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Poly(HEMA)/Cyclodextrin-Based Hydrogels for Subconjunctival Delivery of Cyclosporin A

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ABSTRACT: To enhance the solubility and ocular permeability of immunosuppressive agent, cyclosporine A (CsA), three types of delivery systems were prepared using (2-hydroxypropyl)-β-cyclodextrin (HPβCD), and 2-hydroxyethyl methacrylate (HEMA). Those systems are (i) hydrogels of HPβCD with crosslinking agent ethylene glycol diglycidylether, (ii) poly(HEMA) hydrogels, and (iii) different amounts of HPβCD-containing poly(HEMA) hydrogels indicated as poly(HEMA-*co*-HPβCD). In the presence of HEMA, hydrogels have desired mechanical integrity with lower equilibrium content than that of hydrogels without HEMA. CsA was loaded into the HPβCD-based hydrogels by embedding from its aqueous suspensions in higher amounts than that of the poly(HEMA) hydrogels that were loaded by CsA–HPβCD complex solution. Although the poly(HEMA) hydrogels are releasing total CsA in 3 days, long-term release was realized from HPβCD-based hydrogels. For subconjunctival administration, regarding to the amounts of loaded CsA, release profiles, and mechanical integrity, the most suitable system is poly(HEMA-*co*-HPβCD) hydrogels in high HPβCD content. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40397.

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INTRODUCTION

Cyclosporin A (CsA) is a potent immunosuppressive agent[1,2] obtained from a type of fungus called *Tolypocladium inflatium*.[3] It mediates its immunosuppressive effects by inhibition of calcineurin activity.[4,5] The effect of immunosuppression is reversible when administration is completed.[6] Because of its immunosuppressive properties, CsA is widely used to prevent the rejection of transplanted organs such as heart, kidney, lung, and pancreas.[7,8] In addition to organ transplantation, CsA is also used in the treatment of autoimmune disorders including rheumatoid arthritis and psoriasis and in ophthalmology.[2,9–11]

Recent studies have been shown that CsA is effective in the treatment of corneal graft rejection. However, in ocular systems, CsA has difficulty in administration by virtue of its hydrophobic character and in oily solutions it has limited ocular bioavailability.[12] In ophthalmology, the most popular administration is topical route, however, less than 5% of the topical dose is available to the eye.[13] Therefore, several doses, which worsen the patient's quality of life, are usually required.[14] Systemic administration of CsA is also limited by the common adverse effects seen with CsA like nephrotoxicity, hypertension, hyperlipidemia, and gastrointestinal effects.[15,16] To overcome these limitations in ocular systems, methods for local administration of CsA have been investigated. Subconjunctival delivery devices gain importance due to highest ocular bioavailability and allowing one time administration at the time of surgery.[6]

Over the past 10 years, efforts for the enhancement of the aqueous solubility of hydrophobic drugs and their controlled release have been mainly focused on cyclodextrins (CDs). Because of their unique property, being capable of forming inclusion complexes with hydrophobic drugs and good availability, CDs have been increasingly used in drug delivery systems.[17] Applications of CDs in ophthalmic preparations enhance the solubility and ocular drug permeability.

Cyclodextrins are cyclic oligosaccharides commonly composed of six, seven, and eight α-D-glucose units (α-,β-, and γ-CD, respectively) that have a shape like a truncated cone.[18,19] They have a hydrophobic interior that is capable of encapsulating of poorly water-soluble molecules completely or partially in aqueous environment.[20] The ability to form inclusion complexes depends on the size and polarity of the host molecule. The hydrophilic exterior allows for solubilization, thus making these complexes useful for formulation of hydrophobic drugs.[21] The formation of the inclusion complex greatly modifies the

Table I. Compositions, Equilibrium Swelling Ratios (Q_{eq}), and Amounts of Loaded CsA of Hydrogels Synthesized in This Study

Hydrogels	Composition	Q_{eq}	Amounts of loaded CsA (mg/g dry gel)	
			Experimental	Equation (4)
HPβCD-1	15% HPβCD (w/v)–60 EGDE (v/v)	4.79 ± 0.05	20.84 ± 0.76	11.41
HPβCD-2	30% HPβCD (w/v)–40 EGDE (v/v)	4.92 ± 0.01	22.12 ± 1.12	12.25
HPβCD-3	30% HPβCD (w/v)–60 EGDE (v/v)	2.46 ± 0.01	18.67 ± 0.39	10.11
HPβCD-4	40% HPβCD (w/v)–40 EGDE (v/v)	3.76 ± 0.01	19.43 ± 0.51	11.33
HPβCD-5	40% HPβCD (w/v)–60 EGDE (v/v)	1.94 ± 0.07	16.05 ± 0.80	7.04
Poly(HEMA)	HEMA–EGDMA	0.48 ± 0.01	2.50 ± 0.35	2.05
Poly(HEMA-*co*-HPβCD)–1	2 mL HEMA–0.021 g HPβCD	0.70 ± 0.02	1.77 ± 0.12	nd
Poly(HEMA-*co*-HPβCD)–2	2 mL HEMA–0.042 g HPβCD	0.65 ± 0.03	12.13 ± 0.09	nd
Poly(HEMA-*co*-HPβCD)–3	2 mL HEMA–0.063 g HPβCD	0.61 ± 0.01	13.96 ± 0.26	nd
Poly(HEMA-*co*-HPβCD)–4	2 mL HEMA–0.084 g HPβCD	0.70 ± 0.02	17.13 ± 0.37	nd

chemical and physical properties, mostly in terms of aqueous solubility and stability of the hydrophobic guest molecule.[22,23] The driving forces forming inclusion complexes are hydrophobic and van der Waals interactions, hydrogen bonding, and alteration of surface tensions.[20,24] Additionally, CDs can form chemically crosslinked hydrogels in different ways. CD-based hydrogels can be prepared directly using crosslinkers such as ethylene glycol diglycidylether and hexamethylene diisocyanate. Copolymerization of vinyl monomers (acrylic acid, AA, and 2-hydroxyethyl methacrylate, HEMA) with CDs is an alternative way and it would change the drug-polymer interactions and sustained the drug release.[25,26]

