peaks match both, those of MIP_3, as well as NMIP. They are: 1252, 1129 cm^{-1} (symmetric and asymmetric ester C–O stretch bands), 1636 cm^{-1} (stretching vibration of residual vinylic C=C bonds), and 985 cm^{-1} (out-of-plane bending vibration of vinylic C–H bond).

Thermo gravity analysis (TGA) plots of the unleached and leached MIP particles are plotted in Fig. 4. In unleached MIP_3 particles, TGA revealed two decomposition states. The first mass loss (10% weight loss), is between 100 and 180°C which represents

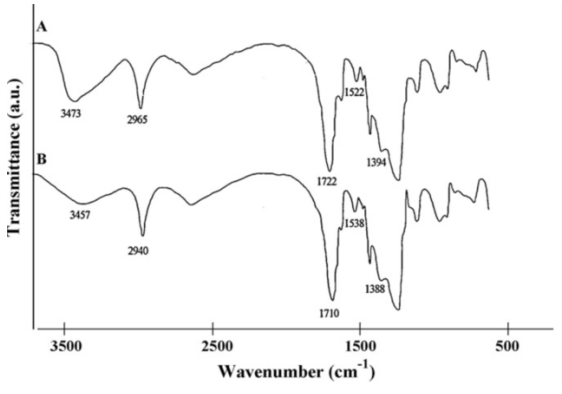


Figure 3. Infrared spectra of the leached (A) and unleached (B) MIP particles.

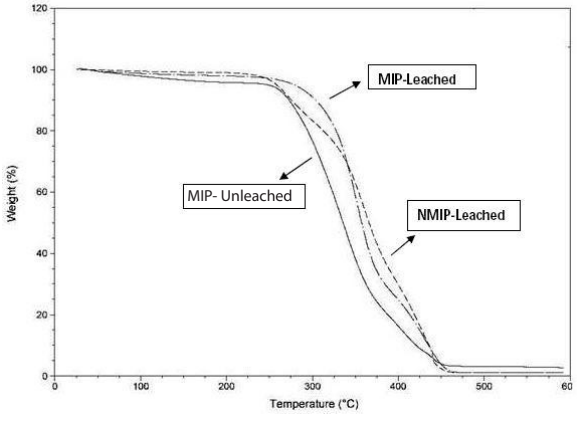
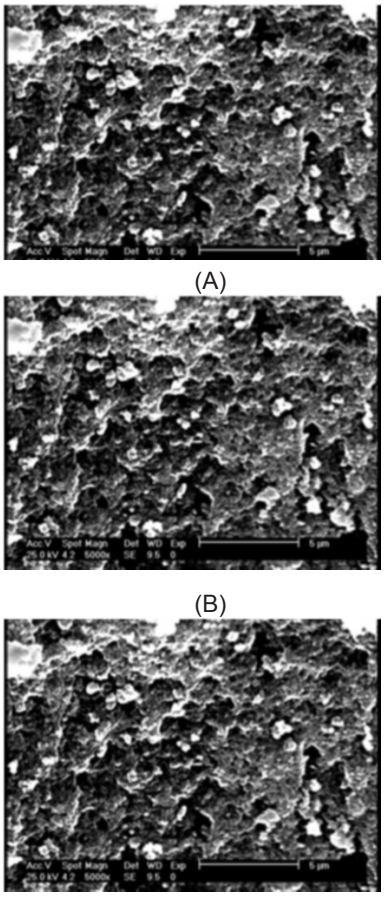


Figure 4. Thermogravity analysis of the NMIP leached, unleached and leached MIP particles.

decomposition of a free monomer and the cross-linker, whereas the second mass loss appears at 185°C and is related to the tramadol hydrochloride decomposition as the melting point of tramadol hydrochloride is ~180-181°C [26]. All the materials were fully decomposed prior to reaching the temperature of 460°C. These observations indicate that the rigidity of unleached and leached MIP_3 particles is more than that of blank materials', since MIPs decompose above ~300°C, whereas the controls begin to decompose at ~250°C. Fig. 4 displays that above 400ºC unleached and leached MIP particles have similar degradation patterns while above 450ºC, complete decomposition of the polymeric matrix occurs.

