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Ophthalmic delivery of Cyclosporine A from Brij-97 microemulsion and surfactant-laden p-HEMA hydrogels

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ABSTRACT

Cyclosporine A (CyA) is an immunosuppressant drug that is used for treating a variety of ocular diseases and disorders. CyA is commonly delivered via eye drops, which is highly inefficient due to a low bioavailability of less than 5%. The bioavailability of ophthalmic drugs can be substantially improved to about 50% by delivering them via contact lenses. This paper focuses on the development of nanostructured poly (2-hydroxyethyl methacrylate) (p-HEMA) hydrogels containing microemulsions or micelles of Brij 97 ($C_{18}H_{35}(OCH_2CH_2)_{10}$) for extended delivery of CyA. Release of CyA from these nanostructured hydrogels was performed *in vitro* to explore the mechanisms of release and the effects of surfactant concentration, processing conditions and storage on the release kinetics. Results show that the surfactant and microemulsion-laden gels can deliver CyA at therapeutic dosages for a period of about 20 days. Release of the drug is diffusion controlled with effective diffusivities decreasing with increasing surfactant loading. The release kinetics are relatively similar for both surfactant and microemulsion-laden gels with comparable surfactant loading. The results also show that these hydrogels retain their effectiveness even after exposure to all the relevant processing conditions including unreacted monomer extraction, autoclaving and packaging, and so these materials seem to be very promising for ophthalmic delivery of CyA and perhaps other drugs.

1. Introduction

Approximately 90% of all ophthalmic drugs are delivered using eye drops (Bourlais et al., 1998). While eye drops are convenient to use, only about 1–5% of the drug applied via eye drops eventually reaches the target tissue, and the remaining 95–99% drug enters the systemic circulation through conjunctival uptake or drainage into the nasal cavity (Lang, 1995). The low bioavailability leads to drug wastage, and more importantly, the systemic uptake of ophthalmic drugs can lead to side effects. To overcome the drawbacks of eye drops, several ophthalmic drug delivery systems have been proposed such as suspension of nanoparticles, nanocapsules, liposomes and niosomes, ocular inserts like collagen shields and Ocusert®, and therapeutic contact lenses. Among these, contact lenses have been widely studied due to their higher degree of comfort and biocompatibility. On insertion of a medicated contact lens in the eye, drug diffuses through the lens matrix into the thin tear film called post-lens tear film trapped between the lens and the cornea, and the drug has a residence time of about 30 min in the eye in the presence of contact lenses (McNamara et al., 1999; Creech et al., 2001). An increase in the residence time leads to a significant increase in the bioavailability. Both, mathematical models and clinical data suggest that the bioavailability for ophthalmic drug delivery using contact lenses can be as large as 50% (Li and Chauhan, 2006).

A large number of drugs, such as cromolyn sodium, ketotifen fumarate, ketorolac tromethamine, dexamethasone sodium phosphate (Karlgard et al., 2003), timolol (Alvarez-Lorenzo et al., 2002), pilocarpine (Hsiue et al., 2001) and fluoroquinolones (Tian et al., 2001), have been studied for uptake and release by soft contact lenses. A number of studies have been conducted for uptake of the drug by soaking the lens in concentrated drug solution followed by *in vitro* or *in vivo* release studies (Hillman, 1974; Ramer and Gasset, 1974; Ruben and Watkins, 1975; Rosenwald, 1981; Arthur et al., 1983; Wilson and Shields, 1989; Schultz et al., 1995; Fristrom, 1996; Schultz and Mint, 2000). The major problem of loading drug by soaking method is that in most cases the loading capacity of the soaked contact lenses is inadequate. The drug loading capacity can be increased by 2–3 times by designing molecularly imprinted soft contact lenses (Hiratani and Alvarez-Lorenzo, 2004; Hiratani et al., 2005). Another commonly used method of entrapping drugs in gels is direct addition of the drug in the polymerizing medium

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(Ende and Peppas, 1997; Colombo et al., 1999; Ward and Peppas, 2001).

While the bioavailability can be increased by using contact lenses soaked in drug solutions, these cannot be used for extended drug delivery because these systems release the entire drug amount in a short period of time (Jain, 1988). The duration of release can be increased by using molecular imprinted lenses or by dispersing colloidal systems into the lenses which either bind a significant amount of drug or provide a substantial barrier to drug release. Here, we explore microemulsion and micelle laden contact lenses for extended release of CyA, which is a commonly used immunosuppressant. It is prescribed for treating a number of ophthalmic diseases such as dry eyes (Calonge, 2001), uveitis in children and adolescents (Walton et al., 1998), vernal keratoconjunctivitis (Gupta and Sahu, 2001) and peripheral ulcerative keratitis (Georganas et al., 1996). In addition to treating ocular disorders, CyA has also shown promise in treating contact lens mediated dry eyes, and so these systems could also be very useful for a large population that is unable to wear contact lenses due to discomfort (Hom, 2006).

