Molecularly Imprinted Polymer Microspheres with Nanopore Cavities Prepared by Precipitation Polymerization as New Carriers for the Sustained Release of Dipyridamole

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ABSTRACT: Imprinted polymers are now being increasingly considered for active biomedical uses such as drug delivery. In this work, the use of molecularly imprinted polymers (MIPs) in designing new drug delivery devices was studied. Imprinted polymers were prepared from methacrylic acid (MAA) (functional monomer), ethylene glycol dimethacrylate (cross-linker), and dipyridamole (DIP) (as a drug template) using precipitation polymerization method. The influence of the template/functional monomer proportion and pH on the achievement of MIPs with nanopore cavities with a high enough affinity for the drug was investigated. The small pores (average 3.9 nm) in the imprinted microspheres show excellent retention properties for the target analyte. The polymeric devices were further characterized by FT-IR, thermogravimetric analysis, scanning electron microscopy, photon correlation spectroscopy, Brunauer-Emmett-Teller analysis, and binding experiments. The imprinted polymers showed a higher affinity for DIP and a slower release rate than the nonimprinted polymers. The controlled releases of DIP from the prepared imprinted polymers were investigated by an *in vitro* dissolution test by measuring the absorbance at 284 nm by means of a UV–Visible spectrophotometer. Loaded imprinted microsphers showed very slow release in various solutions such as phosphate buffer solution (pH 6.8), HCl (pH 1.0) and mixture of HCl and MeOH at 37.0 ± 0.5°C and were able to prolong DIP release for more than two days. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 1586–1593, 2011

Key words: molecularly imprinted polymer; dipyridamole; sustained release; nanopore; carrier

INTRODUCTION

Molecular imprinting technology can provide efficient polymer systems with the ability to recognize specific bioactive molecules and a sorption capacity dependent on the properties and template concentration of the solution.[1,2] Among the different methods available for the preparation of molecularly imprinted polymers (MIPs), the so-called noncovalent approach, which uses only noncovalent interactions between the template and the functional monomers, is probably the most flexible regarding the selection of the functional monomers and the possible template molecules. For these reasons, the noncovalent approach has been the most widely adopted.[1] The procedure for synthesizing an MIP is based on the chemical polymerization of a functional monomer and a cross-linking agent in the presence of a molecule used as a template. After the removal of the imprinted molecule, an imprinted polymer is obtained. This polymer contains sites with high affinity for the template molecule, because of their shapes and the arrangement of the functional groups of the monomer units. The imprinted polymers are used as antibody-like materials for high selectivity and sensitivity, owing to their long-term stability, chemical inertness, and insolubility in water and most organic solvents.

These MIPs have shown to be useful in wide fields such as sensors and biosensors,[3,4] solid-phase extraction,[5,6] affinity chromatograghy,[7,8] enzyme-like catalysts,[9] enantioseparation,[10,11] and pharmaceutical applications.[12]

In the recent years, a progressive increase in the number of articles devoted to the application of MIPs in the design of new drug delivery systems (DDS) is also seen.[13–17] The molecular imprinting technology can provide polymeric materials with the ability to recognize specific bioactive molecules and with a sorption/release behavior that can be made sensitive

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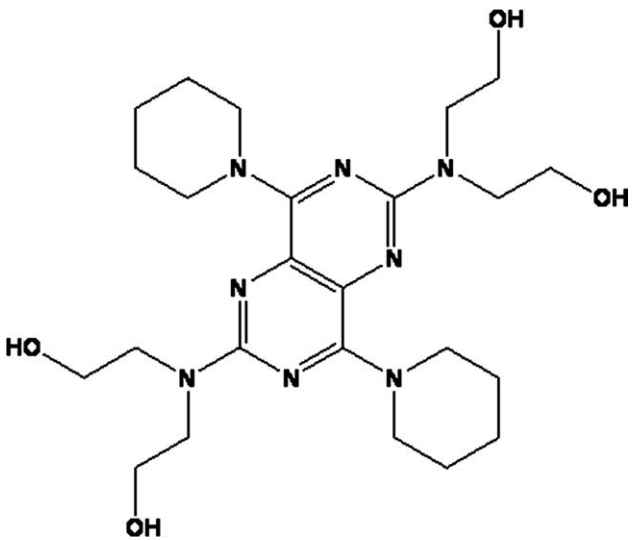

Figure 1 Structure of DIP.

to the properties of the surrounding medium. The potential advantage of imprinted polymers as (DDS) is owing to their cross-linked polymeric nature. They can potentially increase the residence time of the drug within the body by reducing the rate at which the drug is released. In cases where the drug has a narrow therapeutic index, MIP delivery vehicles might keep the concentration of the drug in the body below the concentration where adverse side effects become dominant.