In this study, it was aimed to develop cyclodextrin-based hydrogels as subconjunctival drug carrier systems for Cyclosporine A. That is why, a chemically modified β-cyclodextrin, (2-hydroxypropyl)-β-cyclodextrin (HPβCD), was chosen as due to its low toxicity, larger molecular size, greater hydrophilicity, and no adverse effects in humans.[18,27] We have developed three different carrier systems using HPβCD. In the first system, HPβCD hydrogels were prepared by directly crosslinking with ethylene glycol diglycidylether (EGDE) in different ratios. In the second system, poly(HEMA) hydrogels which have good biocompatibility and mechanical properties[28,29] were synthesized by bulk polymerization/crosslinking with ethylene glycol dimethacrylate (EGDMA). To overcome the limitation of hydrophilic poly(-HEMA) hydrogels for hydrophobic drug loading, CsA–HPβCD inclusion complex was prepared. Lastly, poly(HEMA-*co*-HPβCD) hydrogels which promote the loading and release of CsA were prepared with different HPβCD proportions. Following synthesis of hydrogels, their characterizations, CsA loading, and *in vitro* release studies were performed comparatively for all types of hydrogels.

MATERIALS AND METHODS

Materials

Cyclosporine A (CsA) was a gift from Novartis (Switzerland). HPβCD, ethylene glycol diglycidylether (EGDE, 50% [w/w] in water), 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile (AIBN) were purchased from Aldrich. Phosphate buffer saline (PBS) and sodium hydroxide were obtained from Sigma. Hydrochloric acid and acetonitrile were supplied from Merck (Germany). Acetonitrile was HPLC grade and all other chemicals used were of analytical grade. Water used throughout the study was distilled or ultrapure water with a resistivity of 18.3 MΩ-cm that was obtained by a EASYpure UF water purification system (Barnstead Thermolyne Corporation).

Preparation of Hydrogel Systems

Three different polymeric carrier systems for CsA were developed and characterized (Table I). The preparation methods of cyclodextrin-based hydrogels and poly(HEMA) hydrogels were explained in the following sections.

Synthesis of Hydroxypropyl-β-Cyclodextrin Hydrogels. Different amounts of HPβCD (15, 30, and 40% [w/v]) were dissolved in 0.2*M* NaOH and stirred for 2 min at room temperature for homogeneity. Then, different percentages of crosslinker, EGDE (20, 40, and 60% [v/v]), was added to the HPβCD solutions and stirred for 2 min. The solutions were kept in an incubator (NÜVE ES 500, Turkey) at 50 ± 0.5°C for 12 h. At the end of 12 h, samples which become to gel form were cooled at room temperature for 1 h and then, removed from tubes. Cylindrical pieces of each gel (diameter: 6 mm and thickness: 1 mm, weight: 0.3 g, in dry form) were cut and washed with ultrapure water. Then, they were immersed into 10 mM HCl solution for 12 h and washed with ultrapure water again.[30]

Synthesis of Poly(HEMA) Hydrogels. Poly(HEMA) hydrogels were synthesized by bulk polymerization method. Concentrations of initiator (AIBN) and crosslinker (EGDMA) were adjusted to 0.002 g/mL HEMA and 0.04 mL/mL HEMA, respectively. Casting solution was poured into polypropylene molds and kept in an incubator with temperature of 60 ± 0.5°C for 24 h. To remove the impurities like monomer and initiator, poly (HEMA) hydrogels (diameter: 12 mm, thickness: 1 mm, dry weight: 0.3 g) were washed with distilled water several times.

Synthesis of Poly(HEMA-co-HPβCD) Hydrogels. During the preparation of poly(HEMA-*co*-HPβCD) hydrogels, the amount

of HEMA was kept constant and the effects of the different amounts of HPβCD were observed. EGDMA (3 μL) and different amounts of HPβCD (0.021, 0.042, 0.063, and 0.084 g) were dissolved/dispersed in 2 mL HEMA. Initiator, AIBN (10 mM), was added to the monomer solutions. The solutions were kept at 50 ± 0.5°C for 12 h in an incubator and then, at 70 ± 0.5°C for 24 h. Cylindrical pieces of each gel (diameter: 14 mm and thickness: 1 mm, weight: 0.4 g, in dry form) were cut and immersed in water for 15 min to remove impurities. Samples were first kept in 10 mM NaCl and then, in distilled water for 3 days. In this step, both NaCl and distilled water were replenished in every 12 h.

Drug Loading

Cyclosporine A was loaded into the hydrogel systems by embedding method. After drying in a vacuum incubator (NUVE EV 018, Turkey), cylindrical HPβCD hydrogels and poly(HEMA-*co*-HPβCD) hydrogels were placed in aqueous suspensions of CsA (10 mL of 2 mg/mL) at 30°C for 5 days. Furthermore, to examine the effect of initial concentration of loading solution, poly(-HEMA-*co*-HPβCD) hydrogels were immersed in 1 mg/mL CsA solutions (10 mL) at 30°C for 5 days.

Dried poly(HEMA) hydrogels were loaded with CsA by immersing them in 5 mL complex solution (see Preparation of HPβCD–CsA Inclusion Complex section) at 30°C for 5 days. After loading of CsA, hydrogels were removed from aqueous suspensions and rinsed with distilled water. For *in vitro* release studies, drug loaded hydrogels were dried at room temperature and vacuum incubator.

The amount of loaded CsA was determined by measuring the amount of CsA remaining in the loading solution by HPLC method (see In Vitro Release Study section). The difference between the initial amount of CsA in the solution and the remaining amount was denoted as the proportion of loaded CsA. The experiments were performed in triplicate.