In this paper, we show that contact lenses made from microemulsion and surfactant-laden hydrogels can be used for extended delivery of CyA at therapeutic dosages. Also, for the first time, we show that surfactant-laden hydrogels can go through all the processing steps that a typical contact lens goes through including monomer extraction, autoclaving and packaging, and still provide extended drug release at therapeutic dosages. The results of this study provide strong evidence that microemulsion and/or surfactant-laden contact lenses can be used for extended delivery of various ophthalmic drugs including CyA.

2. Materials and methods

2.1. Materials

2-Hydroxyethyl methacrylate (HEMA) monomer, ethylene glycol dimethacrylate (EGDMA), ethyl butyrate, Dulbecco's phosphate buffered saline (PBS), acetonitrile and polyoxyethylene (10) oleyl ether (Brij 97) were purchased from Sigma–Aldrich Chemicals (St. Louis, MO). 2,4,6-Trimethylbenzoyl-diphenyl-phophineoxide (TPO) was kindly provided by Ciba (Tarrytown, NY). CyA was purchased from LC Laboratories (Woburg, MA). All the chemicals were reagent grade. Acetonitrile was filtered before use and all the other chemicals were used without further purification.

2.2. Methods

2.2.1. Microemulsion formulation

The surfactant solution was prepared by adding the Brij 97 surfactant to de-ionized (DI) water in the required ratio and then stirring the mixture at 600 rpm and at room temperature for a period of about 10 h. Specifically, 1, 1.5, and 2 g of Brij 97 was dissolved in 10 ml DI water to prepare three different surfactant solutions (named M1, M2, and M3, respectively). Separately, 0.4 g of CyA was dissolved in 5 ml of ethyl butyrate to prepare the drug loaded oil phase. Next, 100 μl of the drug loaded oil was added to 5 ml of the surfactant solution, and the mixture was then stirred at 600 rpm (70 °C) for 20 min. The solution was then cooled to room temperature, resulting in formation of a clear microemulsion. Microemulsions without the drug were synthesized by eliminating CyA in the formulation described above.

2.2.2. Particle size analysis

The particle sizes for microemulsions were measured using a Precision Detectors PDDLS/CoolBatch+90T instrument. The data was analyzed with the Precision Deconvolve32 Program. The measurements were obtained at 20 °C and 90° scattering angle, using a 683 nm laser source.

2.2.3. Preparation of microemulsion-laden gels

The microemulsion-laden p-HEMA hydrogels were prepared by free radical solution polymerization with UV initiation. Specifically, 2.7 ml of HEMA monomer was mixed with 15 μl of the crosslinker (EGDMA) and 2 ml of the CyA containing microemulsion. The solution was then degassed by bubbling nitrogen for 10 min. Next, 6 mg of the initiator (TPO) was added, and the solution was stirred at 300 rpm for 10 min to ensure complete solubililization of the initiator. The solution was then poured in a mold that comprised two glass plates separated by a 200 μm (thick gels) or 100 μm (thin gels) thick spacer. The mold was placed on Ultraviolet transilluminiator UVB-10 (Ultra·Lum, Inc.) and the gel was cured by irradiating UVB light (305 nm) for 40 min. The gels loaded with microemulsions M1, M2 and M3 are named M1, M2 and M3 gels, respectively. Microemulsions without any drug were incorporated in the polymerizing mixture for synthesizing gels with no drug and the synthesis protocol was same as described above.

2.2.4. Preparation of surfactant-laden gels

The surfactant solution was prepared as described earlier. Specifically, 0.2, 0.6, 1.5 g of Brij 97 surfactant was dissolved in 10 ml DI water to make three different surfactant solutions (named S1, S2, and S3, respectively). Separately, 3.5 mg of CyA was dissolved in 2.7 ml of HEMA monomer and stirred at 600 rpm for a period of 5 h. Next 15 μl of the crosslinker and 2 ml of surfactant solution were added to the 2.7 ml of drug loaded monomer. The hydrogels were then prepared by adding the mixture to the molds followed by UV curing, as described above. Control, drug loaded p-HEMA gels without surfactants (D1) were prepared by following procedures identical to those described above except that the 2 ml surfactant solution was replaced by 2 ml DI water. Also, surfactant-laden gels without any drug were synthesized in a similar manner as above by not incorporating drug in the monomer mixture.