Recently, we have successfully synthesized new MIPs as suitable artificial recognition materials for the construction of biomimetic sensors for hydroxyzine,[4] and cetirizine,[18] and solid-phase extraction of bromhexine,[19] verapamil,[20] metoclopramide,[21] and tramadol[22] in human serum and urine.

Dipyridamole (DIP) (Fig. 1) is a drug that inhibits thrombus formation when given chronically and causes vasodilation when given at high doses over short time. This drug inhibits the cellular reuptake of adenosine into platelets, red blood cells, and endothelial cells leading to increased extracellular concentrations of adenosine. Recent studies suggest that DIP possesses beneficial properties in vasculature in addition to antiplatelet effects.[23]

In the last years, many efforts have been made to prepare various matrices as drug carriers for DIP. These investigations induced sustained DIP release in poly styrene microspherules and microcapsules,[24] controlled release coevaporators with acrylic polymers,[25,26] formulations of DIP-alginate microspheres containing polymers such as alginate, pectin, and sodium carboxymethyl cellulose (CMC),[27] systems based on poly(lactide-co-glycolide) (PLGA),[28] a combination of poly(lactide-*co*-glycolide) microspheres with ReGel®,[29] poly(3-hydroxybutyrate),[28] and poly(L-lactide) single crystals.[30]

In this article, we present the first devices based on MIPs for controlled release of DIP. The key factors controlling recognition and release by imprinted polymer matrices are discussed. To date, DIP MIPs were synthesized and studied using a bulk acoustic wave sensor technique,[31] a post chemiluminescence reaction of the DIP in the potassium fenicyanide-luminol system,[32] and a chemiluminescence imaging method.[33]

EXPERIMENTAL

Materials

Reagents

Methacrylic acid (MAA) from Merck (Darmstadt, Germany) was distilled in vacuum before use to remove the stabilizers. Ethylene glycol dimethacrylate (EGDMA) and 2,2′-azobisisobutyronitrile (AIBN) from Merck (Darmstadt, Germany), were of reagent grade and were used without any further purification. All the other chemicals were of analytical reagent grade and the solutions were prepared with distillated water. The phosphate buffer solutions, 0.02*M*, were prepared by adding KH_2PO_4 0.02*M* to Na_2HPO_4 0.02*M* to reach the desired pH value. DIP standard powder was a gift from center of quality control of drugs (Tehran, Iran).

MIP and NIP preparation with precipitation polymerization

The molecular imprinted polymers for DIP were prepared from a reagent mixture obtained by mixing of DIP and MAA in chloroform as Table I. The solution was placed in room temperature for 5 h to prearrange template and monomer. EGDMA and AIBN were added to the solution and the mixture was uniformly dispersed by sonication (sonic bath model Ultrasonic UTD35-Falc, Via Piemonte, Italy). After sonication (5 min), it was purged with N_2 for 3 min and the glass tubes were sealed under N_2 atmosphere. It was, then, put into a water bath maintained at 60°C for 22 h. The produced polymer was filtered using a Whatman filter and washed with acetone and methanol before the template removal. The template was removed by washing the MIP successively in 50 mL of a methanol/acetic acid solution (9 : 1, v/v, of 98% methanol and pure acetic acid) for five times, each time for 1.5 h, and then four times in 100 mL of pure water for 1.5 h. The template extraction of the polymer created the cavities, leading to the specific sorption of the template. In addition, the removal of other materials from the polymer took place (e.g. residual monomers or oligomers and initiator fragments). NIPs were also synthesized following exactly the same procedure, but excluding the template DIP from the formulation.

