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Dummy Template Molecularly Imprinted Polymer for Omeprazole and the Study of Its Drug Binding and Release Properties

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ABSTRACT: In this study, we tried to prepare an omeprazole (OMP)-imprinted polymer and study its binding and release properties in an aqueous media. Because of the instability of OMP under polymerization conditions and the inability of the molecule to form effective interactions with monomers, pantoprazole (PANTO) was used as a dummy template for the imprinting process. Different monomers and solvents were evaluated in polymerization. The optimized imprinted polymer was prepared in chloroform as a porogen. Also, 4-vinylpyridine and ethylene glycol dimethacrylate were selected as a functional monomer and a crosslinker, respectively. The optimized imprinted polymer was evaluated in a binding study. The binding and release properties of the polymer were then investigated at different pHs. Our data indicated a higher affinity of the imprinted polymer to PANTO and OMP than that of nonimprinted polymer (NIP). The maximum percentage of OMP released from the imprinted polymer was 36–41%, whereas that for the NIP was 74–85%. These data were related to the 38–43 and 29–34 μg of OMP released from the imprinted polymer and NIP, respectively. Also, the protective effect of the imprinted polymer for OMP at pH 2 was greater than that of the nonimprinted one. This study revealed that the dummy template molecular imprinting was an effective method for preparing selective imprinted cavities in a polymeric matrix, especially for the molecules that were unstable during polymerization or unable to establish effective bonds with the monomers. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 4165–4170, 2013

KEYWORDS: dummy template; molecular imprinting; binding; release; omeprazole; pantoprazole

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INTRODUCTION

Molecular imprinting technology is a rapidly developing technique for the preparation of specific cavities for a template molecule, its analogues, or an enantiomer.[1,2] A molecularly imprinted polymer (MIP) is prepared by the arrangement of functional monomers around the template molecules and then fixing in a suitable solvent with a crosslinker during polymerization.[3–8] MIPs have applications in many fields of chemistry, biology, and pharmaceutics, such as in biosensors,[9] artificial antibodies,[10] sorbents for solid-phase extraction,[3–5,8,11] chromatographic stationary phases,[12] and drug-delivery systems.[13–16] The template is the most important factor in the spatial arrangement of functional monomers during polymerization. The molecule should have some characteristics as a suitable template in the molecular imprinting process. Some of these factors are as follows: (1) it must be stable under polymerization conditions (heat or UV irradiation), (2) a proper template does not bear any polymerizable groups and must not inhibit the polymerization process, and (3) the size of the template is very important. The imprinting of small molecules (pharmaceutics, amino acids, and pesticides) can be easily established, whereas molecular imprinting for big molecules, for example, proteins, is more difficult.[2,4] Sometimes in molecularly imprinted solid-phase extraction, template bleeding from the MIP matrix is the main problem in analysis, and it results the inaccurate quantification of the analyte.[17] In these cases, the use of a template that mimics the chemical structure of the main molecule can solve the problem. The MIP prepared in this method is called a dummy template molecularly imprinted polymer (D-MIP). Although the technique is usually used to remove template leakage during the molecularly imprinted solid-phase extraction procedure, some researchers have indicated that the use of a dummy template during polymerization increased the selectivity of the final MIPs.[18,19]

Omeprazole (OMP) is a proton pump inhibitor (PPI) used in the treatment of dyspepsia, gastroduodenal ulcers, gastroesophageal reflux disease, and some other digestive tract diseases. This drug is usually administered by mouth and inhibits acid secretion in parietal cells. Other PPIs include lansoprozole, pantoprazole (PANTO), rabeprazole, and esomeprazole. They

