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Magnetic Molecularly Imprinted Polymer Nanoparticles Coupled with High Performance Liquid Chromatography for Solid-Phase Extraction of Carvedilol in Serum Samples

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ABSTRACT: Herein, we report a magnetic molecularly imprinted polymers (m-MIPs) using Fe_3O_4 as a magnetic component, carvedilol as a template molecule for the solid-phase extraction (MISPE) as the sample clean-up technique combined with high-performance liquid chromatography (HPLC) and for the controlled release of carvedilol at different pH values of 1.0 (simulated gastric fluid), 6.8 (simulated intestinal fluid), and 7.4 (simulated biological fluid). The adsorption kinetics was modeled with the pseudo-first-order and pseudo-second-order kinetics, and the adsorption isotherms were fitted with Langmuir and Freundlich models. The performance of the m-MIPs for the controlled release of carvedilol was assessed and results indicated that the magnetic MIPs also have potential applications in controlled drug release. Furthermore, the m-MIPs were applied to the extraction of carvedilol from human blood plasma samples. Carvedilol can be quantified by this method in the 2–350 $\mu g\ L^{-1}$ concentration range. The limit of detection and limit of quantification in plasma samples are 0.13 and 0.45 $\mu g\ L^{-1}$. The results from HPLC showed good precision (3.5% for 50.0 $\mu g\ L^{-1}$) and recoveries (between 85 and 93) using m-MIP from human plasma samples. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 41209.

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INTRODUCTION

Biological fluids such as blood, plasma or urine are referred to as complex matrices and normally contain a number of endogenous components. These matrix components may interfere with and may adversely affect the subsequent separation and identification of analyte(s) of interest if they are not removed. Therefore, two-thirds of the total analysis time in chromatographic methods is typically due to the sample preparation steps and these are often the major source of error in the overall analytical process.

A few types of extraction method are used routinely in analytical toxicology such as liquid–liquid extraction (LLE),[1] liquid–liquid micro-extraction (LLE),[2,3] solid phase extraction (SPE),[4] solid-phase micro-extraction (SPME),[5] and molecularly imprinted solid phase extraction (MISPE).[6–8] Currently, the most convenient method for extraction of drugs in biological fluids is SPE due to its simplicity and versatility which leaves LLE, which is labor intensive and solvent consuming, far behind in analytical methodologies. MISPE is currently a growing field in clean up techniques for the analysis of biological samples.

SPE is a simple, fast, portable sampling and sample preparation technique using small volumes of solvent and is solvent-free in several cases. SPE is a very common type of sample extraction and clean up technique for biological fluids in analytical chemistry and a well-accepted sample preparation technique in the analytical chemistry community.[9–13]

A molecularly imprinted polymer (MIP) is a synthetic polymer bearing on its surface the molecular imprint of a specific target molecule (the template). Because of its imprinting according to the size, shape and functional groups of a template molecule, MIP acts as an artificial specific receptor. In fact, the trace analysis of bio-fluids and other complex matrices are usually based on selective sample pre-treatment. However, when a selective extraction from a complex sample has to be performed, the

typical SPE adsorbents lack sufficient selectivity are imperfect. Therefore, seeking more selective SPE adsorbents is important. The SPE approaches using highly selective adsorbents such as MIPs have the potential to overcome this problem.[14] Besides the high selectivity of MIPs, their simplicity of synthesis and less strict operation conditions compared with immune sorbents have led to their outstanding widespread applications.[15–20] The main advantage of molecular imprinting is the possibility of preparing a selective sorbent pre-determined for a particular substance or a group of structurally related substances. The polymer has a permanent memory of the template and is capable of selectively rebinding the template or structurally similar molecules such as the metabolites.

The application of MIPs as sorbents in SPE has been found very useful for selective extraction for the reason that MIPs offer higher selectivity than conventional SPE. MIPs also reduce the influence of the matrix on the resulting chromatogram and high sample enrichment factors are achieved. The MIPs are extensively employed in SPE and they found to be quite effective and selective adsorbents.[21–24]

Currently, magnetic nanoparticles (MNPs) have found numerous biological and environmental applications owing to their unique magnetic properties enabling them to be used by the magnetic field in order to separate the target from the samples.[25,26] Thus, it can be anticipated that the cooperation of the MNPs with MIPs will offer a new approach for separation applications and extend employing of MIPs. In addition, the combination of magnetically susceptible properties of MNPs and the advantage of MIP not only affords the selectivity for the target molecule but also provides the ability of one-step separation. The magnetic molecularly imprinted polymers (m-MIPs) based on chitosan-Fe_3O_4 were used for sorption of carbamazepine from water.[27] The extraction of melamine from milk followed by liquid chromatography-tandem mass spectrometry was performed by m-MIPs.[28] The extraction of chloramphenicol from honey using m-MIPs was also carried out.[29] Moreover, the solid phase extractions of cephalosporin from milk,[30] synephrine from methanol–water media,[31] emodin from kiwi fruit root,[32] five sulphonylurea herbicides,[33] organophosphorus pesticides in fruit samples,[34] and epinephrine in real samples[35] have been conducted.