TABLE I
Polymer Compositions and the Percentage of DIP Bound by Each Matrix after 30 min in Aqueous Media (pH 6.8)

Polymer	Template : Monomer	Dipyridamole (mmol)	MAA (mmol)	EGDMA (mmol)	Recovery (%)
MIP-1	1 : 2	0.65	1.3	13.8	43 (± 1.4)[a]
MIP-2	1 : 4	0.32	1.3	13.8	56 (± 2.3)
MIP-3	1 : 6	0.22	1.3	13.8	79 (± 3.1)
MIP-4	1 : 8	0.16	1.3	13.8	58 (± 2.8)
MIP-5	1 : 10	0.13	1.3	13.8	72(± 4.2)
NIP	-	–	1.3	13.8	34 (± 2.5)

[a] Average of four determinations.

Instrumentation

The DIP concentration was determined by measuring the absorbance at λ_{max} of 284 nm by means of Cary 4000 spectrophotometer UV–Visible of Varian (CA, USA). For each solution, the absorbance was measured three times.

FT-IR spectra (4000–400 cm^{-1}) in KBr were recorded on a Bruker VERTEX 70 spectrometer. Scanning electron microscopy (SEM, PhilipsXL30 scanning microscope, Philips, Netherlands) was employed to determine the shape and surface morphology of the produced particles. Polymeric particles were sputter coated with gold before SEM measurement. Thermogravimetric analysis (TGA) was carried out on a Perkin Elmer TGS-2 instrument at the maximum heating rate of 20°C/min in nitrogen atmosphere. The polymer structures were characterized by means of N_2 adsorption–desorption isotherm measurements at 77 K with a BELSORP-mini, BEL Japan, Inc. The particle size of the particles were measured by PLS (Malvern Zetasizer ZS, Malvern UK). Particles were suspensed in acetonitrile (AN) and measured at a fixed scattering angle of 90°.

Procedures

Binding experiments

For measuring of template binding, 100 mg of particles were suspended in 20 mL of 200 μM DIP solution (pH 6.8). The solution was mixed for 1 h and then particles were filtrated on a paper filter (flow rate = 100 mL min^{-1} by applied vacuum). The supernatant was analyzed by UV spectrophotometer. The amount of DIP bound to particles was calculated by subtraction of the free fraction from the total amount added. The same procedure was followed for NIP particles.

Drug loading by soaking procedure

100 mg of polymers were suspended in 20 mL of DIP solution (200 μM) and soaked for 30 min at room temperature. During this time, the mixture was continuously stirred and then the solvent was removed. Subsequently the MIP particles were dried under vacuum overnight at 40°C.

In vitro release studies

The release studies were carried out using the dissolution method.[34] Two parallel experiments for MIP-3 and NIP matrices were performed. First, MIP-3 and NIP particles (100 mg) loaded with DIP, were dispersed in flasks containing various solutions (20 mL) such as 20 m*M* phosphate buffer solution (pH 6.8), 0.1*M* HCl (pH 1.0) and mixture of HCl 0.1*M* and methanol (10 : 1 v/v) at 37.0 ± 0.5°C in a water bath under magnetic stirring (50 rpm). Samples (2 mL) were drawn from the solution at appropriate time intervals to determine the amount of drug released. Experiments were repeated three times.

RESULTS AND DISCUSSION

Characterization

The IR spectra of NIP, the unleached and leached MIPs displayed similar characteristic peaks, indicating the similarity in the backbone structure of the different polymers (Fig. 2). As a result of the hydrogen binding with the —COOH group of MAA, the O—H stretching and the bending vibrations at 3458 cm^{-1} and about 1392 cm^{-1} in the leached MIP materials were shifted to about 3431 cm^{-1} and 1386 cm^{-1} in the corresponding unleached MIP, respectively. A strong peak at about 1731 cm^{-1} corresponded to $\nu_{C=O}$ in the IR spectra of the leached blank, the unleached and the leached MIP.