Characterization of Hydrogels

Swelling Studies. Hydrogels were dried in air condition (at room temperature) or vacuum incubator until they reached constant weight to investigate as to whether drying condition influenced the swelling kinetics. Dry hydrogels were submerged in 25 mL phosphate buffer solution (PBS, pH = 7.4) at 37 ± 0.5°C and weighed in certain time intervals after wiping of the surface with a soft tissue. Equilibrium swelling ratio of samples was estimated using the following equation:

$$Q_{eq} = \frac{(W_\infty - W_o)}{W_o}, \quad (1)$$

where Q_{eq} is the equilibrium swelling ratio, W_∞ is the weight of the fully swollen hydrogel, and W_o is the weight of the dry hydrogel. The experiments were performed in triplicate.

ATR-FTIR Analysis. Fourier Transform Infrared attenuated total reflectance (ATR-FTIR) spectra were recorded using an ATR-FTIR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA) over the range of 650–4000 cm^{-1}. Hydrogels including CsA and without CsA were freeze-dried (Christ Alpha 2–4 LD, Germany) and powdered in a mortar. ATR-FTIR analyses were performed in duplicate for each of the samples.

Differential Scanning Calorimetry Analysis. Differential Scanning Calorimetry (DSC) studies were performed using a DSC instrument (Perkin Elmer Diamond) to identify the thermal transition temperatures of samples and the possibility of interactions between CsA and hydrogels. Samples were contained in aluminum pans and the scanning rate was 10°C/min over a temperature range of 25–350°C in a nitrogen environment.

Dynamic Mechanical Analysis. For the dynamic mechanical analysis, the TA Instrument Q800 Dynamic mechanical Analyzer (USA) was used. Freeze-dried hydrogels were powdered and mixed with Al_2O_3 (50 : 50, w:w). The frequency of each analysis was 1 Hz and the temperature was ranging from 30 to 200°C with a scanning rate of 3°C/min.

Preparation of HPβCD–CsA Inclusion Complex

Phase solubility studies were performed according to the method described by Higuchi and Connors.[26] Excess amounts of CsA (50 mg CsA) were added to the aqueous solutions of HPβCD in different concentrations ranging from 0 to 12 mM. The suspensions were shaken at 25 ± 0.5°C for 7 days. After equilibrium was reached, samples were filtered through a 0.22 μm cellulose acetate membrane and drug concentration was determined spectrophotometrically (Labomed) at the wavelength of 200 nm. The calibration curve ($R^2 = 0.96$) was used to calculate the concentration of the drug.

To load Cyclosporine A to the hydrophilic poly(HEMA) hydrogels, CsA–HPβCD complex was prepared due to increasing the aqueous solubility of hydrophobic drugs in complex forms.[22] HPβCD (0.15 mM) was dissolved in 5 mL distilled water to obtain a saturated solution. Afterwards, 0.015 mM CsA was added to the HPβCD solution and shaken at 25 ± 0.5°C for 7 days to reach the equilibrium. After filtration through a 0.22 μm cellulose acetate membrane, complex solutions were used for loading.

The apparent affinity constant ($K_{1:1}$), assuming that a 1 : 1 complex was formed, was calculated from the solubility data using the following equation:

$$K_{1:1} = \frac{m}{S_o(1-m)}, \quad (2)$$

where m is the slope of the plot and S_o is drug (CsA) solubility in the absence of cyclodextrin.[31] All samples were prepared in duplicate.

In Vitro Release Study

The drug release experiments were performed in an incubator (GFL 3032, Germany) maintained at 37°C and 30 rpm under sink conditions. CsA-loaded HPβCD hydrogels, poly(HEMA-*co*-HPβCD) hydrogels, and poly(HEMA) hydrogels were placed in 10 mL PBS (pH = 7.4). The samples were withdrawn at pre-established time interval and replaced by the same amount of fresh buffer to keep the liquid volume constant. The amount of CsA released was measured by HPLC. The experiments were performed in triplicate.

The cumulative percentage of CsA released (cumulative release %) from hydrogels was calculated by the following equation:

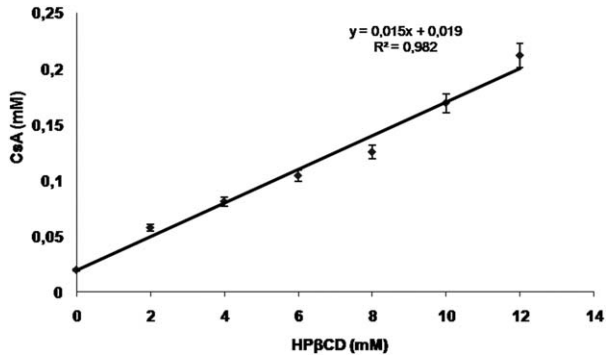

Figure 1. Phase solubility diagram of Cyclosporine A in HPβCD.

$$\text{Cumulative release } (\%) = \left(\frac{W_t}{W_{\text{total}}}\right) \times 100, \qquad (3)$$

where W_t is the amount of cumulative released at time t and W_{total} is the amount of drug loaded.

Amount of drug loaded and released was determined by HPLC (Varian ProStar) equipped with a C18 column (Pursuit, 150 × 4.6 mm, 5 µm, Varian, Part No.A3000150x046) and a UV detector (PDA, Varian, Model 330). The mobile phase consisted of acetonitrile:distilled water (75 : 25, v/v, pH adjusted to 3.1 by phosphoric acid) with a flow rate of 1 mL/min and the column was heated to 72°C. The detection wavelength was set at 200 nm.[32] The calibration curve for area under the peak versus concentration was linear ($R^2 = 0.99$).

