Controlled Release of Prednisolone Acetate from Molecularly Imprinted Hydrogel Contact Lenses

Bizhan Malaekeh-Nikouei,[1] Fatemeh Abbasi Ghaeni,[2] Vahideh Sadat Motamedshariaty,[2] Seyed Ahmad Mohajeri[2]

[1]*Nanotechnology Research Centre, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

Received 6 September 2011; accepted 1 December 2011
DOI 10.1002/app.36625
Published online in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The aim of this work was to study the influence of methacrylic acid (MAA) as a comonomer and the application of a molecular imprinting technique on the loading and release properties of weakly crosslinked 2-hydroxyethyl methacrylate (HEMA) hydrogels, with a view toward their use as reloadable soft contact lenses for the administration of prednisolone acetate (PA). The hydrogels were prepared with HEMA (95.90–98.30 mol %) as a backbone monomer, ethylene glycol dimethacrylate (140 m*M*) as a crosslinker, and MAA (0, 50, 100, or 200 m*M*) as a functional monomer. Different PA/MAA molar ratios (0, 1 : 8, 1 : 6, and 1 : 4) in the feed composition of the hydrogels were also applied to study the influence of the molecular imprinting technique on their binding properties. The hydrogels (0.4 mm thick) were synthesized by thermal polymerization at 60°C for 24 h in a polypropylene mold. The hydrogels were then characterized by the determination of their swelling and binding properties in water. Their loading and release properties were also studied in 0.9% NaCl and artificial lachrymal fluid. Increasing the MAA content of the hydrogel and applying the molecular imprinting technique led to an increase in the loading capacity of the hydrogel. The optimized imprinted hydrogel showed the highest affinity for PA and the greatest ability to control the release process, sustaining it for 48 h. The results obtained clearly indicate that the incorporation of MAA as a comonomer increased the PA loading capacity of hydrogel. Our data showed that the molecular imprinting technique also had a significant effect on the loading and release properties of the hydrogels. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2012

Key words: drug delivery systems; hydrogels; molecular imprinting

INTRODUCTION

The ocular bioavailability of drugs applied on the corneal surface is usually around 5% of the dose.[1–3] The poor bioavailability of conventional ophthalmic preparations is due to factors such as nasolacrimal drainage, ocular protective mechanisms, lacrimation and tear turnover, and metabolic degradation.[1,2,4] The drug in tear fluid is carried from the lacrimal sac and nasolacrimal duct into the nasal cavity. The absorption from the nasal cavity into the blood stream leads to drug waste and undesirable side effects; this is important for certain drugs. Another problem is the rapid variation in the drug-delivery rates from eye drops to the cornea; this limits the efficacy of some ocular drug-delivery systems.[5,6] In addition, the volume of a drop administered by the patient is inconsistent and leads to insufficient or toxic amounts of delivered drugs.[2,5,7–10] To overcome this problem, the residence time or duration of drugs on the eye surface should be extended. Thus, some types of ophthalmic dosage forms, such as ointments, viscous solutions, and therapeutic soft contact lenses, have been proposed to maintain a proper duration of drug contact with the cornea.[2,11–15] One approach is the use of hydrogels as soft contact lenses loaded with the drug by immersion in a drug solution.[16] In comparison with eye drops, the residence time of ophthalmic drugs in the tear film increases in the presence of a soft contact lens.[5,17] Poly(2-hydroxyethyl methacrylate) [poly(HEMA)], which is widely used as a biocompatible polymer for the preparation of soft contact lenses, has been studied for the ocular delivery of several ophthalmic drugs.[5,18,19] Because of the poor exchange of postlens lachrymal fluid, the residence time of the drug on the corneal surface is significantly increased in comparison with that of eye drops. Therefore, the bioavailability of ophthalmic drugs could be enhanced.[1,17] To optimize the loading capacity and release properties of poly(HEMA) contact lenses, some acidic and basic monomers have been incorporated into the main network.[5,20] Furthermore, these

Correspondence to: S. A. Mohajeri (mohajeria@mums.ac.ir).
Contract grant sponsor: Mashhad University of Medical Sciences; contract grant number: 88619.

© 2012 Wiley Periodicals, Inc.

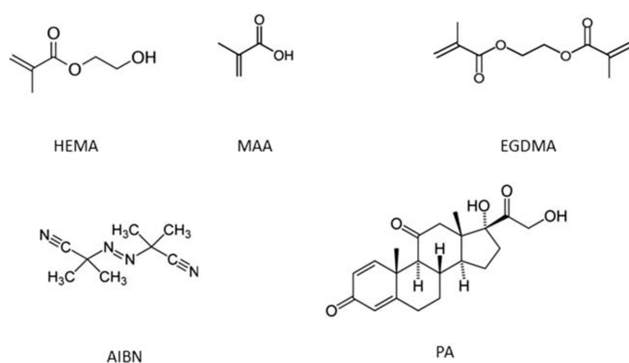

Figure 1 Structures of chemicals used in this study.