TGA plots of the unleached and leached MIP particles revealed two decomposition states: one mass loss between 90°C and 130°C (10% weight loss), assigned to the decomposition of the free monomer and the cross-linker, and one starting at 165°C, related to the DIP decomposition as the melting point of DIP is 162–168°C.[35] All the materials were completely decomposed before reaching the temperature of 460°C. These observations indicated the rigidity of the unleached and leached MIP particles is further than blank materials, as the formers exhibits

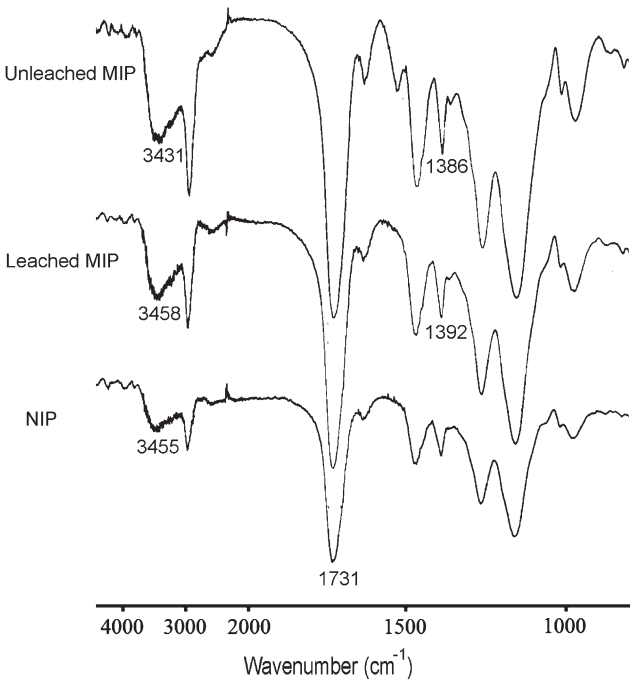

Figure 2 FT-IR spectra of MIP-3 (leached and unleached) and NIP polymeric microspheres.

decomposition above about 300°C, the latter starts its decomposition at about 250°C onward.

Study of morphology and particle size of microspheres

The microscopic morphology of the dry MIP and NIP polymer particles, determined by a scanning electron microscope (SEM) at magnification 5000, is shown in Figure 3 and a porous surface could be clearly observed for the MIP. From the SEM images obtained for the nonimprinted and the imprinted polymers it was not possible to see any differences in their morphology. The diameters of the particles and their size distributions were not affected significantly by the presence of the template drug.

To study the particles size of the polymer nanospheres in solution, MIP-3 and NIP were resuspended in AN and characterized with photon correlation spectroscopy (PCS). The PCS measurements provided valuable information about the hydrodynamic radius and poly dispersity index (PDI) of the colloidal particles. The results showed MIP-3 had 1.74 ($\pm$0.08) μm size and 0.11 PDI and NIP had 1.89 ($\pm$0.15) μm size and 0.12 PDI for three measurements.

Porosity studies on imprinted microsphers

The porosities of the microspheres were determined by nitrogen adsorption/desorption analysis of Brunnauer-Emmet-Teller (BET) adsorption measurements. The total pore volume and specific surface area of the imprinted microspheres are compared with the corresponding control materials (Table I). The corresponding BET surfaces were 207 and 215 m^2g^{-1} for MIP-3 and NIP, respectively. Upon extraction with a good solvent, here methanol-acetic acid (9 : 1, v/v), only the specific surface of the imprinted polymer changed significantly to 266 m^2g^{-1}. Furthermore, these results are complemented by pore size studies on the synthesized imprinted polymer materials. The results revealed that with MIP-3, the average pore dimension is 3.9 nm based on BET analysis.