Carvedilol, 1-(9H-carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino]-2-propanol, is a drug with β- and α1- receptor blocking activity approved for the treatment of congestive heart failure (CHF). In view of its antioxidant activity, carvedilol is efficient to suppress lipid peroxidation, protein oxidation or to inhibit the generation of reactive oxygen species. It is beneficial for patients with CHF due to its protective effect of the heart. Since its use for pharmaceutical purposes is relatively recent, literature reveals that methods available for carvedilol determination are scarce. Different analytical techniques have been employed for the determination of carvedilol in biological fluids especially those applied to the analysis of plasma should be highlighted.[36–43]

Herein, synthesized 2-(methacrylamido) ethyl methacrylate was used as a cross linker to prepare magnetic molecularly imprinted nanoparticles for controlled release and screening of carvedilol in human plasma using combination of MISPE and HPLC method. The resulting m-MIPs could be quickly collected and regenerated by an external magnetic field without tedious centrifugation or filtration. The method for determination of carvedilol in human plasma was rapid with a wide linear range and low detection limit. Effect of pH, adsorption isotherm, adsorption kinetic and selectivity of polymers were examined.

EXPERIMENTAL

Materials

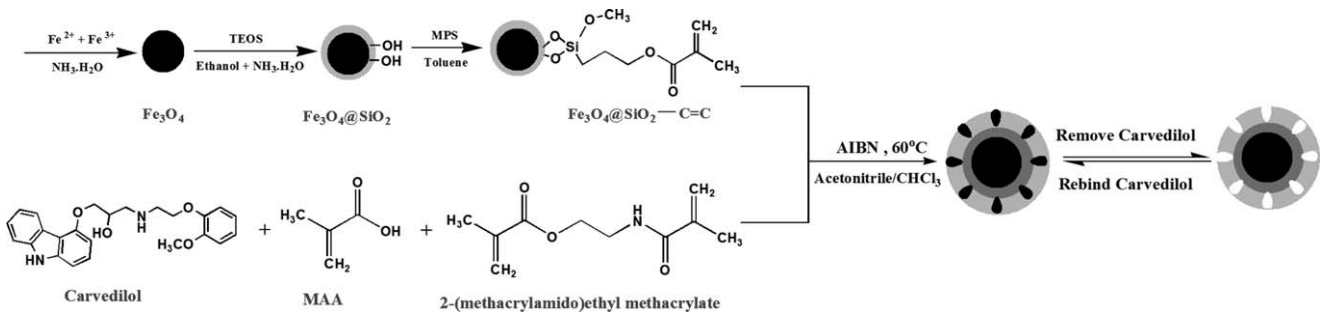
Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$), ammonia solution (28%, w/w) for synthesis of Fe_3O_4 nanoparticles, ethanol, tetraethyl orthosilicate (TEOS), and methacryloxypropyl trimethoxysilane (MPS) were obtained from Merck (Darmstadt, Germany). For preparation of the cross linker, methacrylic acid (MAA), thionyl chloride ($SOCl_2$), and ethanolamine were obtained from Merck (Darmstadt, Germany), Acros organics and Sigma companies. Carvedilol and other drugs obtained from the Ministry of Health and Medical Education (Tehran, Iran). Dialysis tubes (Sigma dialyses tubes Mw cutoff 12 kDa) were heated in an aqueous solution of 2% wt sodium hydrogen carbonate and 0.05% wt ethylene diamine tetra acetic acid (EDTA) and then kept under refrigeration in an aqueous solution of 0.05 wt sodium azide until use. 2,2-Azobisisobutyronitrile (AIBN) and solvents were also obtained from Merck.

Instruments and Chromatographic Conditions

High performance liquid chromatography (HPLC) system consist a Waters 515 pump, a 486Waters UV-Vis detector, a model 7725i Rehodyne injector with a 20 µL sample loop. Chromatographic separation was achieved on an ACE 5 μm, C18 column of 4.6 $\times$ 150 mm^2 column. HPLC data was acquired and processed using a PC and Millennium 2010 Chromatogram Manager software (Version 2.1 Waters). pH measurements were performed with a model 780 Metrohm (Switzerland) pH meter using a combined glass electrode. A Windaus two-channel peristaltic pump model D-38678 was used to pump solvents during the MISPE experiments. Particle size of nanoparticles was determined by transmission electron microscopy (TEM). The TEM investigation of nanoparticles was carried out following negative staining with uranyl acetate. Briefly, a drop of the nanoparticles suspension (1 mg/ mL) was placed on formvar-coated copper grids (Ted Pella, Redding, CA) and allowed to equilibrate. The excess amount of liquid was detached with filter paper and a drop of 2% W/V uranyl acetate was mixed to the grid and air dried after 3 min followed by TEM examination (TEM, Philips/FEI, NY). The diameters of at least fifty individual nanoparticles in the TEM images were determined manually using a digital caliper, which was used to calculate the average particle size. Scanning electron microscopy (SEM) was also used for analyzing morphology of nanoparticles by a Philips XL30 (Philips/FEI, Netherland).

MISPE Conditions

m-MIP and m-NIP columns were prepared by packing 100 mg of the polymers into 2 mL empty cartridge. The cartridge was conditioned sequentially with 1 mL of methanol, 1 mL of



Scheme 1. Schematic illustration of the synthesis of magnetic MIPs.

deionized water, and 1 mL ammonium phosphate (20 mmol L^{-1}) at pH 3.0. Extraction experiments involved loading the column with 5 mL, 50 $\mu g\ L^{-1}$ carvedilol (pH 9.0) at a flow rate of 1 mL min^{-1}. After loading, the column was washed with 1 mL acetone:acetonitrile (3 : 1 v/v) and 1 mL dichloromethane. After washes, SPE cartridge was dried thoroughly by vacuum application. Finally, the elution was performed by passing 3 × 1 mL of acetonitrile:phosphate buffer (90 : 10, pH 9.0) through the cartridge. All fractions were evaporated to dryness at 20°C under a stream of nitrogen and finally recovered in 1 mL of mobile phase. Then, 25 μL of each sample was injected onto the analytical column of HPLC.