comonomers can facilitate *molecular imprinting:* the synthesis of a polymer in the presence of a molecule to prepare specific cavities with a high affinity for desirable species.[16,21] This technique involves the arrangement of functional monomers around template molecules in an appropriate solvent and polymerization in the presence of a crosslinker.[22–26] Molecularly imprinted polymers have enormous potential for application in the pharmaceutical field as novel carriers for drugs.[11,16,25,27–30] The mechanical and optical characteristics of contact lenses restrict the molar concentration of functional monomers and crosslinker (<10 mol %). Thus, the molecular imprinting procedure should be designed carefully for the preparation of contact lenses.[1,25]

Prednisolone acetate (PA) is a glucocorticoid with a potent anti-inflammatory effect.[31] Surgical interventions, such as cataract extraction, and some diseases of the anterior part of the eye disturb the blood aqueous barrier of the anterior chamber.[32,33] Proteins and cells that appear in the chamber fluid are considered indicators of intraocular inflammation. Local corticosteroids, preferably 1% PA, are topically applied to reduce noninfective inflammations.[34–36] Also, low-dose topical 0.5% PA might be used to treat postcataract inflammation.[34] Therefore, ocular bioavailability and anti-inflammatory effects of different topical prednisolone preparations, such as suspensions (eye drops) and high-viscosity eye gels, have been studied.[31,34,37–39] The aim of this work was to prepare hydrogel contact lenses that were able to load and release PA in a sustained way. We also investigated the effect of imprinting on the hydrogel binding properties. The polymers were prepared with 2-hydroxyethyl methacrylate (HEMA) as a backbone copolymerized with methacrylic acid (MAA) as a functional monomer. The loading and release properties of the imprinted hydrogels were studied and compared with blank nonimprinted ones.

EXPERIMENTAL

Chemicals and materials

HEMA, MAA, and ethylene glycol dimethacrylate (EGDMA) were supplied by Aldrich (Milwaukee, WI, USA). 2,2′-Azobisisobutyronitrile (AIBN) was purchased from Acros (Geel, Belgium), and PA was obtained from Sina Darou (Tehran, Iran). The structures of the chemicals used in this study are presented in Figure 1.

Synthesis of the nonimprinted blank hydrogels (NIPs)

EGDMA (140 m*M* or 1.70 mol %, as a crosslinker) and different amounts of MAA (0, 50, 100, and 200 m*M*, equivalent to 0, 0.60, 1.20, and 2.40 mol %, respectively) were dissolved in HEMA (95.90–98.30 mol %, as a backbone monomer). The feed compositions of the hydrogels are shown in Table I. After the addition of AIBN (10 m*M*, as an initiator) and sparging with oxygen-free nitrogen for 5 min, each monomer solution was immediately injected into a mold (0.4 mm thick) made of two polypropylene plates. The mold was then placed in an oven for 24 h at 60°C. After polymerization, each polymer was immersed in boiling water to remove unreacted monomers. The hydrogel film was then punched into disks with a diameter of 14 mm (similar to commercial contact lenses).[16] The hydrogels were immersed in 0.9% NaCl for 3 days, with the medium replaced every 12 h, and we then immersed them in distilled water for 3 days, with the medium replaced again every 12 h. This step was carried out for complete

TABLE I
Feed Compositions of the Hydrogels

Hydrogel	HEMA (mol %)	PA (m*M*)	MAA (m*M*)	PA/MAA	EGDMA (m*M*)	AIBN (m*M*)
NIP_1	98.30	—	0	—	140	10
NIP_2	97.70	—	50	—	140	10
NIP_3	97.07	—	100	—	140	10
NIP_4	95.90	—	200	—	140	10
$MIP_{1:4}$	95.90	50	200	1 : 4	140	10
$MIP_{1:6}$	95.90	33.33	200	1 : 6	140	10
$MIP_{1:8}$	95.90	25	200	1 : 8	140	10

washing and better removal of the unreacted monomers and chemicals from the hydrogels. Finally, the hydrogel was dried at 40°C for 48 h.

Synthesis of the imprinted hydrogels (MIPs)

Three series of imprinted hydrogels were prepared with a procedure similar to that described previously for the synthesis of the NIPs, with the following compositions: 200 m*M* (2.4 mol %) MAA and 140 m*M* (1.70 mol %) EGDMA. This solution was mixed with different amounts of PA to achieve PA/MAA ratios of 1 : 4, 1 : 6, and 1 : 8. The boiling, washing, and drying steps were carried out as described previously. The washing process was continued until no PA was detected in the supernatant.