Optimization of MIP formulations

There are several variables, such as the amount of monomer or nature of cross-linker and solvent that affects the final characteristics of the obtained materials in terms of capacity, affinity, and selectivity for the target analyte. Our template has four hydroxyl groups and could form strong bonds with carboxyl group in MAA, and because of this, we used MAA as functional monomer. This monomer is also a usual monomer for preparing MIPs in DDS.[13–16,36–38] Primary experiments revealed that the imprinted

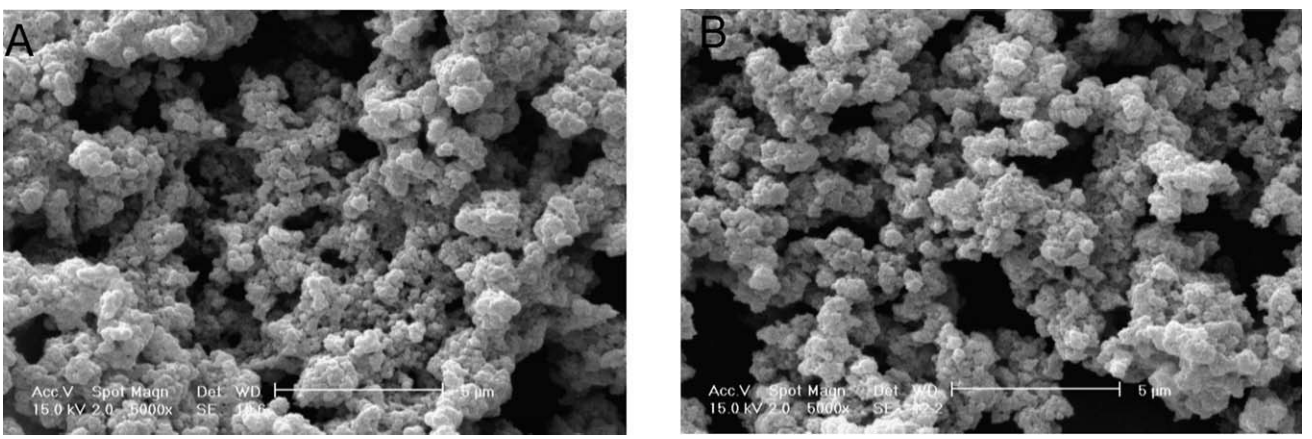

Figure 3 Scanning electron micrographs of (A) NIP and (B) MIP-3 at 5000 magnification.

TABLE II
Results of BET Analysis for Molecularly Imprinted and Control Polymers Prepared by Precipitation Polymerization

Sample		BET surface area ($m^2\ g^{-1}$)	Total pore volume ($cm^3 g^{-1}$)
MIP-3	Unleached	207 ± 10[a]	0.201 ± 11
	Leached	266 ± 14	0.243 ± 12
NIP		215 ± 12	0.201 ± 12

[a] Standard deviation of three measurements.

polymers prepared in chloroform show better molecular recognition ability than AN in aqueous environment. Thus, in chloroform, different formulations for the obtainment of MIPs with improved molecular recognition capabilities have been used (Table II). Generally, proper molar ratios of functional monomer to template are very important to enhance specific affinity of polymers and the number of MIPs recognition sites. High ratios of functional monomer to template result in high nonspecific affinity, whereas low ratios produce fewer complications because of insufficient functional groups.[1] Five molar ratios of the monomer MAA to the template of 2 : 1, 4 : 1, 6 : 1, 8 : 1, and 10 : 1 were used in the experiments. The optimum ratio of functional monomer to template for the specific rebinding of DIP was 6 : 1 (Table I), which had the best specific affinity of 45%. The specific adsorption recovery of DIP at 2 : 1 was 9%, whereas those at 4 : 1, 8 : 1, and 10 : 1 were 22%, 24%, and 38%, respectively. For the polymers with a ratio of 8 : 1 and 10 : 1, an excess of the functional monomer with respect to the template yielded higher nonspecific affinity. Therefore, the typical 1 : 6 : 21 template : monomer : cross-linker molar ratio was used for further studies.

pH effects on drug loading

We synthesized different polymers with different template : monomer ratio and investigated pH effects on drug loading. The effect of pH on the sorption of DIP was investigated by varying the solution pH from 3.0 to 8.0. Several batch experiments were performed by equilibrating 100 mg of the imprinted particles with 20 mL of solutions containing 200 μM of DIP under the desired range of pH. The results for different polymers (Fig. 4) showed that the pH has great effects on loading and we could see a difference about 45% between MIP-3 and NIP, at pH 6.8. This difference in binding indicated specific sites for DIP on MIP-3 particles that leaded to more capture and binding of DIP. Template rebinding on NIP can be explained with the presence of nonspecific recognition, because of physical adsorption, and to random interaction of the template molecule with functional groups in the polymer chains. But we could observe that MIPs show more tendencies to template because of the formation of specific binding sites in addition to nonspecific binding sites. At high pHs, the nitrogen heteroatoms can be protonated and, therefore, negligible amounts of DIP are adsorbed to the polymer.