Table I. Compositions and Comparisons of the Extraction of Carvedilol from Carvedilol Standard Solution (10 mL, 0.05 mM) Using 50 mg of Various Polymers as Sorbents (mean ± SD, $n = 3$)

MMIP	Template (mmol)	MAA (mmol)	Cross link agent (mmol)	AIBN (mmol)	Extraction (%)
m-MIP 1	1	1	8	0.152	50 ± 2
m-MIP 2	1		10	0.152	60 ± 1
m-MIP 3	1		12	0.152	65 ± 1
m-MIP 4	1		14	0.152	65 ± 3
m-MIP 5	1		16	0.152	60 ± 2
m-MIP 6	1	2	8	0.152	75 ± 1
m-MIP 7	1		10	0.152	89 ± 1
m-MIP 8	1		12	0.152	78 ± 2
m-MIP 9	1		14	0.152	70 ± 3
m-MIP 10	1		16	0.152	70 ± 1
m-MIP 11	1	4	8	0.152	66 ± 1
m-MIP 12	1		10	0.152	70 ± 1
m-MIP 13	1		12	0.152	72 ± 2
m-MIP 14	1		14	0.152	68 ± 3
m-MIP 15	1		16	0.152	70 ± 1
m-MIP 16	1	6	8	0.152	55 ± 2
m-MIP 17	1		10	0.152	65 ± 3
m-MIP 18	1		12	0.152	60 ± 1
m-MIP 19	1		14	0.152	60 ± 2
m-MIP 20	1		16	0.152	57 ± 1
m-NIP	-	2	10	0.152	16 ± 2

MIP Preparation

Surface modified magnetic nanoparticles (Fe_3O_4) and crosslink agent (2-(methacrylamido) ethyl methacrylate) were prepared according to previous studies.[44,45] The schematic diagram for the preparation of m-MIPs is presented in Scheme 1. A solution of 2 mmol MAA as a monomer, 1 mmol carvedilol as template in acetonitrile was shaken at room temperature for 2 h to form a template–monomer complex. Afterwards, 0.5 g of dispersed double-bond-functionalized Fe_3O_4 magnetic nanoparticles, 10

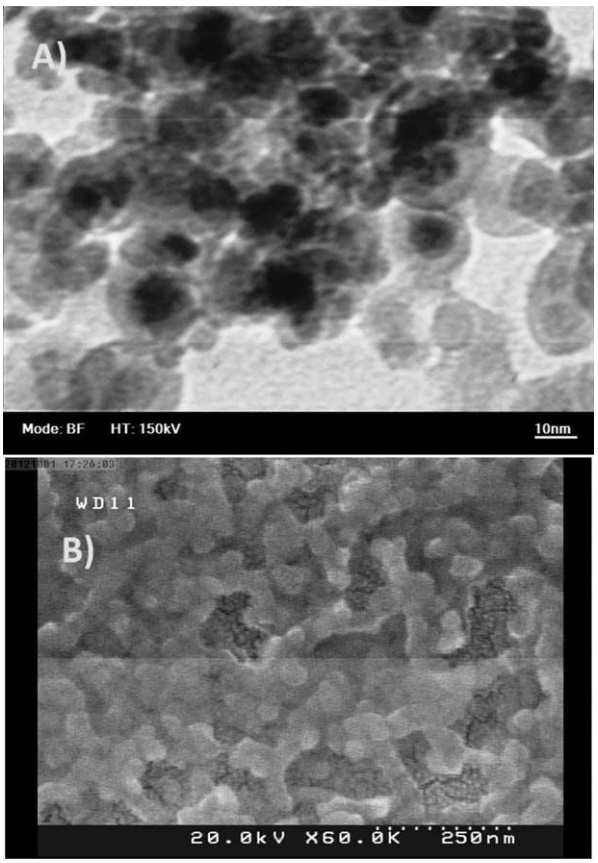


Figure 1. (A) TEM and (B) SEM images of solution containing m-MIPs.

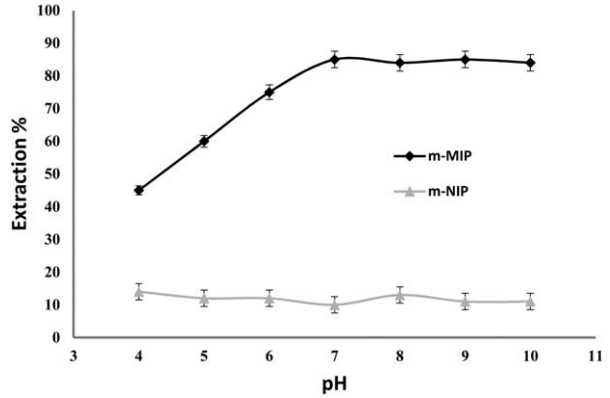


Figure 2. Effect of pH on rebinding efficiency of carvedilol. About 50 mg of the polymers; sample volume: 5 mL; carvedilol concentration: 50 m*M*; room temperature (mean ± S.D., $n = 3$).

mmol 2-(methacrylamido) ethyl methacrylate and 0.1 mmol AIBN were added to the solution and the mixture was sonicated in an ultrasonic bath. After sonication (5 min), it was purged with N_2 and the flask was sealed under N_2 atmosphere. The magnetic MIPs were synthesized in a 100-mL flask equipped with a mechanical stirrer in an oil bath kept at 60°C for 20 h. The resultant polymeric nanoparticles were collected by the external magnetic field and eluted by a mixture solvent of methanol and acetic acid (9 : 1, v/v) for several times to extract the template molecules until the eluent was free from templates as detected by UV-vis spectrometry (at 242 nm). The obtained polymers were finally rinsed with water to remove the remaining solvents and dried in the vacuum for 24 h before use. For a comparison, magnetic non-molecularly imprinted polymers (m-NIPs) were prepared in the absence of the template during the polymerization process and treated in an identical manner.