Swelling in water

The weights of each hydrogel in the dry state (W_D) and after equilibration in distilled water (W_S) at room temperature were measured five times. The water content (Q) of each lens was calculated as follows:

$$Q(\%) = \frac{(W_S - W_D)}{W_D} \times 100$$

Differential scanning calorimetry (DSC)

DSC was carried out with a DSC apparatus equipped with STARe software (Mettler–Toledo SW7.01, Zurich, Switzerland). Thermograms of different samples, including PA and the dry hydrogels with and without PA, were obtained on 10-mg samples placed in sealed aluminum crucibles and heated from 150 to 300°C at a heating rate of 10°C/min in a nitrogen atmosphere. Empty 40-μL crucibles were used as references.

Binding study

Dried discs were placed in 0.01, 0.025, and 0.05 m*M* PA aqueous solutions (10 mL). The solutions were incubated at room temperature for 48 h. The initial and equilibrium concentrations of PA in the solution were measured by ultraviolet–visible spectrophotometry. The amount of PA loaded by each hydrogel was calculated as the difference between the initial and final amounts of drug in solution.

PA release

The dried imprinted and blank hydrogels were placed in 50 mL of PA aqueous solution (0.05 m*M*) for 48 h at room temperature. The amount of drug loaded by each hydrogel was calculated as described previously. The PA-loaded hydrogels were rinsed with water and placed in 10 mL of 0.9% NaCl or artificial lachrymal fluid (6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, 0.084 g/L $CaCl_2 \cdot 2H_2O$, pH 8) at 37°C for 48 h. The experiments were carried out five times. Approximately, 1 mL of each sample was decanted at regular intervals and returned to the vial immediately after analysis. PA determination in the samples was carried out with an ultraviolet–visible spectrophotometer (UV-1700 Pharmaspec model) from Shimadzu (Kyoto, Japan) set at 247 nm. The release profiles were characterized by the fitting of the release data with different *in vitro* models, including zero-order,[40] first-order,[40] Higuchi,[41] and Korsmeyer–Peppas models.[42]

For the zero-order model, the following equation was used:

$$Q_t = Q - Kt$$

where Q_t is the amount of drug released, Q_0 is the initial amount of drug in solution, and K is the zero-order release constant.

The drug release in the first-order model is expressed by another equation:

$$dC/dt = -KC$$

where dC/dt is the rate of change in concentration with respect to time and K is the rate constant.

In the Higuchi model, the release of drugs is dependent on the square root of time:

$$Q = Kt^{1/2}$$

where K is the rate constant.

The first 60% drug-release data were fitted in the Korsmeyer–Peppas model:

$$M_t/M_\infty = Kt^n$$

where M_t/M_∞ is the fraction of drug released at time t, K is the rate constant, and n is the release exponent.

Statistical analysis

Data are expressed as the mean plus or minus the standard error of the mean (SEM). The swelling of the hydrogels and binding and release data were assessed by a one-way analysis of variance followed by a Tukey–Kramer posttest. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Hydrogel preparation

Highly crosslinked imprinted polymers prepared in an organic solvent usually produce fragile polymers

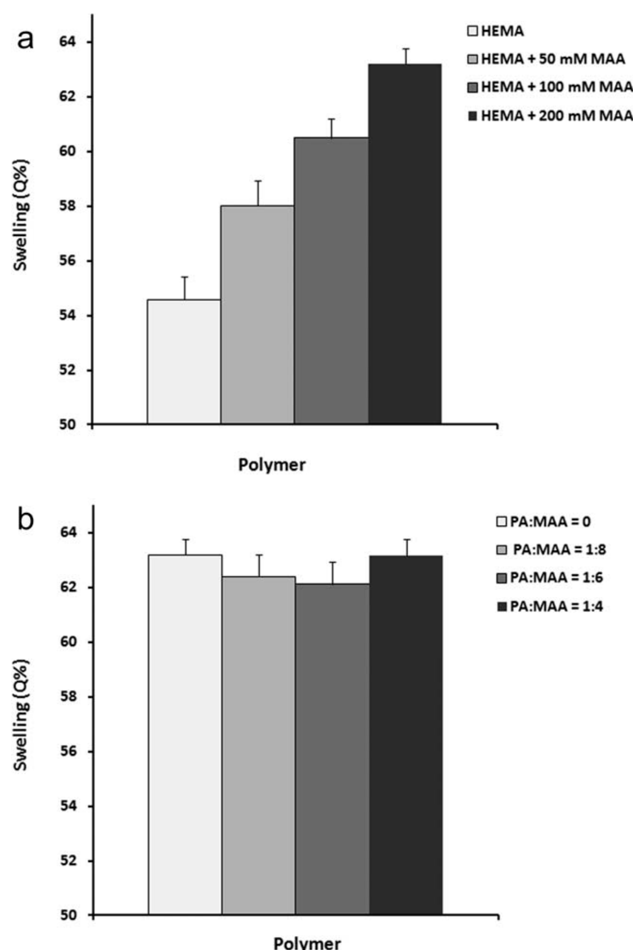

Figure 2 (a) Water contents of NIPs prepared with different concentrations of MAA. (b) Swelling of NIPs and imprinted hydrogels prepared with 200 m*M* MAA. Each datum represents the mean plus SEM ($n = 5$).