The effect of the extraction time on the efficiency of the extraction was also investigated, and the results showed that, the time of the extraction from 1 to 8 h has not any significant effect on the extraction efficiency of the DIP.

Capacity and binding constants of polymers

The capacity of the sorbent is an important factor in DDS. For investigating adsorptions of DIP, the same volumes of DIP solution (20 mL) with different concentrations of DIP were contacted with 100 mg of sorbent in the batch mode. Then, the concentration of the remaining DIP in the solution was determined by UV. The adsorption isotherm that is the number of milligram absorbed per gram of adsorbent (Q) versus the equilibrium concentration of DIP is shown in Figure 5. According to these results, the maximum amount of DIP that can be absorbed by MIP was found to be 108 mg g^{-1} (215 μmol g^{-1}) at pH 6.8. For higher DIP amounts (higher than 215 μmol g^{-1}), a slight increase of the retained DIP was observed on the MIP capacity curve. As all the accessible specific cavities of the MIP are saturated, the retention of the analyte is only because of nonspecific interactions, which can be almost identical for MIP and NIP polymers. The binding constants (K_a) were determined from the Scatchard plots and the K_a of the DIP-imprinted polymer was 205 M^{-1}, whereas that of the nonimprinted polymer was 78 M^{-1}.

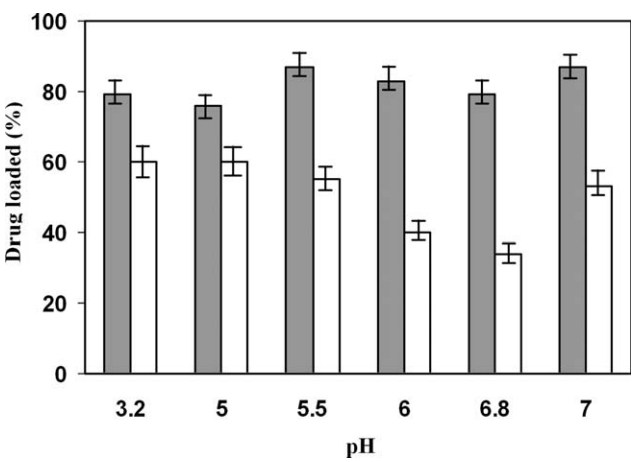

Figure 4 Effect of adsorption pH on the percent recovery of DIP using MIP-3 (first column) and NIP (second column).

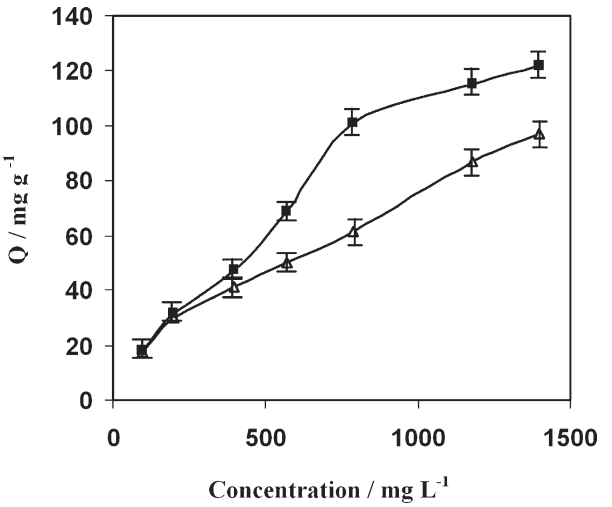

Figure 5 Curve of capacity obtained after the loading of 5 mL aqueous solution spiked with increasing amounts of DIP onto the MIP (■) and NIP (△) particles.