RESULTS AND DISCUSSION

Phase Solubility Studies

Phase solubility studies were performed to examine the effect of HPβCD on CsA solubility in water. Figure 1 shows that phase solubility diagram of CsA in the presence of HPβCD. Solubility diagram exhibits A_L type according to the Higuchi and Connors classification.[26] A_L type diagram is indicative of the solubility of CsA in water increased linearly upon increasing HPβCD concentration. A_L type curve with a slope lower than 1 (slope = 0.0151) which is characteristic of the formation of 1 : 1 mol–mol complexes.[33] CsA solubility in the absence of HPβCD (S_o) calculated 23.5 µg/mL confirms the literature value (23 µg/mL).[1] The apparent stability constant ($K_{1:1}$) of the complex was calculated to be 786 M^{-1} using eq. (2).

Hydrogel Synthesis

HPβCD Hydrogels. It is known that cyclodextrins have the potential to enhance hydrophobic drug release. Because of higher solubility in water and good tolerance in human body,[27,34] HPβCD was used in our study. The hydrogels in different HPβCD ratios were synthesized by crosslinking with EGDE. The cross-linking agent, EGDE, has two epoxy groups and these groups have similar reactive characteristics and can simultaneously react with hydroxyl groups of cyclodextrins directly and form cross-linked structures. At three different HPβCD concentrations, that is, 15, 30, and 40% (w/v), crosslinker concentration was varied as 20, 40, and 60% (v/v). In the compositions of 15% HPβCD–20% and 40% EGDE; 30% HPβCD–20% EGDE and 40% HPβCD–20% EGDE gelling were not observed. This observation indicates that both the amounts of crosslinking agent and HPβCD are vital for the formation of network structure. Swelling of HPβCD hydrogels in water and increasing amounts of crosslinkers from 40 to 60% leads to more stiff structure as a result of increasing crosslinking density (Table I).

Poly(HEMA) Hydrogels. Poly(HEMA) hydrogels were synthesized by bulk polymerization. The concentrations of EGDMA and AIBN were determined regarding the previous studies to obtain optimum mechanical hydrogel strength.

Poly(HEMA-co-HPβCD) Hydrogels. The drug release performance of hydrogels is affected by not only the gel structure, that is, the structure of the reactive areas and the crosslinking ratio, but also the gel–drug interaction plays an important role. Hydrogels have two main drawbacks for drug delivery, that is, exiguous loading capacity for poorly water-soluble drugs, and fast release property for water-soluble drugs.[30,31] Therefore, to minimize such those limitations for poorly water-soluble drug, CsA, and for its water-soluble inclusion complex form, we decided to incorporate CDs into the poly(HEMA) hydrogels during synthesis. As polymer synthesis was performed in the absence of EGDE, crosslinker for HPβCD, it could be considered that CD molecules are freely dispersed in the poly(HEMA) network. Thus, poly(HEMA-*co*-HPβCD) hydrogel can increase the interaction of carrier system with the CsA and a suitable system for sustained drug release can come true.

In our study, the amount of HEMA is kept constant as 2 mL and by varying the amounts of HPβCD (0.021, 0.042, 0.063, and 0.084 g), four different poly(HEMA-*co*-HPβCD) hydrogels given in Table I were synthesized. It was observed that the structure of poly(HEMA-*co*-HPβCD) hydrogel is much harder than homopolymer HPβCD hydrogels. The varying amount of HPβCD does not make any significant physical changes in gels.

Loading of CsA into Hydrogel Implants

Loading of CsA into HPβCD Hydrogels. The amounts of CsA loaded into the hydrogels were determined by HPLC according to the difference between the initial amount of CsA in the loading solution and the remaining amount of CsA after loading. Conversely, if "eq. (4)," which is suggested by Kim et al.,[35] is used to find the amount of loaded drug, it was concluded that the amounts of CsA loaded into the HPβCD hydrogels are approximately half the actual amounts determined by the analysis (Table I). This result shows that the amount of drugs loaded into the hydrogels depends on not only the concentration of the loading solution, but also the interest of CsA to HPβCD.

$$\text{Loaded amount of drug} = \left(\frac{V_s}{W_p}\right) \times C_o \qquad (4)$$

In the equation, V_s is the absorbed water volume by the hydrogel, W_p is the dry weight of hydrogel, and C_o is the drug concentration in loading solution.

The highest amount of loaded CsA (22 mg/g of dry gel) is obtained for hydrogels in 30% HPβCD–40% EGDE

composition, while the lowest amount of CsA is 16 mg/g dry gel for 40% HPβCD–60% EGDE containing hydrogel (Table I).

Loading of CsA into Poly(HEMA) Hydrogels. Loading of CsA into poly(HEMA) hydrogels is performed in CsA–HPβCD complex solution. Water molecules fill into the CD spaces while the complex is formed. Then, the hydrophobic molecules disperse in aqueous medium and water is replaced with hydrophobic molecules and an inclusion complex, which does not contain any covalent bonds, is formed. The free drug molecules in complex solution and the drug molecules trapped into the CD space are in equilibrium.[36]

Initially, 0.015 mM of CsA is in the complex solution. The amount of CsA loaded into the poly(HEMA) hydrogels was determined as 2.50 ± 0.35 mg/g dry gel (Table I).

Loading of CsA into Poly(HEMA-co-HPβCD) Hydrogels. Loading of CsA into the poly(HEMA-*co*-HPβCD) hydrogels was performed in two different concentrations, that is, 1 and 2 mg/mL, to examine the effect of the initial CsA concentration. The amount of CsA loaded from 2 mg/mL CsA loading solution is a little more than that of 1 mg/mL CsA loading solution. In the case of 1 mg/mL, the loaded amounts of CsA are 1.27 ± 0.03, 10.42 ± 0.21, 12.13 ± 0.39, and 15.19 ± 0.31 mg/g dry gel for poly(HEMA-*co*-HPβCD)−1, 2, 3, and 4 hydrogels, respectively.