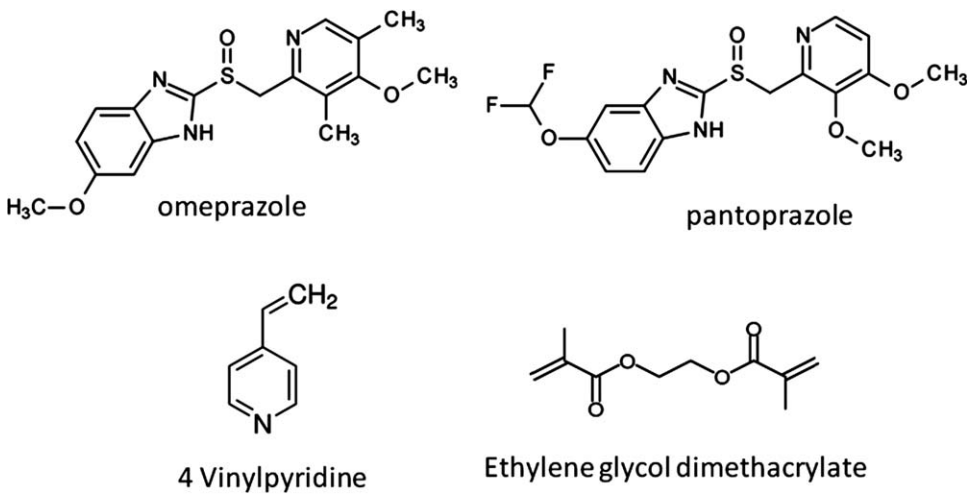

Figure 1. Chemical structures of the drugs and monomers used for the preparation and evaluation of D-MIP.

are benzimidazole derivatives that differ in their substitution patterns. PPIs are basic compounds with a pK_a around 4.0.[20–22] In this study, we tried to prepare an MIP for OMP and study its binding and release properties. The template was unstable and easily decomposed during polymerization. Also, it could not form effective interactions and bonds with functional monomers to prepare imprinted cavities. Thus, the MIPs did not work specifically. Finally, we used PANTO as a dummy template in polymerization. The PANTO was more stable under polymerization conditions and could interact properly with monomers to prepare suitable MIPs. The results show that the D-MIP could selectively bind the OMP in aqueous media.

EXPERIMENTAL

Materials

OMP and PANTO sodium sesquihydrate were obtained from Hetero (India). Methacrylic acid (MAA; purity = 99%), acrylamide methacrylamide (MAAM; purity = 98%), 2-hydroxyethyl methacrylate (HEMA; purity ≥ 99%) and 4-vinylpyridine (4VP; purity = 97%) were purchased from Aldrich. Ethylene glycol dimethacrylate (EGDMA; purity = 98%), toluene, acetonitrile, methanol, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), tetrahydrofuran, chloroform, and acetic acid were of high-purity grade or high-performance liquid chromatography (HPLC) grade and were ordered from Merck (Germany). 2,2′-Azobisisobutyronitrile (AIBN; purity = 98%) was obtained from Acros (Belgium). The PANTO base was prepared by the acidification of an aqueous solution of PANTO sodium sesquihydrate with HCl and the separation of sediment from the supernatant by a centrifuge (3000 rpm, 10 min). The precipitate was washed three times with distilled water and finally dried *in vacuo* at room temperature. The structures of the chemicals used in this study are presented in Figure 1.

Methods

Synthesis of Polymers. ***OMP as the template.*** At first, we tried to prepare MIPs with OMP as the template molecule in a conventional bulk polymerization method.[7] Different functional monomers (MAA, MAAM, HEMA, and 4VP) and solvents (chloroform, tetrahydrofuran, DMF, DMSO, toluene, methanol, and water) were applied for the preparation of MIPs. EGDMA was used as the crosslinker in all compositions. The synthesis was carried out under thermal (60°C) or UV (365 nm) polymerization. AIBN was the initiator of the polymerization process. The nonimprinted polymers (NIPs) were prepared in a manner similar to the MIP synthesis in the absence of the template.

PANTO as the template. In the second strategy, PANTO was selected as a pseudo template for the preparation of D-MIP. As described before, this method has been tested several times successfully by other researchers.[18,23,24] For the preparation of D-MIP, PANTO (0.4 mmol) and 4VP (1.6 mmol) were dissolved in chloroform (5 mL) in a screw-capped glass tube. The solution was set at room temperature (15 min) for better template–monomer complex formation. Then, EGDMA (9.2 mmol) and AIBN (10 mg) were added. After the solution was sparged with oxygen-free nitrogen for 5 min, the tube was sealed and placed under UV radiation (365 nm) for 6 h. The resulting polymer monolith was crushed, ground mechanically, and passed through a 200-mesh sieve (particle sizes < 75 μm was collected). The polymer particles were washed several times with a methanol/acetic acid (80:20 v/v) mixture (10 × 40 mL) and methanol (15 × 40 mL) followed by centrifugation (3000 rpm) to remove the supernatant solution. The washing step was continued until no PANTO or other compound could be detected by HPLC in the supernatant. Finally, the polymer was dried at 40°C for 24 h. The blank NIP was prepared in the absence of PANTO with the same procedure as described previously.