Binding Experiment

For measuring of template binding, 50 mg of polymer nanoparticles were added to 10 mL carvedilol solution (pH 9.0) of various initial concentrations in a conical centrifugation tube and sealed. The mixtures were thermo stated at 25°C for 24 h under continuous stirring and then magnetic materials were separated via an external magnetic field. Then, the residual concentration of carvedilol in solution was established using HPLC-UV at 242 nm. The quantity (Q) of the template bound to m-MIP or m-NIP was calculated according to the following equation[46]:

$$Q = [(C_0 - C_t) \times V]/W$$

where C_0 and C_t (mg/L) are the initial concentration and the residual concentration of carvedilol, respectively; V (L) is the initial volume of the solution, and W (g) is the weight of the m-MIPs or m-NIPs.

Extraction Procedure for Human Plasma Samples

Drug-free human plasma was obtained from the Iranian blood transfusion service (Tehran, Iran) and stored at −20°C until use after gentle thawing. Due to possibility of protein-bonding for carvedilol and reducing the recoveries processes, it is necessary to have some treatments with plasma before extraction with m-MIP nanoparticles. So, the plasma samples were diluted with 25 m*M* ammonium acetate (pH 9.0), then centrifuged 30 min at 6000 rpm to remove excess of proteins. Then the supernatant

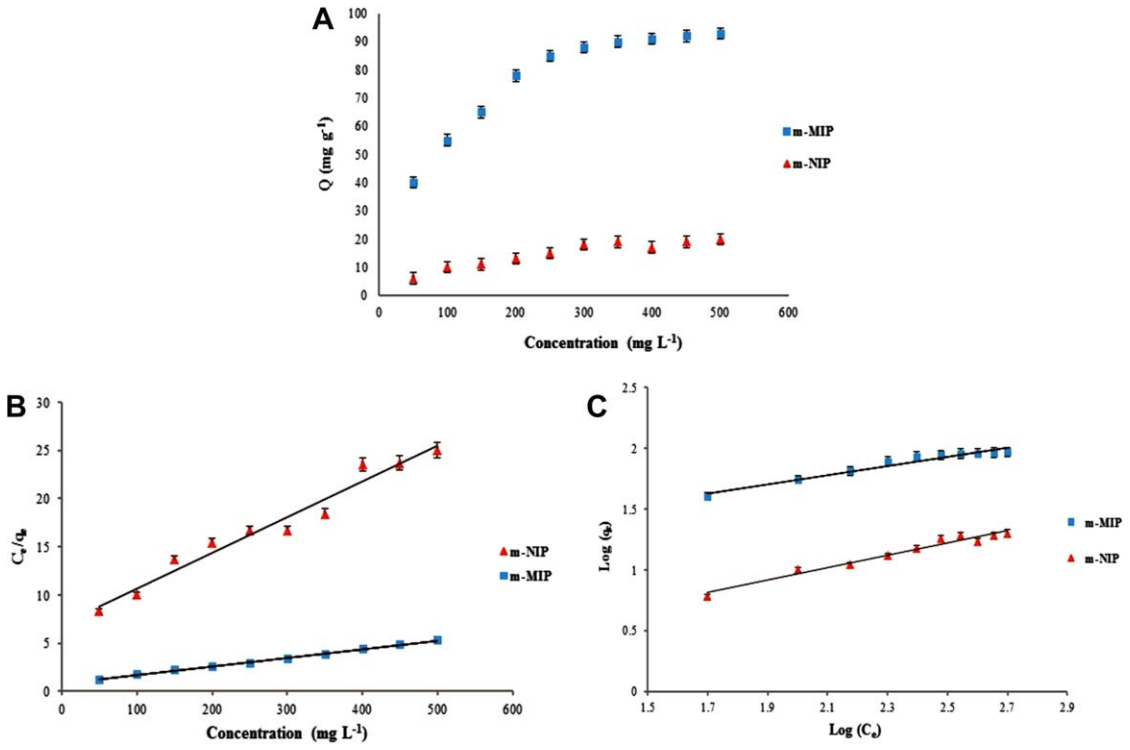


Figure 3. (A) Adsorption isotherms of carvedilol onto m-MIPs and m-NIPs nanoparticles. (B) The adsorption isotherms fitted with Langmuir model. (C) The adsorption isotherms fitted with Freundlich model. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Isotherm Parameters for Carvedilol Adsorption by m-MIPs and m-NIPs Nanoparticles

	Langmuir			Freundlich		
	q_m (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F (L mg^{-1})	1/n	R^2
m-MIPs	112.36	0.0107	0.9958	9.8423	0.3752	0.9548
m-NIPs	27.1	0.0052	0.9633	0.8697	0.5151	0.9651

was filtered through a cellulose acetate filter (0.2 μm pore size, Advantec MFS, CA). The filtrate was collected in glass containers and stored at −20°C until the analysis was performed. About 2 mL of the filtered supernatant was collected to be directly percolated through the m-MIP or the m-NIP.