Morphology of MIP_3 and $NMIP_3$ particles determined by a SEM, is shown in Figs. 5A, B and C at magnification 5000. There are remarkable differences seen in the morphologies of the polymers and a porous surface is observed for the MIP_3.



(A)

(B)

(C)

Figure 5. Scanning electron micrographs: (A) unleached MIP; (B) leached MIP; (C) leached NIP.

3.2. Optimal MIP formulation and progenic solvent

Various parameters, such as amount of monomer or nature of cross-linker and solvent, influence final characteristics of the obtained materials in terms of affinity and selectivity for the target analyte, Solvents play an important role in the formation of a porous structure of MIPs, which belong to a larger class of materials known as macroporous polymers [27,28]. Porosity and surface area are determined by the type of solvent, referred to as "porogen," used in the polymerization. Porosity arises from the phase separation from the porogen and the growing polymer during polymerization. Porogens with low solubility phase separate early and tend to form larger pores and results in materials with lower surface area. Conversely, porogens with higher solubility phase separate later in the polymerization, providing materials with smaller pore size distributions and greater surface area. It does not appear, however, that binding and selectivity in MIPs are dependent on porosity. In fact, optimal results are often obtained when chloroform is used as porogen [29].

As Fig. 6 shows, the primary experiment revealed that the imprinted polymer prepared in chloroform exhibits a better molecular recognition compared to that of acetonitrile (ACN) and dimethyl formaldehyde (DMF) in aqueous environment. Thus, chloroform is chosen as a suitable solvent for optimization of functional monomer to template ratio in order to improve molecular recognition capabilities. Generally, proper mole ratios of functional monomer to template are very important to enhance specific polymers and a number of MIP recognition sites. A pre-polymer complex can be increased by increasing the template concentration. This is an interesting prospect because the template can in theory be increased to very high concentrations without having any change on the composition of monomers in the final polymer. This is because the template is not covalently incorporated into the final polymer and is removed at the end of the imprinting process [29]. On the other hand, high ratio of

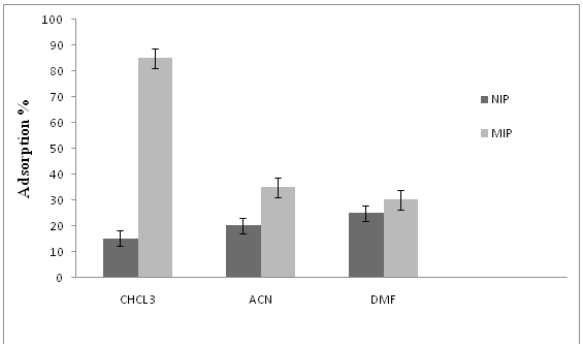


Figure 6. Recovered samples obtained using the MIP and NMIP polymers synthesized in different organic solvents. Batch experiments with 50 mg or polymer particles; sample volume, 5 mL; pH, 7.5; tramadol concentrations, 50 μgL^{-1} (mean±S.D., n=3)

functional monomers to template results in a non-specific affinity, while low ratios produce fewer complication due to low number of functional group [30].

As Table 1 depicts, different ratios of monomers MAA to template were used in the experiment. The optimal ratio of functional monomer to template for tramadol by bulk polymerization was 6:1 and it had the best specific affinity and highest recovery of 85%. In comparison, NMIP recovery was only 15%. Excess of the functional monomer with respect to the template yielded higher non-specific affinity. Fig. 7 shows the maximum difference between MIP and NMIP in this mole ratio. Therefore, a typical 1 : 6 : 60 template : monomer : cross linker mole ratio was used in further studies.