Binding Study. Dried polymer particles (10 mg) were placed in 2 mL of OMP or PANTO solution. The solution was gently shaken (70 rpm) and incubated at room temperature for 17 h. The solution was centrifuged (3000 rpm for 10 min), and the supernatant was analyzed by HPLC. The amount of drug loaded by each polymer was calculated as the difference between the initial and the final amounts of drug in solution. Each test was carried out four times, and the mean plus or minus the standard error of mean (SEM) is reported. Binding tests were done in water (pH 10).

Drug Loading and Release Study. Dried polymer particles (10 mg) were suspended in 2 mL of aqueous solution (pH 10) of OMP. An OMP concentration of 0.3 mg/mL was used to load the polymers in the OMP release study. The solution was shaken and incubated at room temperature for 17 h. After centrifugation (at 3000 rpm for 10 min), the concentration of OMP in the supernatant was measured by HPLC. The amount of OMP loaded by each polymer was calculated as described before. Then, the solvent was removed, and subsequently, the drug-loaded polymers were dried overnight at room temperature.

The release studies were carried out in normal saline–methanol (70:30 v/v) at pH values of 6, 7, and 8. The dried OMP-loaded MIP and NIP were placed in 2.5 mL of saline–methanol (70:30 v/v) with different pH values (6, 7, and 8) at 25°C. The solution was filtered at different times (5, 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, and 150 min), and fresh medium was added immediately. The amount of OMP released in the filtered solution was determined by HPLC.

Protective Effect of the Polymers at pH 2. Because of the instability of OMP in acidic media, the release test could not be done at pH 2. Thus, the protective effect of MIP and NIP for OMP against acidic conditions was evaluated. The dry MIP and NIP (10 mg) were incubated with OMP (300 μg/mL) in 2 mL of aqueous solution (pH = 10) for 6 h. The drug-loaded polymer particles were then filtered and dried overnight at room temperature. The polymers were incubated in 2.5 mL of normal saline–methanol (70/30, v/v at pH 2) for 30 min. The polymers were then filtered and eluted with 4 mL of methanol to remove OMP. The amount of undecomposed OMP in saline–methanol (70/30, v/v) and eluting methanol was determined by HPLC.

HPLC. The identification and quantification of OMP and PANTO were performed on a Younglin Acme 9000 system (South Korea), consisting of an SP930D solvent delivery module, an SDV50A solvent mixing vacuum degasser, a CTS30 column oven, a UV730 dual-wavelength ultraviolet–visible detector, and a ODSA C18 (4.6 × 150 mm, 5 μm) column. The data analysis was carried out by Autochro-3000 software. The injection volume was 20 μL, the flow rate was 1.5 mL/min, and the column temperature was adjusted to 30°C. The UV detector was set to 290 nm. An isocratic method was used for the chromatographic analysis of the compounds. The composition of the mobile phase was as follows: 50% water, 30% methanol, and 20% acetonitrile.

Statistical Analysis. The Student *t* test was used to assess the significance of the differences between the MIP and NIP. Results with *p* values of less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Polymer Synthesis

The choice of a suitable template, functional monomer, solvent, and polymerization conditions (heat or UV) is very important in the optimization of the molecular imprinting process. These factors affect template–monomer interactions during polymerization. A proper template molecule should be stable during polymerization and, in terms of compatibility with radical polymerization, should be chemically inert under the polymerization conditions. Also, it must be able to interact effectively with monomers during polymerization to form imprinted cavities. Thus, alternative imprinting strategies may need to be sought if the template can participate in radical reactions or is, for any other reason, unstable under the polymerization conditions.[2] OMP was unstable under heat polymerization (60°C), acidic conditions (with MAA as the functional monomer), and in some organic solvents (e.g., chloroform). It was decomposed into a black product, which inhibited polymerization. Although OMP was stable in some solvents (e.g., DMSO), the final MIP was not specific for the template. Nonpolar solvents were more suitable for the imprinting process.[6] DMSO and other polar solvents disrupted the weak template–monomer interactions during polymerization. Our findings indicated that the OMP–monomer bonds were not strong enough to prepare specific binding sites. Thus, PANTO was applied as a dummy template for this purpose. It was more stable under the polymerization conditions and could interact more effectively with the monomers. Different functional monomers (HEMA, MAA, MAAM, acrylamide, and 4VP) and solvents with different polarities (chloroform, methanol, toluene, DMSO, and DMF) under UV or heat polymerization conditions were applied to prepare the PANTO–MIP. The results show that the optimized MIP was synthesized with 4VP as a functional monomer in chloroform under UV polymerization. The chemical structure of the drugs indicated that the electronegativity of the $OCHF_2$ group in PANTO was significantly higher than that of the OCH_3 group in the OMP structure. Thus, the polarity of the NH group in PANTO was greater than that in OMP. Therefore, compared to OMP, as an H-bound donor via NH groups, PANTO could more effectively interact with an H-bound acceptor monomer (e.g., 4VP). Also, the pyridine ring in 4VP formed proper π–π interactions with the aromatic rings in the PANTO structure. The dummy molecularly imprinting process is illustrated in Figure 2.