In Vitro Controlled Release of Carvedilol

Magnetic MIP nanoparticles were tested *in vitro* as devices for carvedilol delivery and their targeting ability to plasma and colon were considered. Release studies were performed for drug entrapped during m-MIPs synthesis, for adsorption of drug into the soxhelted m-MIP and m-NIP nanoparticles by incubation in the drug solution in two parallel experiments. The first one was performed at a pH of 1.0 (simulated gastric fluid) for 2 h and then at a pH of 6.8 (simulated intestinal fluid) using the pH change method. In the second experiment, the release profile was evaluated at a pH of 7.4 (simulated biological fluids). These conditions were maintained throughout the experiment. Samples were drawn from the dissolution medium at appropriate time intervals to determine the amounts of drug released by UV-Vis.

RESULTS AND DISCUSSION

As illustrated in Scheme 1, the synthesis of the m-MIPs is a multistep procedure, which involves synthesis of Fe_3O_4 MNPs, silica-shell deposition (Fe_3O_4@SiO_2), modification of Fe_3O_4@-SiO_2 with MPS, preparation of a cross linker (2-(methacrylamido) ethyl methacrylate) and m-MIPs. At first, Fe_3O_4 MNPs were prepared by the co-precipitation method. Then, the surface of Fe_3O_4 MNPs was coated with silica by the TEOS. The SiO_2 shell provided a biocompatible and hydrophilic surface, and prevented oxidation of Fe_3O_4. Furthermore, silanol groups were beneficial to chemical modification on the surface of Fe_3O_4@SiO_2. Thus, double bonds were introduced onto Fe_3O_4@SiO_2 using MPS to ensure tight growth of the imprinted layer. We have prepared 2-(methacrylamido) ethyl methacrylate by from the reaction of ethanol amine with methacryloyl chloride in a 1 : 2 mole ratio in dichloromethane. Finally, m-MIPs were synthesized using modified Fe_3O_4 MNPs, MAA, cross linker, carvedilol and AIBN in acetonitrile/chloroform (5 : 1 v/v). The resultant polymers were used to human plasma assay.

Optimal MIP Formulation

Various amounts of monomer and cross-link agent to optimize different formulations of MIPs with enhanced molecular recognition capabilities have been used. Generally, appropriate mole ratios of functional monomer and cross-link agent to template are very valuable to enhance specific polymers and a number of MIP recognition sites. Several molar ratios of the functional monomer MAA and cross-link agent to template were used in this study. As Table I depicts, the optimum ratio of functional monomer and cross-link agent to template for the specific rebinding of carvedilol was 2 : 10 : 1 (m-MIP 7), which had the best extraction of 89%, while that of the corresponding m-NIP was low at 16%.

TEM and SEM

The particle size method was always used to characterize nanoparticles since it makes it possible to understand the dispersion and aggregation process. In addition, particle size affects biological handling of nanoparticles and the sub-hundred nanometer particle size is helpful in the drug delivery system, since nanoparticles in this size range have been shown to have higher cellular and tissue uptake. The carvedilol loaded magnetic MIP nanoparticles prepared in this study present a clear appearance with an average particle size of around 25 ± 1.2 nm [Figure 1(A)]. Figure 1(B) also shows SEM image of nanoparticles shapes which are in agreement with the TEM image (presenting a spherical morphology).

Effect of pH on Drug Loading

The effect of pH on the sorption of carvedilol was examined by varying the pH of solutions from 4.0 to 10.0. Several batch experiments were performed by equilibrating 50 mg of the imprinted particles with 100 mL of the solutions containing 0.05 m*M* of

Table III. Kinetic Parameters for Adsorption of Carvedilol Sorption

		Pseudo-first-order model			Pseudo-second-order model		
Concentrations (mg L^{-1})	$q_{e,exp}$ (mg g^{-1})	$q_{e,cal}$ (mg g^{-1})	K_1 (min^{-1})	R^2	$q_{e,cal}$ (mg g^{-1})	K_2 (g mg^{-1} min^{-1})	R^2
100	56.0	58.13	0.0188	0.9991	62.36	0.00258	0.981
200	79.5	77.46	0.0179	0.9992	81.21	0.00156	0.993
400	91.0	90.74	0.0176	0.9991	95.52	0.00141	0.9989