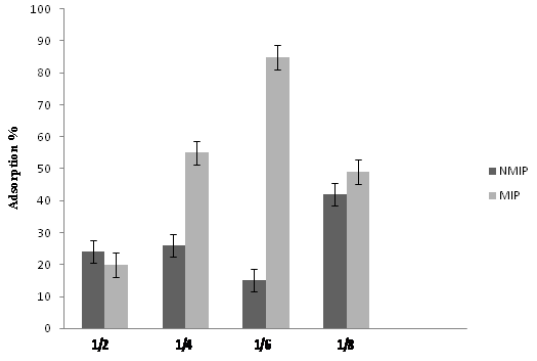


Figure 7. Recovered samples obtained using the MIP and NIP polymers synthesized at different template / monomer ratios (mean±S.D., n=3)

3.3. Effect of pH on drug loading

Different polymers with different template : monomer ratios were synthesized and pH effects were investigated on drug loading. The effect of pH on the sorption of tramadol was examined by varying the pH of a solution between 2.0 - 10 pH. Several experiments were performed by equilibrating 50 mg of the imprinted particles with 5 mL of solutions containing 50 µg L^{-1} of tramadol under the desired range of pH. The results for different polymers (Fig. 8) display that the pH greatly affects loading in that the percentage of tramadol recovery increases up to 7.5 pH and then it decreases by further increase of pH. A difference in response of about 70% between MIP_3 and $NMIP_3$ was seen at pH 7.5. Lesser effects were observed at lower and higher pH values and which may have been attributed to the protonation of the functional group of tramadol and to the deprotonation of carboxyl groups of the polymer, respectively.

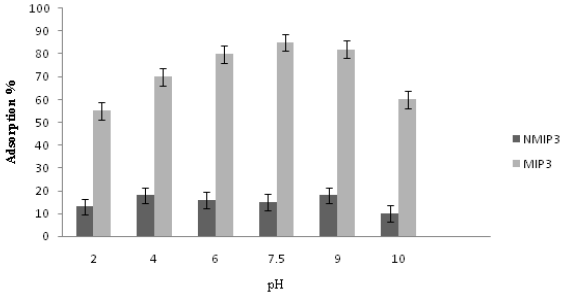


Figure 8. Effect of pH on rebinding efficiency of tramaddol. 50 mg of the imprinted polymers; sample volume: 5 mL; tramadol concentration: 50 µg L^{-1}; temperature 20°C (mean±S.D., n=3)

3.4. Effect of the extraction time on drug loading

As shown in Fig. 9, the percentage of tramadol recovery for MIP was increased by increasing the extraction time and it had reached the maximum value in 10 min. A major difference appears at the 10th minute. We believe that high adsorption rate in the first 10 min results from the preferential and rapid adsorption of template molecules onto the recognition sites in the cavities of MIPs. When these imprinted sites are occupied, it becomes difficult for tramdol to implant into the MIPs. This may be causing the adsorption to slow down. Based on these results, the optimal extraction time of 10 min was selected and used in all subsequent studies.

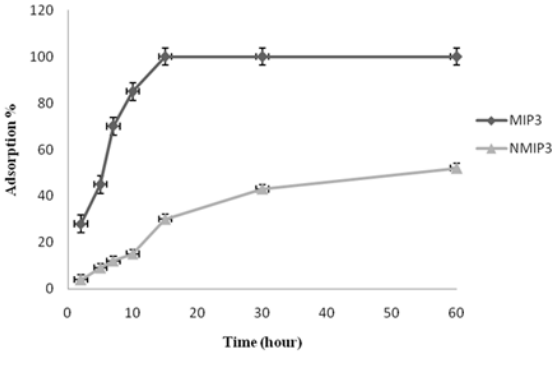


Figure 9. Effect of time on rebinding efficiency of tramaddol. 50mg of the imprinted polymers; sample volume: 5 mL; tramadol concentration: 50 µg L^{-1}; pH 7.5; temperature 20°C (mean±S.D., n=3).