The amount of loaded CsA into the poly(HEMA-*co*-HPβCD) hydrogels is also increased by increasing the HPβCD content in gel composition. In addition, the loaded amount of drug is not proportional with the swelling ratios. These two results indicate that the complex formation between CsA and HPβCD is more effective on the amount of drug loaded, due to the lower interest of CsA to the poly(HEMA).[31] In similar to the HPβCD hydrogels, the actual amounts of loaded CsA into poly(HEMA-*co*-HPβCD) hydrogels are almost twice the calculated amounts. The amount of loaded CsA into the poly(HEMA-*co*-HPβCD)−1 hydrogel which has lowest HPβCD content is much less than that of other copolymers. It was considered that, below certain amounts of HPβCD, the interaction with CsA could not properly occur.

Characterization of Hydrogels

Swelling Behavior. Swelling studies were performed to examine the effect of cross-linking agent, drying conditions, and gel type on hydrogels' swelling behavior. Equilibrium swelling ratios indicate that drying environment (air condition or vacuum incubator) has no effect on swelling behavior of hydrogels. By comparison with three different types of hydrogels (HPβCD, poly(HEMA), and poly(HEMA-*co*-HPβCD) hydrogels), the highest equilibrium swelling ratio belongs to the HPβCD hydrogels and in descending order poly(HEMA-*co*-HPβCD) and poly(HEMA) followed. The hydrogels reach the equilibrium nearly in 24 h.

The water uptake of HPβCD hydrogels significantly decreased by increasing EGDE content from 40 to 60% (v/v) in hydrogel composition due to the high cross-linking density which led to making water diffusion to the gel structure difficult. In addition, while EGDE ratio is constant, increasing HPβCD content in hydrogel reduced the equilibrium swelling ratio (Table I).

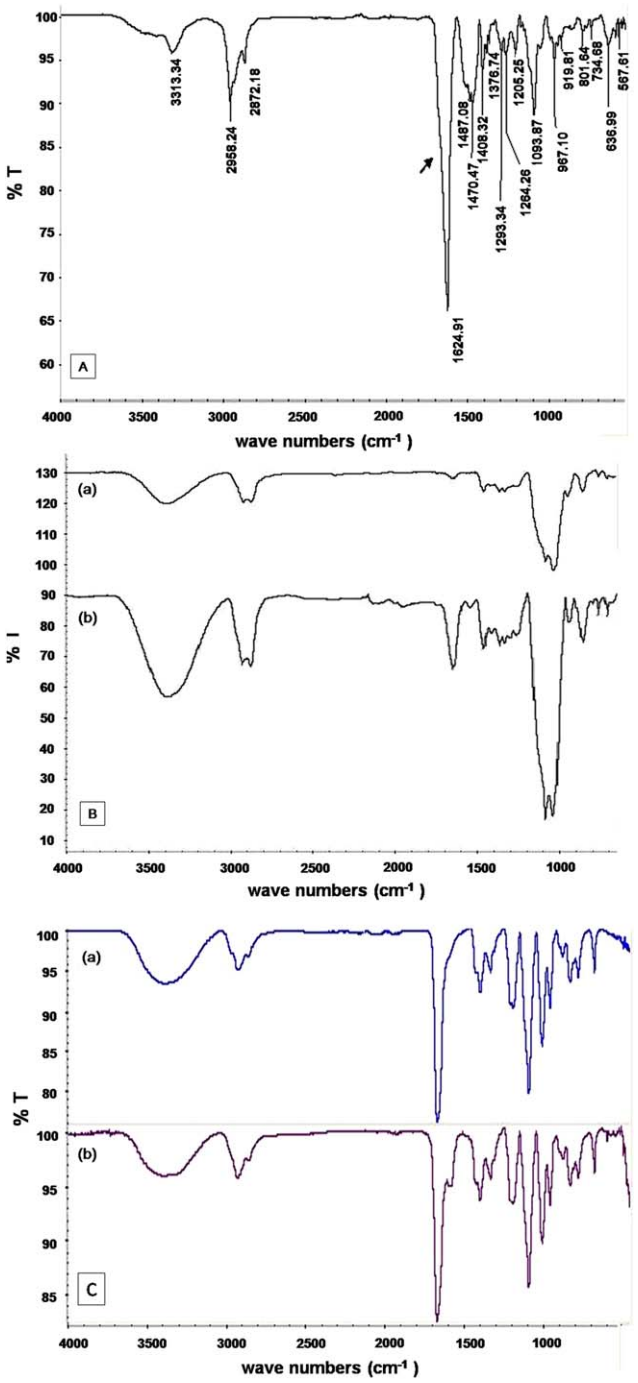

Figure 2. FTIR spectra of (A) CsA; (B) HPβCD hydrogel (a) HPβCD-2 hydrogel, (b) CsA loaded HPβCD-2 hydrogel; (C) poly(HEMA-*co*-HPβCD) hydrogel (a) poly(HEMA-*co*-HPβCD)−2 hydrogel, (b) CsA loaded poly(HEMA-*co*-HPβCD)−2 hydrogel. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Equilibrium swelling ratios of poly(HEMA-*co*-HPβCD) hydrogels were slightly higher than that of poly(HEMA) hydrogels (Table I) as HPβCD molecules were freely dispersed in the poly(HEMA) network.

FTIR Studies. The FTIR spectrum of CsA [Figure 2(A)] showed characteristic bands at 3313 cm^{-1} for NH stretching vibrations,

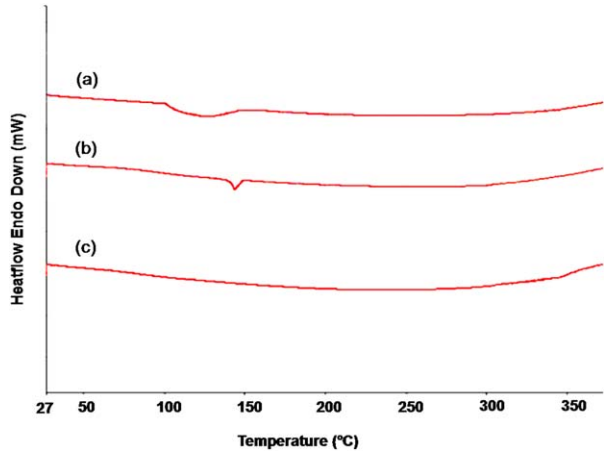

Figure 3. DSC thermograms of (a) HPβCD (powder), (b) CsA–HPβCD mixture, and (c) CsA–HPβCD complex. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

2958 cm^{-1} for CH stretching vibrations, and 1625 cm^{-1} for C═O stretching vibrations. Asymmetric C═O stretching band at 1625 cm^{-1} denotes the carbonyl groups in CsA structure are not identical. The strongest absorption band at 1625 cm^{-1} shows H-bonded C═O group, while the shoulder at highest frequency indicates non-H bonded C═O stretching.