Drug release profiles

Our release studies were carried out in four media. MIP-3 matrices, which are the most effective in template recognition, were tested *in vitro* as devices for DIP delivery and the results were compared with NIP particles. We studied DIP release from polymer particles in HCl 0.1*M*, phosphate buffer (0.02*M*, pH 6.8) and in the mixture of HCl 0.1*M* and methanol (10 : 1, 24 : 1 v/v), respectively. The purpose of this study was to observe a considerable difference between the MIP and NIP in drug release and the investigation of the type of release media on release profiles. The resulted profiles are shown in Figure 6. As shown in the resulted profile in phosphate buffer with pH 6.8, we can see the difference between MIP and NIP but because of the low solubility of DIP at high pHs, MIP, and NIP could not release the total drug. In NIP about 34% but in MIP just 15% of drug

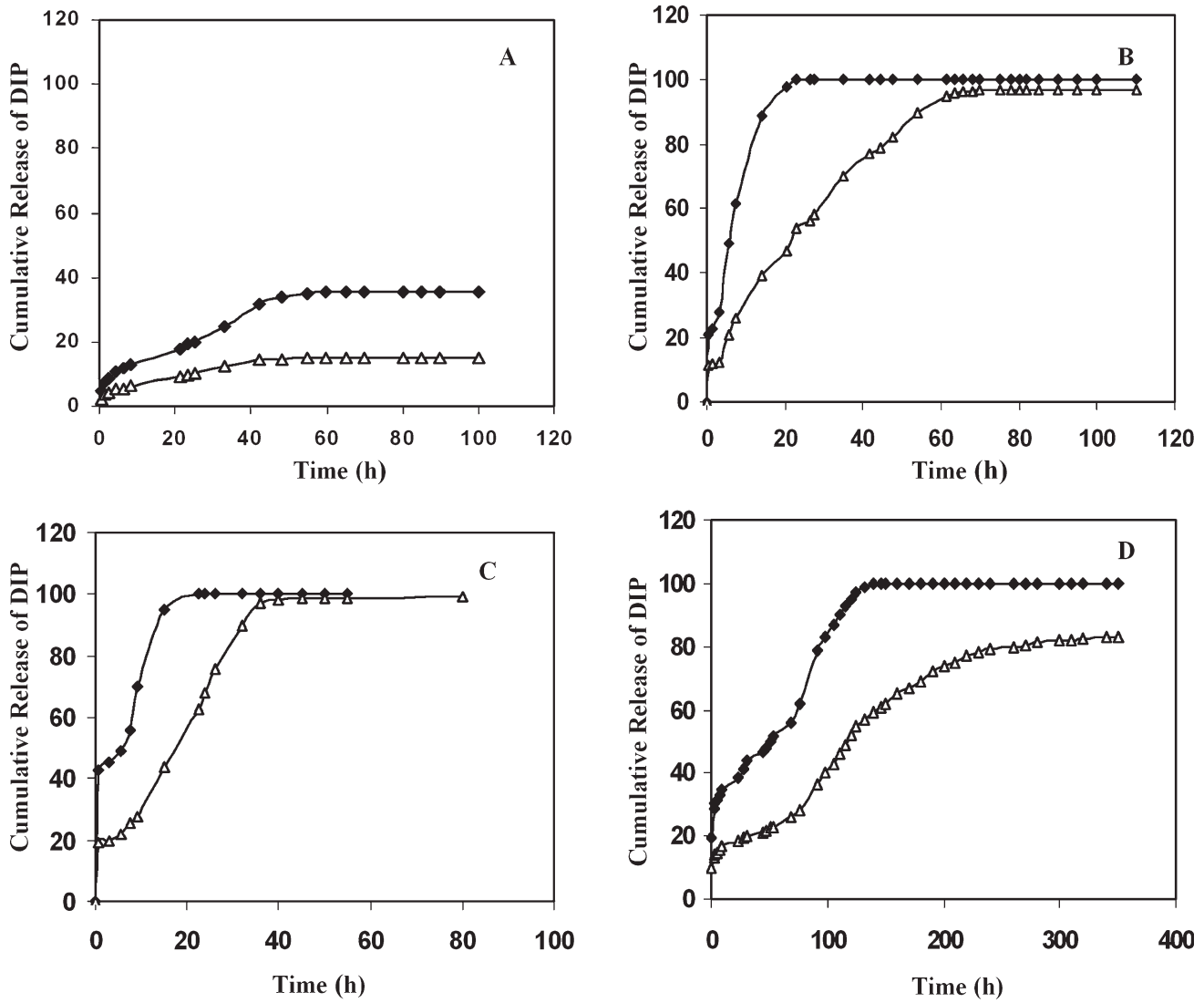

Figure 6 DIP release profiles from MIP-3 (◆) and NIP (△) loaded with 10 mmol of drug in (A) phosphate buffer pH 6.8, (B) HCl 0.1*M*, (C) HCl 0.1*M*, and methanol (10 : 1, v/v), and (D) HCl 0.1*M* (2 h) and then phosphate buffer pH 6.8.

is released after 48 h. It can be explained that this solution is not enough powerful to overcome the strong interactions between drug and specific binding sites, so this solution is not proper for releasing of this drug. In HCl 0.1*M*, because of that DIP is a weak base and its solubility in acid media is high, MIP and NIP release the total drug, but drug release in NIP is faster than the MIP. In the NIP, drug release completed in 20 h but in MIP it completed after 65 h. The initial quick release of DIP in NIP and MIP is related to physical adsorption and nonspecific bonds. But after this time, we have slower release for the MIP because of specific binding sites, which interact strongly with the DIP. In other mediums, we added methanol with the ratio 1 : 10 and 1 : 24 to HCl 0.1*M*. As shown in the Figure 6(C), in the mixture of HCl 0.1*M* and methanol (10 : 1 v/v), the initial release in both MIP and NIP is high with regard to other mediums and release is completed in shorter time in MIP and NIP. Because the DIP has a very high solubility in methanol, this solution is very powerful to overcome specific interactions between DIP and specific binding sites. In NIP drug released in less than 16 h and in MIP it completed in less than 36 h and because of this, the difference between MIP and NIP is less than the first media (HCl 0.1*M*). So the best solution for release is HCl 0.1*M*, because