with a high affinity for the template used in analytical applications.[23,24,43] However, the molar concentration of the crosslinker monomer is very low in the hydrogel polymerization process.[11,16,30] In this study, we did not use any solvent, taking advantage of the liquid state of HEMA. Because of the low molar percentage (1.7%) of EGDMA (crosslinker) in the polymer composition, all of the hydrogels had suitable flexibility with high optical clarity both before and after swelling in the aqueous solutions. The PA structure (Fig. 1) showed that the drug was potentially able to interact effectively with MAA through hydrogen bonds; this is a main requirement for the achievement of an imprinted hydrogel. MAA is a weak acid that can interact with hydrogen-bond donors and acceptors through its carboxylic group.[16,25] Thus, MAA was used as a comonomer with HEMA in the hydrogel preparation. MAA as a functional monomer effectively arranged around the PA as a template, and its carboxylic group interacted with the OH and =O groups of PA via hydrogen bonds in the polymerization solution. After polymerization, the MAA molecules were fixed in a special structure and made an imprinted cavity for PA.

Swelling in water

Hydrogels were prepared with different amounts of MAA, whereas the concentration of EGDMA in the monomer composition was fixed (Table I). Four sets of NIPs were prepared through changes in the MAA/EGDMA ratio (Table I). The imprinted hydrogels were also prepared with 200 m*M* MAA and different PA/MAA ratios (1 : 8, 1 : 6, and 1 : 4). All of the hydrogels obtained had good mechanical strength and optical transparency. The weight of each hydrogel was between 33 and 37 mg. As shown in Figure 2(a), the water content increased with increasing amount of MAA in the NIPs ($p < 0.05$). No difference in the water content was seen between the imprinted polymers and NIPs [200 m*M* MAA, $p > 0.05$, Fig. 2(b)]. The data indicated that the molecular imprinting process had no influence on the swelling and water contents of the hydrogels. Other researchers have also indicated that the imprinting process does not change the water affinity or swelling properties of hydrogels.[16,25]

DSC

Figure 3 shows the thermograms of PA and the hydrogels with and without PA. PA had an endothermic peak at 238°C, which was coincident with the drug melting point. The DSC thermograms of the dry hydrogels with and without PA were very similar. The elimination of the melting peak of PA in the thermograms of the hydrogel prepared with PA clearly showed the formation of a solid solution or solid homogeneous mixture and indicated good compatibility and interaction between the PA and hydrogel. The same observation was reported in the study of Alvarez-Lorenzo et al.[16] for timolol.

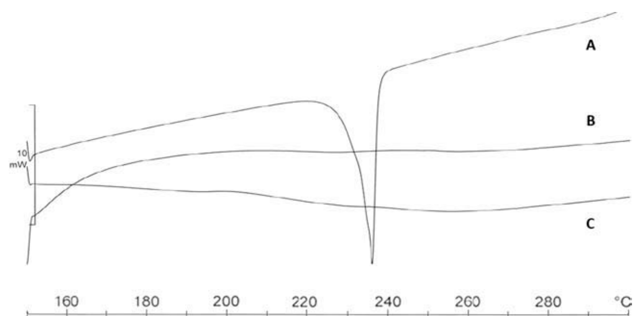

Figure 3 DSC thermograms of (A) PA powder and hydrogel prepared (B) without and (C) with PA.

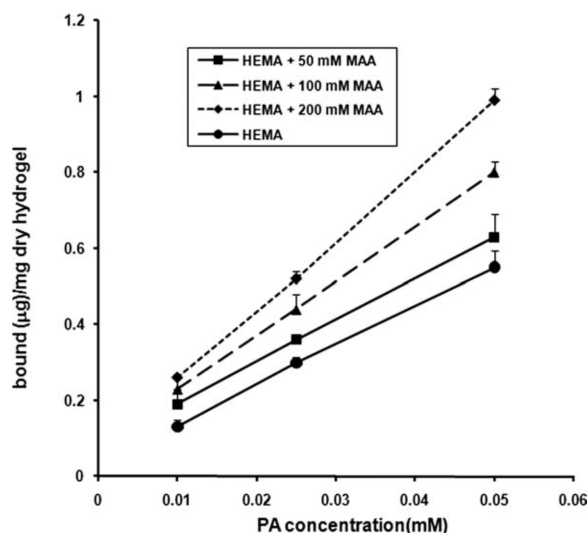

Figure 4 PA binding to nonimprinted poly(HEMA–MAA) hydrogels copolymerized with different concentrations of MAA. Each datum represents the mean plus SEM ($n = 5$).