HPβCD (powder) showed absorption bands at 3353 cm^{-1} for OH stretching, 2927 cm^{-1} for CH stretching, 1456 cm^{-1} for CH_2 and CH_3 bending, and 945 cm^{-1} for skeletal vibration involving α-1,4 linkage (data were not shown). The OH stretching band observed at 3330 cm^{-1} for the physical mixture of CsA–HPβCD, whereas 3334 cm^{-1} for CsA–HPβCD complex (data were not shown). OH stretching band shifted to 3335 cm^{-1} for HPβCD hydrogels due to losing the effect of H-bonded OH groups corresponding to crosslinking [Figure 2(B-a)]. CsA loaded HPβCD hydrogels showed OH stretching vibrations at 3354 cm^{-1} [Figure 2(B-b)].

The spectrum of poly(HEMA) hydrogels showed stretching vibrations at 3399 cm^{-1} (OH), 2946 cm^{-1} (aliphatic CH), 1716 cm^{-1} (C═O), and 1487 and 1451 cm^{-1} (asymmetric CH_2 and CH_3 bending). For CsA–HPβCD complex loaded poly(HEMA) hydrogels, the absorption of the OH stretching shifted to 3398 cm^{-1} (data were not shown).

The FTIR spectrum of the poly(HEMA-*co*-HPβCD) hydrogels is similar to the poly(HEMA) [Figure 2(C-a)]. The broad band at 3399 cm^{-1} is for OH stretching and sharp band at 1716 cm^{-1} is for C═O stretching. For CsA-loaded copolymers, OH stretching vibrations were seen at 3391 cm^{-1} which can be attributed to hydrogen bonding interaction between drug and copolymer [Figure 2(C-b)].

DSC Analysis. One of the important parameters that affect the diffusion of the solvent and solute in controlled release systems is the glass-transition temperature (T_g). Below the glass-transition temperature, the movements of the polymeric chains are limited; as a result, the diffusion rate of the drug in the structure is low. Over the glass-transition temperature, the polymer becomes rubbery and the diffusion rates of drugs from the polymer structure increase.[37,38]

To determine the glass-transition temperatures (T_g and T_m) of the samples that includes and non-includes CsA, differential scanning calorimetry (DSC) analyses were performed. The DSC results demonstrate an endothermic peak for CsA at 129.08°C, which corresponds to the melting point. The melting point of CsA reported by Malaekeh-Nikouei et al.[9] is 130°C.

The DSC data for amorphous HPβCD (powdered) show a broad endothermic peak around 130°C corresponding to the loss of water molecule [Figure 3(a)]. In the thermogram of the CsA–HPβCD physical mixture prepared in 1 : 1 (mol/mol) ratio, the endothermic peaks of CsA and HPβCD overlap. The sharp peak at 140°C is related to the melting peak of CsA [Figure 3(b)]. There is no peak observed in the thermogram that belongs to the CsA–HPβCD complex, which was prepared by lyophilizing [Figure 3(c)]. The disappearance of the CsA characteristic peak was related to forming a complex between CsA and HPβCD.[27] The formed complex was also amorphous.

Any significant peak was not detected for the CsA loaded or unloaded HPβCD hydrogels, poly(HEMA-*co*-HPβCD) hydrogels, and poly(HEMA) hydrogels. Therefore, to obtain more sensitive results, dynamic mechanical analysis (DMA) was approved.

Dynamic Mechanical Analysis. Dynamic mechanical analysis (DMA) is particularly useful for measuring transitions in polymers that cannot be detected by other techniques. DMA measures stiffness and damping, which are reported as modulus and tan δ. At the glass transition, the storage modulus decreases dramatically and the loss modulus reaches a maximum.

DMA thermograms for poly(HEMA) and poly(HEMA-*co*-HPβCD) hydrogels were given in Figure 4(A,B), respectively. Glass transition temperatures according to storage modulus, derivative storage modulus, loss modulus, and tan δ for poly(-HEMA) and poly(HEMA-*co*-HPβCD) hydrogels were listed in Table II. According to the "storage modulus–T" behavior, T_g value of poly(HEMA) is 119.90°C and this value is increased in the presence of CsA–HPβCD complex. After drug released, T_g value decreased below 119.90°C surprisingly. Similar result is seen at "derivative storage modulus–T" relationship. Poly(-HEMA) demonstrates the ability of inter- and intra-molecular hydrogen bonding. Although the drug is releasing, dynamics of hydrogen bond collapsed.

For poly(HEMA-*co*-HPβCD) "storage modulus–T" behavior is evaluated, and T_g value is presented at 110.76°C. Glass transition temperature of drug loaded hydrogel is increased about 4°C (114.48°C) due to the forming of hydrogen bonding between hydrogel and drug.