Binding Study

The data showed that the amounts of PANTO binding to MIP and NIP (at 20 μg/mL) were 18.4 and 7.8 μg/10 mg of polymer, respectively. Thus, the imprinting process was successful. In the next step, OMP binding (at different concentrations) to MIP and NIP were studied in water. Its binding to MIP was significantly higher than to NIP at all concentrations (Figure 3). These data indicated that compared to NIP, the prepared D-MIP had superior binding properties for OMP. In a study by Yin et al.,[23] diphenolic acid and bisphenol were applied as dummy templates for MIP synthesis. The results indicate that tetrabromobisphenol A binding, as the main molecule, to the MIPs were significantly greater than that to the NIPs. In another study,[17] diisononyl phthalate was used as a dummy template, and a highly selective MIP was synthesized for five phthalate esters. Our binding study showed that dummy template molecular imprinting is an effective method for the preparation of high-affinity polymeric binding sites for OMP.

Drug Release

Because of the instability of OMP under acidic conditions, the release studies were performed only at pH values of 6, 7, and 8. Figure 4 shows that the maximum percentage of OMP released from D-MIP was 36–41%, whereas that for NIP was 74–85%.

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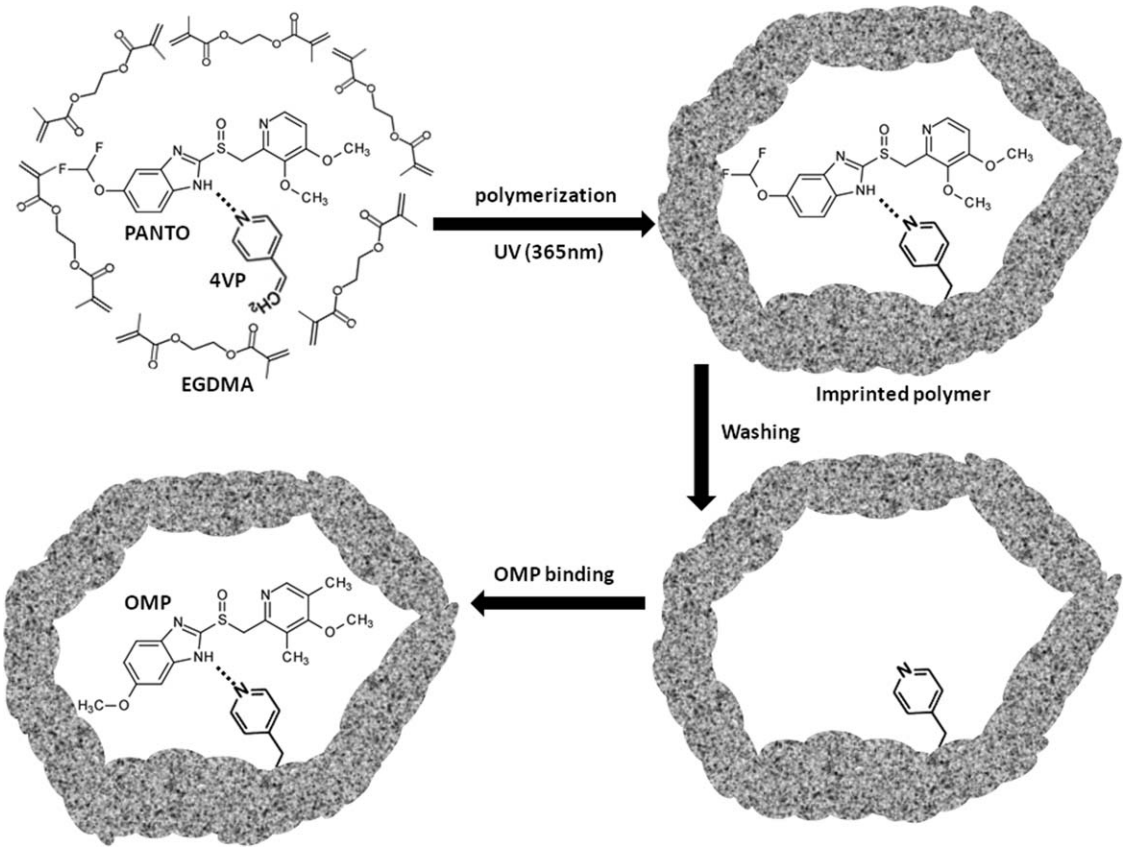

Figure 2. Schematic representation of the dummy template molecular imprinting procedure.