3.5. Choice of loading, washing, and eluent solutions

Polymers can bind specifically or non-specifically. Specific interactions may be originating from the imprinting procedure, which can create selective recognition sites for the template. The non-specific interactions were assessed by measuring binding of the non-imprinted polymer. In order to investigate the usefulness of a washing step, various aqueous media including acetonitrile, acetone, tetrahydrofuran and dimethyl formamide were assessed

to obtain the maximum recovery of the analytes. A tramadol solution was employed for loading separately on MIP and NMIP cartridge, followed by desorption with washing solvent. The results showed that washing with tetrahydrofuran had no clear effect on the retention of tramadol on both MIP and NMIP cartridges. In contrast, polar organic solvents, such as acetonitrile and dimethyl formamide largely affected the retention of tramadol on both, MIP and NMIP cartridges. Since acetone could elute interferences, it was chosen as the washing solution (Table 2). For the recovery of strongly bound tramadol, the polymers were eluted with 3×1 mL of 10% (v/v) AcOH/MeOH. The recovery of tramadol in NMIP cartridge with acetone, was decreased down to 15%, while recovery of tramadol by the MIP cartridges was maintained at 85%.

3.6. Adsorption capacity of polymers

One of the important factors we studied was the capacity of a sorbent to quantitatively remove a specific amount of drug from the solution [31,32]. In the measurement of adsorption capacity of MIP_3 and $NMIP_3$ absorbents, 50 mg samples of the absorbents were added to 10 mL tramadol solutions at concentrations of 1-500 µg L^{-1} The suspensions were mechanically shaken at room temperature, followed by centrifuging and removal of absorbents. The remaining tramadol in the supernatant was measured by HPLC-UV. The adsorption isotherm, which is the number of milligram adsorbed per gram of adsorbent (Q) versus the equilibrium concentration of tramadol, is shown in Fig. 10. According to these results, the maximum amount of tramadol that can be absorbed by MIP_3 is 81 mg g^{-1} (270 µmol g^{-1}) at 7.5 pH. As all the accessible specific cavities of the MIP_3 particles are saturated, the retention of the analyte is mainly due to non-specific interactions, which can be identical for MIP and NMIP polymers.

Table 2. Recovery (%) obtained after the loading of 50 mg of MIP and NMIP

Steps	Fractions	Recovery (%)	
		MIP	NMIP
1A	Washing, 1 mL, acetonitrile	10 ± 3[a]	14 ± 4
1B	Washing, 1 mL, acetone	6 ± 2	8 ± 3
1C	Washing, 1 mL, tetrahydrofuran	2 ± 1	4 ± 1
1D	Washing, 1 mL, dimethyl formamide	17 ± 4	25 ± 4
2	Elution (after step 1B), 3×1 mL, 10% (v/v) AcOH/MeOH	85 ± 2	15 ± 1

[a] Average of three determinations.

3.7. MIP selectivity

Chromatographic evaluation and equilibrium batch rebinding experiments are the methods most commonly used to investigate selectivity of the imprinted materials [33,34]. For equilibrium batch rebinding experiments, a known mass of template in solution is added to a vial containing a fixed mass of polymer. Once the system has reached the equilibrium, the concentration of free template in solution is measured and the mass of template absorbed to the MIP is calculated [35,36]. Dextromethorphan, Pseudoephedrine and diphenhydramine as antitussive (cough suppressant) drugs, hydroxyzine as antihistamines and cetrizine as an active metabolite of hydroxyzine were selected to investigate selectivity of the MIPs. Their molecular structures are shown in Fig. 2. Individual solutions of all compounds were prepared in the concentration of 50 µgL^{-1}. Extraction solvent was 10% (v/v) AcOH/MeOH.