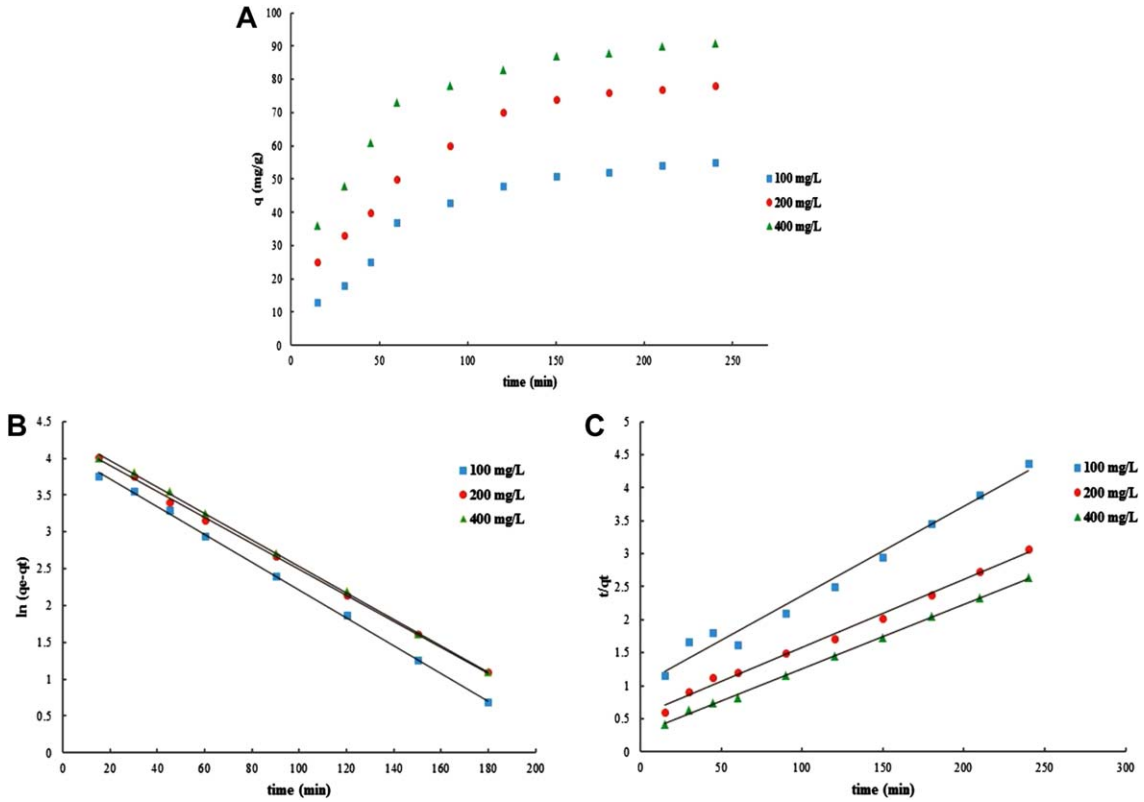


Figure 4. (A) Kinetic adsorption curves of m-MIPs nanoparticles for carvedilol at different initial concentrations. (B) The pseudo-first-order model and (C) the pseudo-second-order model for the sorption of carvedilol onto m-MIPs. Sorbent dose: 50 mg; solution volume: 15 mL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

carvedilol under the desired levels of pH. The results for different polymers (Figure 2) displayed that pH have great effects on loading. The binding behavior of carvedilol was not greatly affected at pH >7. Although, lesser effects were observed at lower pH values and which may have been attributed to the protonation of the functional group of carvedilol and consequence breakdown of the hydrogen bonds at the pH <7.0. Finally, as the recovery rates of carvedilol flatten at around the pH 9.0, therefore, this pH value was chosen for later SPE experiments.

Adsorption Isotherms

The carboxylic acid in the cavities of the MIP has very high hydrophilic affinity with respect to carvedilol. On the other hand, the hydroxyl and the amines of carvedilol also possess hydrogen bonding with the carboxylic acid group of the MIP. In short, the hydrogen bonding and the hydrophilic forces are the major interaction between carvedilol and MIP. The adsorption isotherms (Q) of carvedilol for m-MIP and m-NIP particles are shown in Figure 3(A). The adsorption capacity of m-MIP was much higher than that of m-NIP and the imprinting efficiency (IE) is about 4.65, as considered by the ratio of the maximum adsorption quantity of MIP to that of NIP. This illustrates that the imprinted layer possesses recognition ability of carvedilol and can be attributed to the complementary

Table IV. Distribution Ratio (KD) and Selectivity Coefficient (α) Values for Magnetic Imprinted and Nonimprinted Polymers (Mean ± SD, $n = 3$)

	m-MIP		m-NIP	
Compound	K_D	α	K_D	α
Carvedilol	900		22	
Bromhexine	51	17.65	24	0.92
Aspirin	85	10.59	20	1.10
Meloxicam	25	36.00	19	1.16
Diphenhydramine	37	24.32	21	1.05

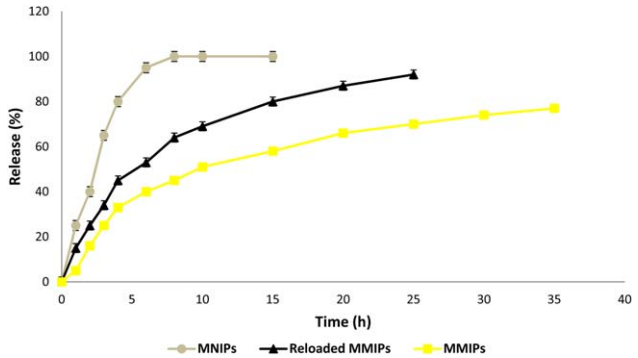


Figure 5. Release profile of carvedilol from m-MIPs and m-NIPs in simulated gastric fluids for 2 h and then in simulated intestinal fluid. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