Binding study

NIPs

Different proportions of functional monomers (e.g., acrylic acid, 4-vinyl pyridine, MAA) can be used to increase the drug-loading capacity and water content of conventional (i.e., nonimprinted) hydrogels.[1,16,25,30] In this work, we applied MAA to increase the affinity of PA for hydrogels. After boiling in water, washing in 0.9% NaCl and distilled water, and drying at 40°C, the binding properties of the NIPs were studied. Once immersed in PA aqueous solutions, the poly(2-hydroxyethyl methacrylate–methacrylic acid) [poly(HEMA–MAA)] hydrogels showed a significantly greater affinity for the drug than did the poly(HEMA) hydrogel (Fig. 4). Increasing the MAA content of the copolymers led to an increase in the loading capacity of the hydrogel. The differences between HEMA + 100 m*M* MAA and HEMA + 200 m*M* MAA groups with HEMA groups were significant ($p < 0.05$), whereas no significant difference was seen between HEMA and HEMA + 50 m*M* MAA groups ($p > 0.05$). These results indicate the role of MAA in the PA-loading capacity of the hydrogels, which was probably due to its electrostatics attraction and hydrogen-bond interaction with various groups of drugs.

Imprinted hydrogels

According to the preliminary binding results, MAA was chosen as the functional monomer for the molecular imprinting procedure. The poly(HEMA–MAA) hydrogel prepared with 200 m*M* MAA had the highest affinity for binding PA. Thus, the imprinting process was carried out with 200 m*M* MAA. For successful imprinting, an adequate template/functional monomer molar ratio had to be used.[1,24,44] Therefore, PA/MAA molar ratios of 1 : 4, 1 : 6, and 1 : 8 were applied to elucidate the importance of the PA/MAA molar ratio in the binding properties of the poly(HEMA–MAA) hydrogels. NIPs were also prepared in the absence of PA. The transparency, appearance, and viscoelastic and swelling properties of the imprinted hydrogels were similar to those of the nonimprinted ones. Figure 5 shows the binding ability of the imprinted hydrogels for PA. The bindings of PA to the 1 : 4 and 1 : 6 PA/MAA hydrogels were higher than that of the blank polymer ($p < 0.05$) whereas there was no statistical difference between the blank and 1 : 8 PA/MAA hydrogels ($p > 0.05$). The data indicated that the imprinting process enhanced the binding affinity of the hydrogels. The maximum binding capacity was obtained when the hydrogels were prepared with a PA/MAA molar ratio of 1 : 4. These data showed that the optimum PA/MAA ratio for the preparation of the PA imprinted cavities was 1 : 4. It seemed that in this ratio, the special arrangement of MAA around PA in the binding sites was optimized, and the affinity of these imprinted cavities for PA was higher than that of the other imprinted hydrogels. Thus, the imprinted poly(HEMA–MAA) hydrogel prepared with 200 m*M* MAA and a PA/MAA ratio of 1 : 4 had a higher binding affinity for PA than other imprinted and nonimprinted ones.

PA loading and release

Our results show that the nonimprinted poly (HEMA–MAA) hydrogels prepared with 200 m*M*

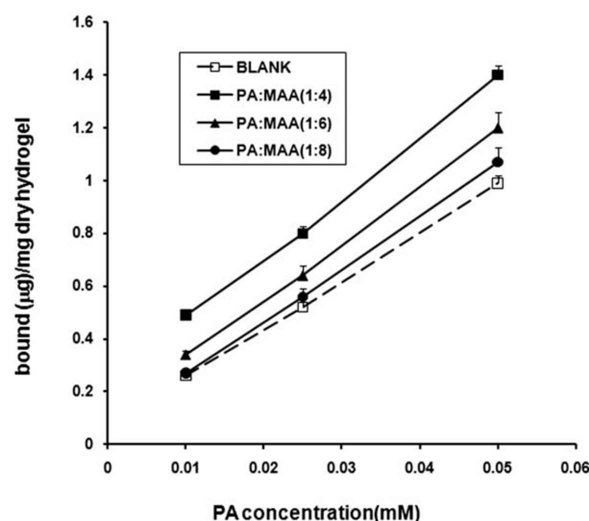

Figure 5 PA binding to nonimprinted and imprinted poly(HEMA–MAA) hydrogels copolymerized with 200 m*M* MAA. Each datum represents the mean plus SEM ($n = 5$).

MAA were able to load 39 μg/disc, whereas the 1 : 8, 1 : 6, and 1 : 4 imprinted ones were able to load 40, 46, and 58 μg/disc, respectively. The formation of imprinted cavities was the only reason for the higher affinity of PA for the imprinted hydrogels compared to the nonimprinted one. The results indicate that the PA/MAA molar ratio played an important role in creating imprinted binding sites. The optimized imprinted hydrogel was prepared at a PA/MAA molar ratio of 1 : 4. The average volume of an eye drop is 40 μL, and the conjunctival sac volume of a human is about 30 μL.[45] If 5% of the instilled eye drop of a 1% solution is effectively absorbed through the cornea, the total amount of PA ocularly available each 24 h when instilled every 8 h is about 60 μg. This dose matches up with the amount of PA loaded by the optimized imprinted hydrogel (PA/MAA = 1 : 4). These data indicated the role of MAA and imprinting in the loading of PA by the hydrogels.