These data were related to the 38–43 and 29–34 μg of OMP released from D-MIP and NIP, respectively. The higher amounts of drug released from D-MIP compared to from NIP were due to the higher amounts of drug loaded into D-MIP. However, due to the presence of imprinted binding sites, the percentage of OMP released from D-MIP was significantly less than that of NIP. No difference was observed in drug release properties of D-MIP and NIP at different pH values. This may have been due to the low solubility of OMP in aqueous media. Also, 4VP is a basic functional monomer with a pK_a of 5.62. This monomer was not ionized at these pH values (6, 7, and 8). Thus, the water diffusion into the polymer (polymer swelling) and dissolution of the drug was significantly lower in these conditions (especially in D-MIP because of the presence of imprinted binding sites compared to NIP). However, this monomer was ionized at pH values of less than 5.62, and this may have led to higher water diffusion and thereby drug dissolution and release under acidic conditions. These results indicate stronger interactions between OMP and D-MIP. The presence of imprinted cavities in D-MIP was the main reason for this higher affinity.

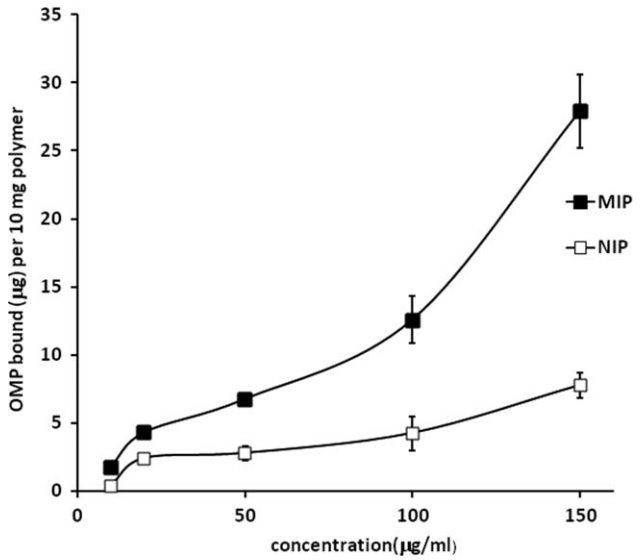

Figure 3. OMP binding to D-MIP and NIP in water (pH 10) at different concentrations. Each datum represents the mean plus or minus SEM ($n = 4$).

Protective Effect of the Polymers at pH 2

Because of instability of OMP under acidic conditions, a drug-release study was not performed at pH 2. However, the protective effect of the polymers in the aforementioned conditions was evaluated. According to our data, compared to NIP, MIP had a stronger protective effect for OMP at this pH. After 30 min of incubation of the drug-loaded MIP and NIP at pH 2, the amount of undecomposed drug in MIP was 5.86 ± 0.54 μg (12.6% of the loaded drug), whereas that for NIP was 3.54 ± 0.27 μg (7% of the loaded drug). Meanwhile, the amount of undecomposed OMP in a standard solution of drug under