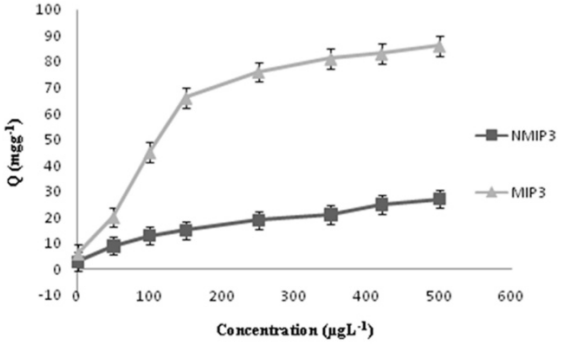


Figure 10. Curve of adsorption capacity obtained after the loading of 5 mL aqueous solution spiked with increasing amounts of tramadol onto the MIP particles (mean±S.D., n=3).

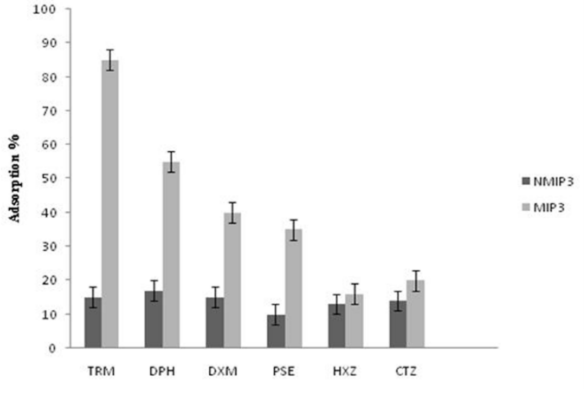


Figure 11. Adsorption of tramadol (TRM), diphenhydramine (DPH), dextromethorphane (DXM), pseudoephedrine (PSE), hydroxyzine (HXZ) and cetrizine (CTZ) with MIP_3 and $NMIP_3$ at 50 µg L^{-1} concentration. V=5 mL; pH 7.5 at 25°C (mean±S.D., n=3)

Extraction yields of the selected compounds with the MIP and NIP are shown in Fig. 11. Noticeably, the yields of analogues with the MIP were much higher than that of the NIP. It was also revealed that the tramadol based-MIPs possess better affinity to the template molecule. This affinity is mainly caused by the hydrogen bonding between the functional groups present in all drugs and carboxylic groups in the MIP. That may be due to relative structural similarity of extractions of drugs to the template molecule of MIP.

3.8. Drug release profiles

The purpose of this study was to observe a considerable difference between the MIP and NMIP in drug release and the investigation of pH and temperature effects on release profiles. MIP_3 matrices, which are the most effective ones for template recognition, were tested *in vitro* as devices for tramadol delivery and the results were compared to those with with $NMIP_3$ particles. We studied tramadol release from polymer particles in HCl (3 pH) and phosphate buffer (5.0 pH and 7.4 pH), respectively.

3.8.1 Effect of pH

The release of tramadol from MIP_3 and $NMIP_3$ was investigated as a function of pH of the media (Fig. 12). At 3.0 pH, the release of both polymers was faster than that at pH 5.0 and 7.4, with 100% release occurring within 2 h for $NMIP_3$ and 5 h for MIP_3. However, release of the polymers was delayed up to 5 h for $NMIP_3$ and up to nearly 12 h for MIP_3 in the 5.0 pH buffer. Release of tramadol at pH 7.4 was delayed more compared to release at other pHs, with 10 h release for $NMIP_3$ and up to 24 h release for MIP_3.