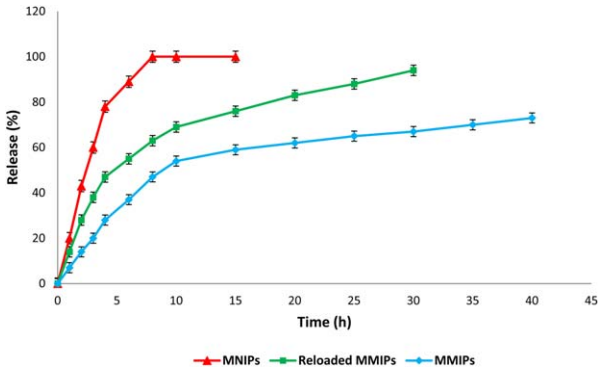


Figure 6. Release profile of carvedilol from m-MIPs and m-NIPs in plasma simulating fluids. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cavities created by carvedilol templates. Results of equilibrium studies were analyzed using Langmuir and Freundlich isotherms to find best fitted model which can describe the adsorption process. The original forms of the Langmuir and Freundlich isotherms, given by eqs. (1) and (2), respectively,

$$q_e = \frac{K_L q_m C_e}{1 + K_L C} \tag{1}$$

$$q_e = K_F C_e^{1/n} \tag{2}$$

where q_m is the maximum amount of adsorption (mg g^{-1}), K_L is the affinity constant (L mg^{-1}), K_F is a constant, which is a measure of adsorption capacity and n is a measure of adsorption intensity. Corresponding linear forms of Langmuir and Freundlich isotherms can be expressed as eqs. (3) and (4), respectively,

$$\frac{C_e}{q_e} = \frac{1}{K_L q_m} + \frac{C_e}{q_m} \tag{3}$$

$$\log q_e = \frac{1}{n} \log C_e + \log K_F \tag{4}$$

As shown in Figure 3(B,C), the two isotherms can be fitted by Langmuir and Freundlich models. It was observed that the equilibrium data were best matched by Langmuir isotherm with good correlation coefficient values and the detailed values were reported in Table II.

Table V. Assay of Carvedilol in Human Plasma by SPE-HPLC Procedure

Sample	Spiked value (μg L^{-1})	Recovery % ± SD[a] m-MIP	m-NIP
Human serum	5	85	11
	10	91	13
	25	88	10
	50	93	10

[a] Average of three determinations.

Adsorption Kinetics

The kinetic data obtained were analyzed using pseudo-first-order rate eqs. (5) and (6) and pseudo-second-order rate eqs. (7) and (8), respectively.[47]

$$q_t = q_e - q_e e^{-k_1 t} \tag{5}$$

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{6}$$

$$q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e t} \tag{7}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{8}$$

where q_e and q_t (mg g^{-1}) are the amount of carvedilol adsorbed on the sorbent at the equilibrium and at time t (min), respectively. k_1 (min^{-1}) and k_2 (g mg^{-1} min^{-1}) are the rate constant of adsorption.

All the linear regression correlation coefficients and rate constants of adsorption were presented in Table III. As illustrated in Table III, the first-order model was more suitable to describe the sorption kinetic data than the second-order model because of the favorable fit between experimental and calculated values of q_e in the first-order model (all R2 values above 0.999 at different initial concentrations). The plots of the experimental data of the sorption capacity against time along with the model values for the pseudo-first-order and pseudo-second-order model were shown in Figure 4(A–C), respectively. It can be seen from Figure 4, the adsorption rate enhanced suddenly in the initial stage and then became slow until equilibrium and the equilibrium adsorption amount increased with an increase of initial concentration.

Study of MIP Selectivity

In order to evaluate the selectivity of the synthesized m-MIP, Carvedilol, and several drugs with the ability to form hydrogen bonds with the m-MIP nanoparticles were selected in this section. The initial concentration of drugs (5 mL, 0.05 mM) was extracted by 50 mg of m-MIPs and m-NIPs at pH 9.0. The distribution ratio (K_D) was calculated using the equation:

$$K_D = \frac{(C_i - C_f)V}{C_f m}$$

where V, C_i, C_f, and m represent the volume of the solution (mL), drug concentration before and after adsorption (mM) and mass of the polymer, respectively. The selectivity coefficient (α) is defined as:

$$\alpha = \frac{K_D(\text{carvedilol})}{K_D(\text{foreign compound})}$$

The results are listed in Table IV. The higher K_D obtained for carvedilol strongly confirmed a higher selectivity of m-MIPs this template and demonstrated the possibility of m-MIP as a selective adsorbent for the extraction of carvedilol from complex matrixes such as biological fluids.

Table VI. Comparison of Developed Method with Existing Methods for the Determination of Carvedilol

Method	LOD	LOQ	LOL	Samples	References
HPLC-fluorimetry	–	0.4 ng mL^{-1}	242 ng mL^{-1}	Human plasma	40
HPLC-fluorimetry	–	1 ng mL^{-1}	80 ng mL^{-1}	Human plasma	41
HPLC-fluorimetry	3.6 ng mL^{-1}	–	1000 ng mL^{-1}	Rat plasma	42
HPLC	–	0.01 ng mg^{-1}	0.35 ng mg^{-1}	Human cardiac tissue	43
HPLC–MS/MS	–	0.1 ng mL^{-1}	200 ng mL^{-1}	Human plasma	44
Spectrophotometry	–	4 μg mL^{-1}	36 μg mL^{-1}	Bulk and formulations	45
Differential pulse voltammetry	0.1 μg mL^{-1}	0.25 μg mL^{-1}	10 μg mL^{-1}	Tablets dosage form	46
Chemiluminometry	8.7×10^{-9} mol L^{-1}	1.2×10^{-7} mol L^{-1}	3.0×10^{-6} mol L^{-1}	Pharmaceuticals	47
Proposed method	0.13 μg L^{-1}	0.45 μg L^{-1}	350 μg L^{-1}	Human plasma	This study

In Vitro Release Studies

Carvedilol incorporation during the process of generation of the magnetic nanoparticles and hence trapping of the drug in the polymeric matrix (m-MIPs), or adsorption of carvedilol into soxhelted magnetic MIP nanoparticles by incubation in the drug solution (reloaded m-MIPs) and m-NIPs were followed to evaluate release behavior of particles (Figures 5 and 6). It obviously shows that the non-imprinted polymers do not have specific binding sites in which the drug is bound with non-covalent interactions, whereas reloaded m-MIPs and m-MIPs, due to their specific network structure, still retain a significant percentage of drugs. This observation supports a model of retention mechanism, which assumes that the selective sites have stronger interaction with the drug than the non-selective sites.