In Vitro Release Studies

As the drug release systems produced within the scope of this study are designed for human applications, release studies were performed in PBS medium (37°C, pH = 7.4). The cumulative release values are calculated with CsA concentrations obtained

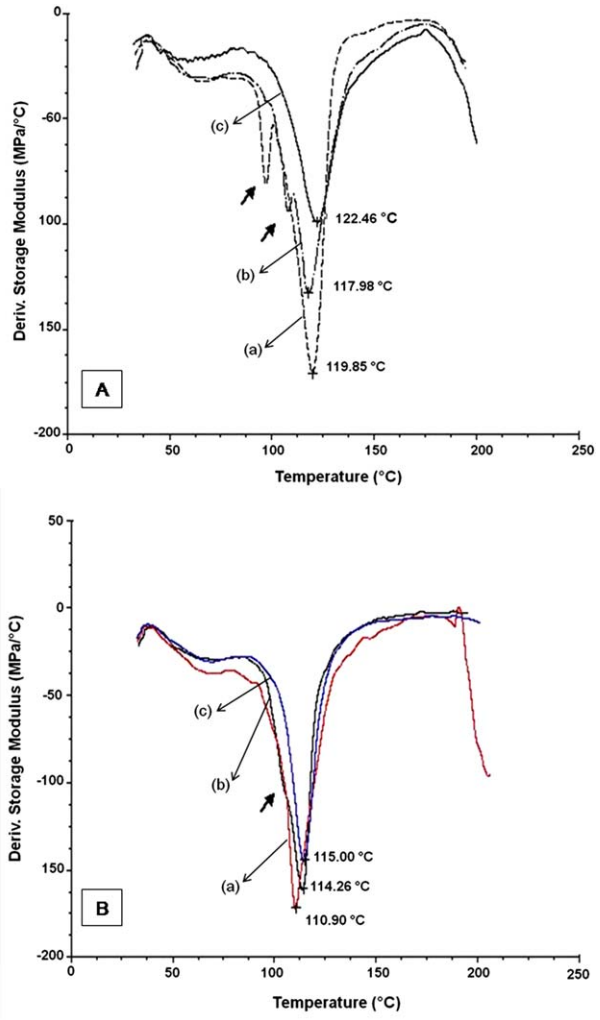

Figure 4. A. Derivative storage modulus–T thermograms of A (a) poly(-HEMA), (b) poly(HEMA) after release (c) complex-loaded poly(HEMA); B (a) poly(HEMA-*co*-HPβCD), (b) poly(HEMA-*co*-HPβCD) after CsA release, (c) CsA-loaded poly(HEMA-*co*-HPβCD). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

from HPLC analysis and they were expressed as a cumulative release amount (mg/g dry gel) and release percentage.

Release from HPβCD Hydrogels. Fifty-five to sixty-five percentage of CsA was released from HPβCD hydrogels during 2 months period (Figure 5). Among the hydrogels highest amount of CsA release was obtained from the hydrogel with 30% HPβCD–40% EGDE compositions (HPβCD-2) due to its high CsA content and also high cumulative release % at the end of the 60 days.

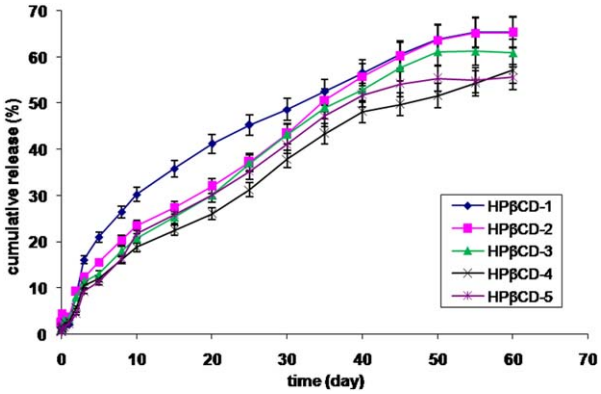

Figure 5. Cyclosporine A release profiles from HPβCD hydrogels (PBS, $T = 37°C$, pH = 7.4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The easy diffusion and the interest to the network structure of the drug molecule play an important role in the release amounts from hydrogels and loading amounts into hydrogels.[25] It was seen that, when the amount of EGDE is kept constant and HPβCD is increased, the release rate decreases. As the increased amount of HPβCD may cause an increase in the hydrophobic interactions between CsA and network structure and may delay the release. The release profiles are close to each other for 30 and 40% (w/v) HPβCD but, the initial release rate is higher than the others for 15% HPβCD (w/v).

For HPβCD hydrogels, when the amount of HPβCD is kept constant and the crosslinking agent concentration is increased, percentage of release decreases. This result could be associated with the increase in the crosslinking ratio. Constant amount of HPβCD means the elimination of HPβCD effect for release amounts. Hence, closer release profiles are an expected result when the HPβCD amount is kept constant.

Release from Poly(HEMA) Hydrogels. A burst-release occurred from poly(HEMA) hydrogels, as it can be seen in Figure 6. Ninety percentage of CsA was released, when the release was completed in 3 days. This result could be explained by the

Table II. Glass Transition Temperatures According to Storage Modulus–T, Derivative Storage Modulus–T, Loss Modulus–T, and tan δ–T Behaviors for Poly(HEMA) and Poly(HEMA-*co*-HPβCD) Hydrogels

	Storage modulus (mid-point) (°C)	Derivative storage modulus (°C)	Loss modulus (°C)	tan δ (°C)
Poly(HEMA)	119.90	119.85	121.57	128.14
CsA-complex loaded poly(HEMA)	122.21	122.46	124.01	134.32
Drug released poly(HEMA)	118.38	117.98	125.86	129.85
Poly(HEMA-*co*-HPβCD)−2	110.76	110.90	116.12	122.86
CsA loaded poly(HEMA-*co*-HPβCD)−2	114.48	115.00	115.95	120.21
Drug released poly(HEMA-*co*-HPβCD)−2	113.86	114.26	115.57	118.71

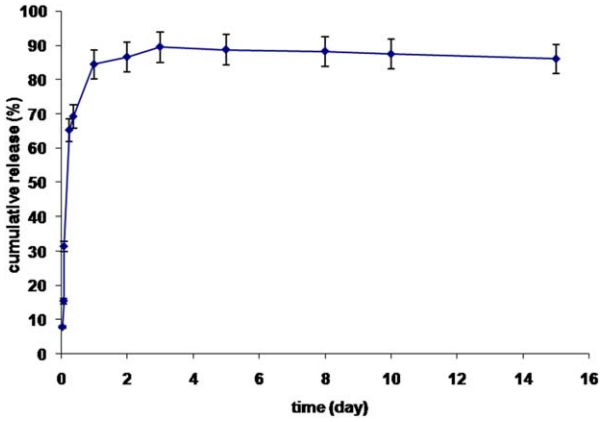

Figure 6. Cyclosporine A cumulative release (%) profile from poly(-HEMA) hydrogels (PBS, $T = 37°C$, pH = 7.4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

retention of CsA–HPβCD complex on the surface instead of diffusing into poly(HEMA). Also, there is a possibility of a sudden dilution of CsA–HPβCD complex in the release medium, which may result the degradation of complex structure. Therefore, there is not a significantly long release as it happened in HPβCD hydrogels.