Initial quick release of tramadol in $NMIP_3$ and MIP_3 is related to physical adsorption and non-specific interactions. However, after this time we have slower release for the MIP_3 because of specific binding sites, which interacted strongly with tramadol. Drug release was reduced with the reduction of pH. However, in all cases release of MIP_3 was retarded for a time longer than that of $NMIP_3$. At pH 7.4 the difference in release was the highest. Since solubility of tramadol (pKa=9.41) in acidic pH is higher than solubility in basic pH, it leads to an acceleration in the release of drug into more acidic media. Thus, improved drug release solubility promoted drug release and decreased the difference between MIP_3 and $NMIP_3$ at lower pH. These studies explained faster drug release into acidic media mainly based on the degree of ionization of tramadol (pKa=9.41). Smaller pH had the effect on the release of tramadol in MIP_3 and $NMIP_3$. The matrices could not control the release of tramadol, thus the two polymers were rapidly released. On the other hand, at 7.4 pH, controlled release occurred better than at lower pH because polymers release was slower and matrices remained intact.

3.8.2. Effect of temperature

The experiment proved that decreasing the temperature from 37 to 25°C did not affect the property of matrix in controlling the MIP and NMIP release. As seen in Fig. 13. slower release of tramadol occurred in both polymers at room temperature (25°C). Nevertheless, the difference between MIP_3 and $NMIP_3$ was still apparent at 25°C.

4. Conclusion

Molecularly imprinted polymers for the selective recognition and the controlle and sustained release of tramadol were successfully synthesized. The applicability of this type of materials towards Drug Delivery System was then evaluated. Altering factors such as drug : monomer ratio, pH, time, and type of solvent, enabled modification of recognition and high affinity for tramadol. After drug loading, *in vitro* release experiments were performed and the results showed the ability of MIP polymers to control

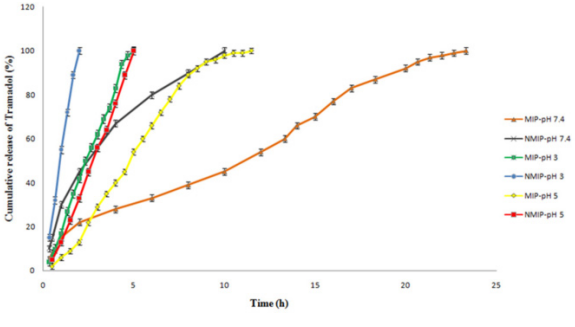


Figure 12. Release profile of 50 mg tramadol imprinted polymer at 37°C and various pH 3.0, 5.0 and 7.4. Media volume: 5 mL (mean±S.D., n=3)

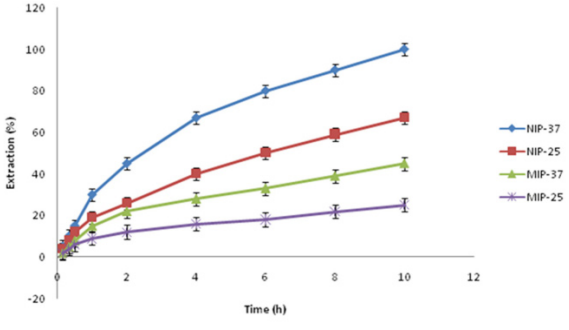


Figure 13. The effect of temperature on release profile of 50 mg tramadol imprinted polymer in pH 7.4 (mean±S.D., n=3)

tramadol release, supporting a release mechanism in which the release rate of the drug from the matrices depends on the selective interaction between the drug and imprinted cavities, pH and temperature of dissolution medium. As a result, the rate of release was considerably different. MIP is a very promising polymeric device for the selective and controlled release of tramadol that can also be used with non-imprinted polymers.