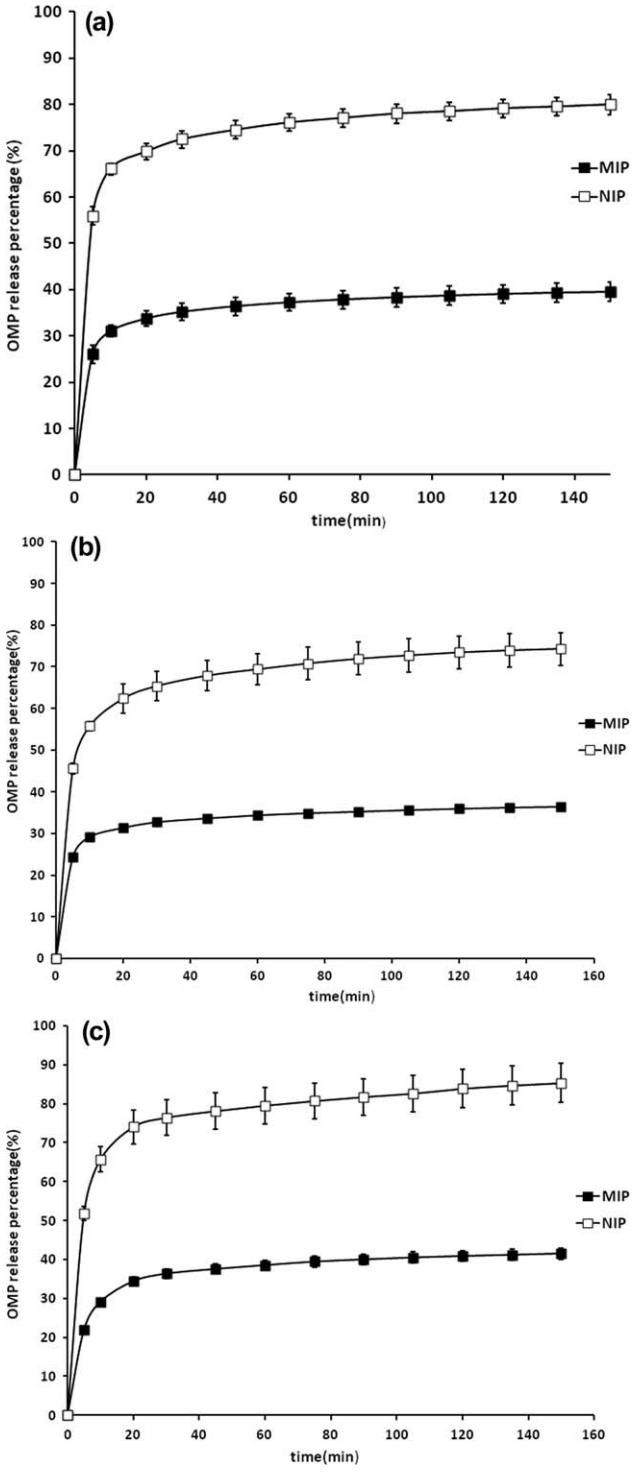

Figure 4. Percentage of OMP released from D-MIP and NIP at pH (a) 6, (b) 7, and (c) 8. Each datum represents the mean plus or minus SEM ($n = 4$).

the same conditions without the protective effect of MIP or NIP ranged between 2.29 and 3.32 μg. At low pH values, 4VP ($pK_a = 5.62$) is usually ionized. Therefore, the chance of water diffusion into the polymer and swelling was increased under these conditions. Thus, the OMP dissolution was enhanced in low-pH media. Because of the ionization of 4VP at pH 2 and water diffusion into the polymer, effective noncovalent and hydrogen interactions could not form between OMP and the polymers. Thus, the OMP release increased under these conditions in both MIP and NIP. In our previous study, the ionization of carboxylic groups in MAA (as the functional monomer in the preparation of a diclofenac-imprinted polymer) at pH value of 8 caused an increase in the amount of diclofenac released (90%) from MIP, whereas the release data at pH 1.5 was 14%. The ionization of carboxylic groups in MAA caused higher polymer swelling and water diffusion into the polymer at pH 8. Therefore, the amount of diclofenac released at pH 8 was significantly higher than that at pH 1.5.[7] However, 4VP is a weak basic monomer and would be more ionized in acidic media (pH 2). Therefore, water diffusion into the polymers, drug release, and then the decomposition of OMP under acidic conditions were significantly greater than those at higher pH values. Under these conditions, imprinted cavities could play an important role in protecting OMP against decomposition in aqueous media. Our data indicated a stronger protective effect of D-MIP for OMP under acidic conditions compared to NIP.

CONCLUSIONS

In this study, we applied molecular imprinting technology to prepare a polymeric matrix with specific cavities for OMP. Because of the instability of the drug under the polymerization conditions and its weak noncovalent interactions with the monomers, PANTO was selected as a dummy template for the imprinting process. 4VP and EGDMA were selected as the functional monomer and crosslinker, respectively. The results indicate that the optimized MIP could bind OMP and PANTO selectively. The OMP release study revealed that the amount of OMP released from MIP was significantly less than that of NIP in the aqueous media. Also, the protective effect of MIP for OMP at pH 2 was significantly greater than that of NIP. This study showed that the preparation of D-MIP was an efficient method for the preparation of selective binding sites for OMP and could be used for other unstable template molecules.

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