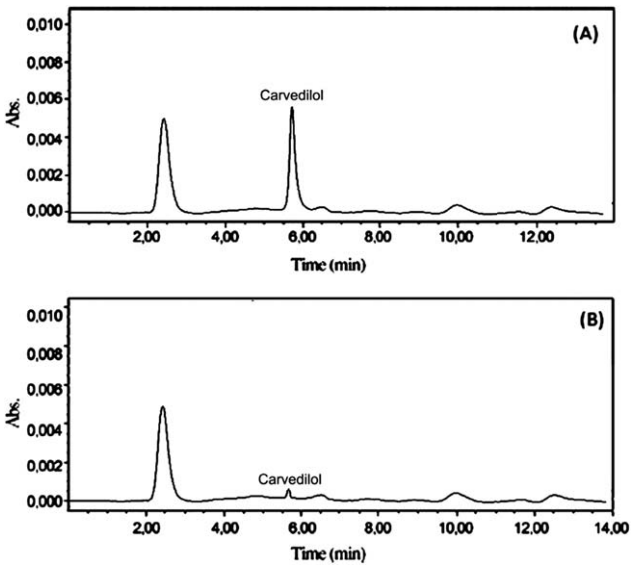


Figure 7. HPLC chromatograms obtained after percolation of 2 mL human serum spiked with 50.0 µg L^{-1} of carvedilol with a cleanup step comprising the (A) m-MIP and (B) m-NIP monitored at 242 nm; conditions: column ACE 5 µm, C18 4.6 mm × 250 mm at +40°C, eluent acetonitrile: phosphate buffer (0.01 mol L^{-1}, pH 3.0) 90 : 10 at flow rate of 1.0 mL min^{-1}.

Additionally, the increased release of carvedilol from reloaded m-MIPs may arise due to higher loading of drug in this polymer, whereas in the case of reloaded nanoparticles the release was fast. It is of great importance to note that the drug release in the m-MIPs was in a controlled manner. This observations can be explained by the fact that the most loading of reloaded m-MIPs comes from surface adsorption which shows burst release, but in case of m-MIPs, carvedilol incorporated in the MIP cavities were released which is controlled by different phenomenon.

The experimental data at a pH of 7.4 also revealed a better controlled release of drugs from the m-MIPs samples than that obtained from m-NIPs (Figure 6). The explanation of the drug retention is the same as that proposed for the experiments carried out in simulating gastrointestinal fluids, and the complete release in m-MIPs are again not complete even after 40 h.

Real Sample Analysis

To demonstrate the potential of MIP for the sample clean up, the MIP was applied to the purification of spiked carvedilol in human plasma. Aqueous media was employed for the loading solution, and the wash procedure was assessed for obtaining maximum recovery of the analytes using acetone. The chromatograms obtained for plasma samples are shown in Figure 6. This efficient method allowed cleaner extracts to be obtained and interfering peaks arising from the complex biological matrices to be suppressed. Results from the HPLC analyses in Table V showed that the MIP extraction of carvedilol for plasma samples have good precision (3.5% for 50.0 μg L^{-1}) and recoveries (between 85 and 93). Typical chromatograms (presented in Figure 7) reveal that the MIP can be used for the sample clean up and when MIP sorbent was used, a board peak in chromatogram was omitted. The limit of detection (LOD) and limit of quantification (LOQ) for carvedilol in plasma samples were 0.13 and 0.45 μg L^{-1}. The number of replicate for experiment was been three times.

CONCLUSION

In this study, carvedilol imprinted magnetic nanoparticles were successfully prepared by using molecular imprinted technique for the selective adsorption of carvedilol from human plasma samples and the release profile of obtained m-MIPs in

simulated gastric, intestinal and plasma fluids were studied. This indicated a model of retention mechanism which assumes that the selective sites have stronger interaction with the drug than the non-selective sites. Furthermore, reloaded m-MIPs showed faster release than m-MIPs because the most loading of reloaded m-MIPs comes from surface adsorption which causes burst release, but in case of m-MIPs, template incorporated in the m-MIP cavities which release is in controlled manner. Moreover, the recoveries for the spiked human plasma samples were between 85 and 93% in 5–50 $\mu g\ L^{-1}$, respectively. It could be concluded that the technique has great potential in developing selective extraction method for other compounds. In addition, m-MIPs have promising advantages such as less cost of organic solvents and could be separated quickly by an external magnetic field. For carvedilol adsorbed onto magnetic nanoparticles, the pseudo-first-order model yielded a better fit than those by the pseudo-second-order model. Isotherm study showed that the experimental equilibrium data could be well fitted by the Langmuir adsorption model.

In Table VI, the m-MIP for carvedilol is compared with other methods previously reported, regarding some analytical characteristics. The m-MIP shows low LOD and LOQ.

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