Release from Poly(HEMA-co-HPβCD) Hydrogels. The graphic that shows the cumulative release percentage of CsA from poly(HEMA-*co*-HPβCD) hydrogels is given in Figure 7. The released amount of CsA from hydrogels except hydrogel 1 (2 mL HEMA–0.021 g HPβCD) is determined close to each other.

The CsA release rate from poly(HEMA-*co*-HPβCD) hydrogels decreases as the HPβCD amount increases. It could be explained by the hydrophobic interactions between CsA and HPβCD. As the increase in the amount of HPβCD increases the possibility of complex formation, the decrease of the drug release rate is an expected behavior. The hydrogel in 2 mL HEMA–0.021 g HPβCD composition (hydrogel 1) completes the release in the first 30 days. However, CsA release continues up to 60 days for other compositions.

The frequently used model to describe the release kinetics from hydrogels is power law given in eq. (5),

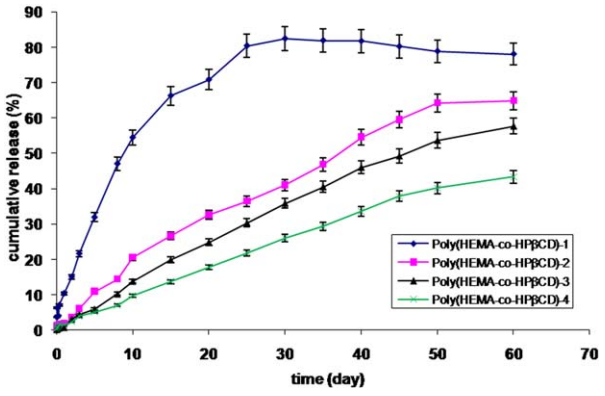

Figure 7. Cyclosporine A cumulative release (%) profile from poly(HEMA-*co*-HPβCD) hydrogels (PBS, $T = 37°C$, pH = 7.4). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Summary of Release Exponents (n), Kinetic Constants (k), and Correlation Coefficients (R^2) for Hydrogels Obtained from Power Law Fit for Release Kinetics

Hydrogels	n	k	R^2
HPβCD-1	0.60	0.009	0.94
HPβCD-2	0.49	0.016	0.94
HPβCD-3	0.60	0.008	0.98
HPβCD-4	0.63	0.006	0.99
HPβCD-5	0.72	0.004	0.98
Poly(HEMA)	1.25	0.073	0.98
Poly(HEMA-*co*-HPβCD)−1	0.50	0.027	0.91
Poly(HEMA-*co*-HPβCD)−2	0.73	0.003	0.96
Poly(HEMA-*co*-HPβCD)−3	0.82	0.002	0.97
Poly(HEMA-*co*-HPβCD)−4	0.70	0.002	0.97

$$\frac{M_t}{M_\infty} = kt^n, \tag{5}$$

where M_t and M_∞ are the amount of drug released at time t and infinite time; k is a constant incorporating structural and geometric characteristics of the system; and n is the diffusional exponent, which can be indicative of the mechanism of diffusion type. The power law equation is thought to be a superposition of two processes, Fickian and Case II diffusion. As the transport varies from Fickian ($n = 0.5$) to Case II diffusion ($n = 1$), the value of n varies as well. In between these two processes, anomalous diffusion is characterized by intermediate values of n ($0.5 < n < 1$). These values of n also depend largely on the geometry of the polymer system. For cylinder system; $n = 0.45$ (Fickian diffusion), $0.45 < n < 0.89$ (anomalous diffusion), and $n = 0.89$ (Case II diffusion). Equation (5) has however been shown to be valid only for the first 60% of the total amount of drug released regardless of the geometry of the polymer.

To establish CsA release kinetics for three different polymeric systems, diffusional exponents were calculated using eq. (5). As listed in Table III for HPβCD and poly(HEMA-*co*-HPβCD) hydrogels; n values are between 0.45 and 0.89 which indicates anomalous transport for cylindrical systems. For poly(HEMA) hydrogel, the transport mechanism may be of supercase II because diffusional exponent (n) is bigger than 0.89.

CONCLUSION

In this study, we compared the CsA loading and releasing performances of three types of hydrogelic carrier systems, that is, HPβCD hydrogels, poly(HEMA) hydrogels, and various amounts of HPβCD-containing poly(HEMA) hydrogels. Among them, the lowest drug loading (2.5 mg/g dry gel) was obtained for poly(-HEMA) hydrogels, however, the amount of CsA loaded to the HPβCD including hydrogels reached to 22 mg/g of dry hydrogel. When the hydrogels were compared regarding the CsA release profiles, the desired long-term release (more than 3 months) could not be obtained from poly(HEMA) hydrogels as the release is completed in the first 3 days. After 2 months, 55–65% and 38–78% of loaded CsA were released from HPβCD hydrogels and

poly(HEMA-*co*-HPβCD) copolymers, respectively. In conclusion, poly(HEMA-*co*-HPβCD) hydrogels which has high HPβCD content should be proposed for the subconjunctival delivery of CsA.

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