The prepared hydrogels were able to sustain drug release for more than 2 days. The release profile of PA was biphasic [Fig. 6(a,b)], with an initial rapid phase followed by a continuous and slower phase. The release rate from the hydrogels was dependent on the drug-to-polymer ratio, and remarkable differences in the release were observed as a function of the PA/MAA molar ratio. The lowest release rate was achieved by the imprinted hydrogel with a PA/MAA ratio of 1 : 4. In this study, two commonly used media, 0.9% NaCl and artificial lacrimal fluid, were used as release media. The behavior of the imprinted polymers was similar in both test media. In both media, the amounts of drug released from the 1 : 4 and 1 : 6 PA/MAA hydrogels were significantly lower than that released by NIP_4 (NIP; nonimprinted blank hydrogel, see table 1) after 48 h ($p < 0.05$), whereas the difference between the blank and 1 : 8 PA/MAA hydrogels was not significant.

Controlled drug-delivery systems are designed to maintain the drug concentration in targeted tissues.[40] There are number of model-dependent methods that explain the kinetics of drug release from dosage forms. In this study, different methods, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, were used. The Higuchi model was first described for drug release from planar matrix systems,[41] and then, it was extended for porous systems.[46] Drug release from polymeric and swelling-controlled release systems is described by the Korsmayer–Peppas model.[42] In this model, the value of n shows the drug-release mechanism. The results obtained by the fitting of the PA release curves [Fig. 6(a,b)] to various models are summarized in Table II. The best linearity was found in Higuchi's model, which describes the release of drugs from an insoluble matrix as the square root of

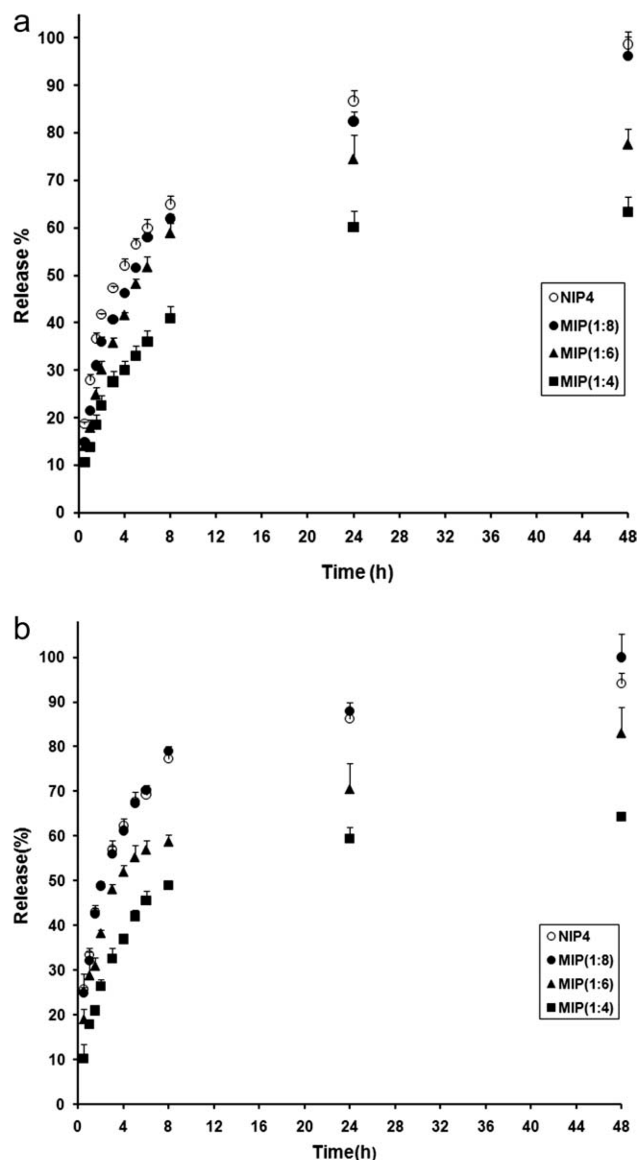

Figure 6 PA release profiles in (a) NaCl 0.9% and (b) artificial lachrymal fluid from poly(HEMA–MAA) hydrogels synthesized with 200 m*M* MAA and 140 m*M* EGDMA with different PA/MAA molar ratios: (○) 0, that is, NIPs, (●) 1 : 8, (▲) 1 : 6, and (■) 1 : 4. The hydrogels were previously loaded by immersion in 0.05 m*M* PA solution ($n = 5$).

a time-dependent process based on Fickian diffusion. Also, the values of n in the Korsmeyer–Peppas model were close to 0.5. To determine the mechanism of drug release, first, 60% drug release data were fitted to the Korsmeyer–Peppas model. The n values confirmed that the main release mechanism was Fickian diffusion due to a chemical potential gradient. Alvarez-Lorenzo et al.[16] showed that the timolol release was dependent on both the medium and the nature and amount of comonomer in the hydrogels. A comparison of the release constants in the zero-order, first-order, and Higushi models showed that the PA release rate in artificial lachrymal fluid was higher than that in the 0.9% NaCl