we could see a considerable difference between the MIP and the NIP which confirmed the existence of specific binding sites beside the nonspecific binding sites and we have a total drug release for the MIP in a reasonable time. The release profile of the drug under strongly acidic and neutral conditions is illustrated in Figure 6(D). Under acidic conditions, the nonimprinted showed a faster release of DIP than the imprinted polymers, with the nonimprinted polymer releasing 29% of its load compared to the imprinted polymer, which lost 13% of the bound drug after 2 h. When the pH was raised to 6.8, the nonimprinted polymer released the rest of its drug load within 120 h, whereas the imprinted polymer had only released 55% of the bound DIP in the lasting assay time.

This significant difference in the release of MIP-3 compared to its NIP indicated the existence of specific sites in MIP-3 that had strong interaction with DIP molecules. In addition, the drug release in MIP or NIP is related to morphology, size distribution, and wet ability property of particles. The polymer particles prepared by precipitation method were fine spherical particles and high surface availability.

CONCLUSIONS

Imprinted polymers are well-established as molecular recognition materials but are now being increasingly considered for active biomedical applications such as drug delivery. In this work, we developed uniformly sized MIPs as a sustained-release system for the delivery of DIP. In this study, some highlights of new research into molecularly imprinted drug delivery and controlled release systems are presented. The key factors controlling recognition and release by imprinted polymer matrices included mole ratios of monomer to DIP and medium nature and pH are discussed. In this case, the monomer/DIP ratio of 6 : 1 showed the best specific affinity of 45% and because of the existence specific binding sites, we obtained proper release profiles compared with the controlled polymers. Changes in the release behavior are observed depending on both the nature and pH of the releasing media. In the mixture of HCl 0.1*M* and methanol (10 : 1 v/v), the DIP is released faster than HCl 0.1*M*. The porosities of the microspheres were revealed upon extraction with a good solvent, here methanol-acetic acid (9 : 1, v/v), only the specific surface of the imprinted polymer changed significantly. This study indicates that the selective binding characteristic of MIPs is promising for the preparation of novel controlled release drug dosage form.

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