References

[1] J. Olson, P. Martin, I.D. Wilson, Anal. Commun. 35, 13H (1998)
[2] M. Komiyama, T. Takeuchi, T. Mukawa, H. Asanuma, Molecular Imprinting (Wiley-VCH, Weinheim 2003)
[3] R.A. Bartsch, M. Maeda, (Eds.), Molecular and Ionic Recognition with Imprinted Polymers, ACS Symposium Series 703 (American Chemical Society, Washington, DC 1998)
[4] B. Sellergen (Eds.), Molecular Imprinted Polymers (Elsevier, Amesterdam 2001)
[5] T. Takeuchi, J. Haginaka, J. Chromatogr. B 728, 1 (1999)
[6] K. Haupt, K. Mosbach, Chem. Rev. 100, 2495 (2000)
[7] K.C. Ho, W.M. Yeh, T.S. Tung, J.Y. Liao, J. Anal. Chim Acta, 542, 90 (2005)
[8] M. Javanbakht et al., Anal. Chim. Acta 612, 65 (2008)
[9] P.T. Vallano, V.T. Remcho, J. Chromatogr. A 887, 125 (2000)
[10] A. Kamal, B.A. Kumar, M. Arifuddin, S.G. Dastidar, Bioorg. Med. Chem. 11, 5135 (2003)
[11] F. Puoci, M. Curcio, G. Cirillo, F. Lemma, U.G. Spizzirri, N. Picci, Food Chem. 106, 836 (2008)
[12] W. Chen, D.K. Han, K.D. Ahn, J.M. Kim, Macromol. Res. 10, 122 (2002)
[13] R. Suedee, T. Srichana, G. Martin, J. Control. Release 66, 135 (2000)
[14] H. Sambe, K. Hoshina, R. Moadel, W. Wainer, J. Haginaka, J. Chromatogr. A 1134, 88 (2006)
[15] C.J. Allender, C. Richardson, B. Woodhouse, C.M. Heard, K.R. Brain, Int. J. Pharm. 195, 39 (2000)
[16] C. Alvarez-Lorenzo, A. Concheiro, Biotech. Ann. Rev. 12, 225 (2006)
[17] H. Hiratani, C. Alvarez-Lorenzo, J. Control. Release 83, 223 (2002)
[18] C. Alvarez-Lorenzo, A. Concheiro, J. Chromatogr. B 804, 231 (2004)
[19] C. Alvarez-Lorenzo, F. Yanez, R. Barreiro-Iglesias, A. Concheiro, J. Control. Release 113, 236 (2006)
[20] B. Sellergen, C.J. Allender, Adv. Drug Deliv. Rev. 57, 1733 (2005)
[21] C.J. Allender, C. Richardson, B. Woodhouse, C.M. Heard, K.R. Brain, Int. J. Pharm. 195, 39 (2000)
[22] M. Dale, The Complete Drug Reference (Pharmaceutical Press, London, 2006)
[23] M. Turchan, P. Jara-Ulloa, S. Bollo, L.J. Nunez-Vergara, J.A. Squella, A. Alvarez-Lueje, Talanta 73, 913 (2007)
[24] Y.H. Zhai et al., Anal. Chim. Acta 593, 123 (2007)
[25] R. Suedee, T. Srichana, T. Rattananont, Drug Delivery 9, 19 (2002)
[26] A.C. Moffat, Clarkes Analysis of Drugs and Poisons in Pharmaceuticals, 3d edition (Pharmaceutical Press, the publications division of The Royal Pharmaceutical Society of Great Britain, 2004) Vol. 2.
[27] A. Guyot, In: D.C. Sherrington, P. Hodge (Eds.), Synthesis and Separations Using Functional Polymers (John Wiley & Sons, New York 1989) 1-36
[28] L. Lloyd, J. Chromatogr. A. 544, 201 (1991)
[29] D.A. Spivak, J. Adv. Drug Deliv. Rev. 57, 1779 (2005)
[30] A. Rachkov, N. Minoura, J. Chromatogr. A 889, 111 (2000)
[31] R. Panahi, E. Vasheghani-Farahani, S.A. Shojaosadati, Biochem. Eng. 35, 352 (2007)
[32] M. Javanbakht, N. Shaabani, B. Akbari-Adergani, J. Chromatogr. B 877, 25 (2009)
[33] P. Martin, G.R. Jones, F. Stringer, I.D. Wilson, Analyst 128, 345 (2003)