TABLE II
PA Release Rate Constants in 0.9% NaCl and Lachrymal Fluid Obtained by the Fitting of the Release Profiles to the Zero-Order, First-Order, Higuchi, and Korsmeyer–Peppas Equations for the NIP_4 and Imprinted Hydrogels Synthesized in the Presence of Different PA/MAA Molar Ratios

	Zero order		First order		Higuchi		Korsmeyer–Peppas	
Hydrogel	K	R^2	K	R^2	K	R^2	K	R^2
				In NaCl				
NIP_4	4.627	0.924	0.355	0.851	11.705	0.977	1.935	0.984
$MIP_{1:4}$	3.982	0.983	0.383	0.944	9.818	0.987	5.715	0.983
$MIP_{1:6}$	4.129	0.970	0.379	0.929	10.222	0.982	3.698	0.977
$MIP_{1:8}$	4.194	0.923	0.395	0.859	10.577	0.969	2.923	0.977
				In lacrymal fluid				
NIP_4	5.011	0.964	0.313	0.916	12.504	0.991	1.144	0.992
$MIP_{1:4}$	4.936	0.968	0.431	0.879	12.298	0.992	4.903	0.988
$MIP_{1:6}$	5.072	0.976	0.345	0.923	12.505	0.980	2.044	0.980
$MIP_{1:8}$	4.930	0.954	0.322	0.907	12.320	0.984	1.217	0.985

medium. MAA was totally ionized at alkaline pH. This ionization eliminated its hydrogen-bonding capacity. Also, the ionization of MAA caused more water diffusion into the hydrogel and more dissolution of the drug. Therefore, the drug release increased at higher pH values. Obviously, in comparison with 0.9% NaCl, MAA was more ionized in artificial lachrymal fluid (pH 8). Thus, the release rate in this media was higher than that in the 0.9% NaCl solution.

In artificial lacrimal fluid, the amount of PA release from $MIP_{1:4}$ (imprinted hydrogel with a PA/MAA ratio of 1:4) was about 64% over a time period of 48 h, whereas 78% of the drug was released from NIP_4 within 8 h. The imprinted hydrogel prepared with a PA/MAA molar ratio of 1 : 4 showed the greatest ability to control the release of the drug, sustaining it for 2 days. In summary, a content of 200 m*M* MAA in the hydrogels synthesized in the presence of a 1 : 4 PA/MAA molar ratio seemed to be optimum for obtaining the highest number of imprinted binding sites with the maximum affinity.

CONCLUSIONS

The use of MAA as functional monomer and the application of the molecular imprinting technique increased the loading capacity of the poly(HEMA) hydrogel and enabled it to sustain the release for 2 days. The PA/MAA ratio significantly affected the structure of the imprinted cavities. Therefore, this ratio is an important variable in optimization of the imprinting process of hydrogels as ocular drug-delivery systems. $MIP_{1:4}$, with a PA/MAA molar ratio of 1 : 4 and an MAA concentration of 200 m*M*, was the optimal hydrogel for the loading and release of PA in aqueous solution. These results open the possibility of preparing hydrogel soft contact lenses as efficient drug-delivery systems.

The authors declare that there was no conflict of interest in this study. The results described in this article are part of a PharmD student thesis.

References

1. Alvarez-Lorenzo, C.; Yanez, F.; Barreiro-Iglesias, R.; Concheiro, A. J Controlled Release 2006, 113, 236.
2. Ali, M.; Horikawa, S.; Venkatesh, S.; Saha, J.; Hong, J. W.; Byrne, M. E. J Controlled Release 2007, 124, 154.
3. Geroski, D. H.; Edelhauser, H. F. Invest Ophthalmol Vis Sci 2000, 41, 961.
4. Gupta, S.; Vyas, S. P. Sci Pharm 2010, 78, 959.
5. Xinming, L.; Yingde, C.; Lloyd, A. W.; Mikhalovsky, S. V.; Sandeman, S. R.; Howel, C. A.; Liewen, L. Contact Lens Anterior Eye 2008, 31, 57.
6. Dorigo, M. T.; De Natale, R.; Miglioli, P. A. Chemotherapy 1995, 41, 1.
7. Lin, H. R.; Sung, K. C. J Controlled Release 2000, 69, 379.
8. German, E. J.; Hurst, M. A.; Wood, D. Ophthalmic Physiol Opt 1997, 17, 196.
9. Sklubalova, Z.; Zatloukal, Z. Pharmazie 2005, 60, 917.
10. Sklubalova, Z.; Zatloukal, Z. Drug Dev Ind Pharm 2006, 32, 197.
11. Hiratani, H.; Fujiwara, A.; Tamiya, Y.; Mizutani, Y.; Alvarez-Lorenzo, C. Biomaterials 2005, 26, 1293.
12. Herrero-Vanrell, R.; Fernandez-Carballido, A.; Frutos, G.; Cadorniga, R. J Ocul Pharmacol Ther 2000, 16, 419.
13. Bochot, A.; Fattal, E.; Gulik, A.; Couarraze, G.; Couvreur, P. Pharm Res 1998, 15, 1364.
14. Srividya, B.; Cardoza, R. M.; Amin, P. D. J Controlled Release 2001, 73, 205.
15. Hornof, M.; Weyenberg, W.; Ludwig, A.; Bernkop-Schnurch, A. J Controlled Release 2003, 89, 419.
16. Alvarez-Lorenzo, C.; Hiratani, H.; Gomez-Amoza, J. L.; Martinez-Pacheco, R.; Souto, C.; Concheiro, A. J Pharm Sci 2002, 91, 2182.
17. Hehl, E. M.; Beck, R.; Luthard, K.; Guthoff, R.; Drewelow, B. Eur J Clin Pharmacol 1999, 55, 317.
18. Ribeiro, A.; Veiga, F.; Santos, D.; Torres-Labandeira, J. J.; Concheiro, A.; Alvarez-Lorenzo, C. Biomacromolecules 2011, 12, 701.
19. Xu, J.; Li, X.; Sun, F. J Biomater Sci Polym Ed 2010, 21, 271.
20. Andrade-Vivero, P.; Fernandez-Gabriel, E.; Alvarez-Lorenzo, C.; Concheiro, A. J Pharm Sci 2007, 96, 802.
21. Sellergren, B.; Shea, K. J. J Chromatogr A 1993, 654, 17.

22. Mohajeri, S. A.; Ebrahimi, S. A. J Sep Sci 2008, 31, 3595.
23. Mohajeri, S. A.; Hosseinzadeh, H.; Keyhanfar, F.; Aghamohammadian, J. J Sep Sci 2010, 33, 2302.
24. Mohajeri, S. A.; Karimi, G.; Khansari, M. R. Anal Chim Acta 2010, 683, 143.
25. Hiratani, H.; Mizutani, Y.; Alvarez-Lorenzo, C. Macromol Biosci 2005, 5, 728.
26. Mohajeri, S. A.; Karimi, G.; Aghamohammadian, J.; Khansari, M. R. J Appl Polym Sci 2011, 121, 3590.
27. Byrne, M. E.; Park, K.; Peppas, N. A. Adv Drug Delivery Rev 2002, 54, 149.
28. Allender, C. J.; Richardson, C.; Woodhouse, B.; Heard, C. M.; Brain, K. R. Int J Pharm 2000, 195, 39.
29. Cirillo, G.; Curcio, M.; Parisi, O. I.; Puoci, F.; Iemma, F.; Spizzirri, U. G.; Picci, N. Pharm Dev Technol 2004, 15, 526.
30. Hiratani, H.; Alvarez-Lorenzo, C. J Controlled Release 2002, 83, 223.
31. Struck, H. G.; Bariszlovich, A. Graefes Arch Clin Exp Ophthalmol 2001, 239, 737.
32. Sawa, M.; Sakanishi, Y.; Shimizu, H. Am J Ophthalmol 1984, 97, 197.
33. Liesegang, T. J.; Bourne, W. M.; Brubaker, R. F. Ophthalmology 1984, 91, 399.
34. Lorenz, K.; Dick, B.; Jehkul, A.; Auffahrt, G. U. Graefes Arch Clin Exp Ophthalmol 2008, 246, 1617.
35. Raizman, M. Arch Ophthalmol 1996, 114, 1000.
36. Roberts, C. W. Ophthalmology 1996, 103, 636.
37. Roberts, C. W.; Nelson, P. L. J Ocul Pharmacol Ther 2007, 23, 182.
38. Johansen, S.; Rask-Pedersen, E.; Prause, J. U. Acta Ophthalmol Scand 1996, 74, 259.
39. Olejnik, O.; Weisbecker, C. A. Clin Ther 1990, 12, 2.
40. Dash, S.; Murthy, P. N.; Nath, L.; Chowdhury, P. Acta Polym Pharm 2010, 67, 217.
41. Higuchi, T. J Pharm Sci 1963, 52, 1145.
42. Ritger, P. L.; Peppas, N. A. J Controlled Release 1987, 5, 37.
43. Mohajeri, S. A.; Ebrahimi, S. A. J Sep Sci 2008, 31, 3595.
44. Baggiani, C.; Anfossi, L.; Giovannoli, C.; Tozzi, C. Talanta 2004, 62, 1029.
45. Kobayakawa, S.; Ooki, K.; Tsuji, A.; Tochikubo, T. J Infect Chemother 2009, 15, 209.
46. Grassi, M.; Grassi, G. Curr Drug Delivery 2005, 2, 97.