2.2.5. CyA detection by HPLC

CyA concentration was measured using a HPLC (Waters, Alliance System) equipped with a C_{18} reverse phase column and a UV detector (Kim et al., 1997). The mobile phase composition was 70% acetonitrile and 30% DI water, and the column was maintained at 60 °C. The flow rate was fixed at 1.2 ml/min and the detection wavelength was set at 210 nm. The retention time for CyA under these conditions was 4.55 min, and the calibration curve for area under the peak vs. concentration was linear ($R^2 = 0.995$).

2.2.6. Drug release kinetics from gels loaded with CyA by drug addition to the monomer

After polymerization, each gel was removed from the glass mold and was cut into smaller pieces that were about 1.5 cm × 1.5 cm (for thick gels) and 1.5 cm × 3 cm (for thin gels) in size and about 40 mg in weight. Drug release kinetics was measured by soaking the gel in 3.5 ml PBS buffer which was replaced every 24 h and all the measurements were done at room temperature. The volume of the release medium was chosen to be 3.5 ml to approximately match the *in vivo* conditions of tear turnover. Additionally, some experiments were conducted without PBS replacement till the gel and the release medium equilibrated. These experiments were conducted to explore the rate limiting step in the drug transport by conducting drug release from gels with two different thicknesses. The dynamic drug concentrations in the release medium were measured for both sets of the drug release experiments by HPLC method described above. The injection volume in HPLC was set as 20 μl which was

significantly lower than the total fluid volume (3.5 ml) to ensure negligible volume changes during the equilibrium experiments.

2.2.7. Drug uptake and release kinetics from gels loaded with CyA after polymerization

In the release protocol described above, CyA was loaded in the hydrogels by dissolving it into the oil phase of the microemulsion. It is conceivable that the process of gel formation may lead to partial loss of drug activity and some irreversible entrapment of the drug. To eliminate the possible loss of activity due to the polymerization process, it was decided to conduct experiments in which the microemulsions (without drug) were entrapped in the gel, and the drug was loaded by soaking the gels into aqueous drug solutions. Specifically, drug was loaded by soaking the gels, about 40 mg in weight, in 4 ml of 11.5 μg/ml drug solution. To determine the time needed for uptake of drug by the microemulsion-laden gels, the duration of soaking period was varied between 5 and 15 days. The release kinetics was subsequently measured by following the same protocols as described in the previous section and these results are discussed in Section 3.2.

2.2.8. Packaging solution for drug release

To explore the effect of packaging solution on drug release from hydrogels containing drug incorporated inside the microemulsions, it was decided to soak the drug containing microemulsion-laden gels (M2) in 1.5 ml of a packaging medium for specific durations and then measure drug release kinetics from these gels. The duration of soaking in packaging solutions was varied from 10 to 100 days to evaluate the effect of storage on these hydrogels. Also, three different compositions of packaging solutions were explored. The first packaging medium was simply DI water, and the second and the third were 0.85% and 4.25% (w/w) salt solutions, respectively. The salt concentration of 0.85% (0.14 M) was chosen to match the typical concentration in commercial packaging solution (Lum et al., 2004), and higher (4.25%) and lower (DI water) salt concentration was used to observe the effect of salt on equilibrium amount of CyA released in the packaging solution. Drug release from these gels after packaging period were carried out in 3.5 ml of PBS with daily PBS replacement as described earlier, and release kinetics from gels used for packaging studies are discussed in Section 3.3.

2.2.9. Processing conditions in contact lens manufacturing

To evaluate the suitability of the Brij 97 microemulsion and surfactant-laden gels as contact lenses, it was decided to subject these gels through processing conditions similar to those used in contact lens manufacturing. The gels were first subjected to an extraction stage in which the unreacted monomer was extracted from the gels by soaking gels in 10 ml of DI water at 50 °C. The DI water was replaced every 5 min, and this step was repeated 5 times. After extraction, each gel was soaked in 4 ml of drug solution (12 μg/ml) for a period of 12 days. Each gel was then soaked in 1.5 ml of DI water and autoclaved for 15 min at 121 °C followed by storage at room temperature for a period of 10 days. In the final step, each gel was submerged in 3.5 ml of PBS, which was replaced every 24 h, and the concentration of the drug was measured to determine the release kinetics. The results from this study are discussed in Section 3.6.

2.2.10. Surfactant release

To measure the surfactant release from the gels, surfactant-laden gel samples were soaked in 3.5 ml of DI water. The DI water was replaced after regular intervals and the surface tension of the solution was measured by the Wilhelmy plate method to determine the concentration of Brij 97. We used a sand blasted platinum plate attached to a Scaime France Microbalance which was further connected to a Stathan Universal transducer (SC001). The transducer was calibrated by using DI water (σ = 72 mN/m) and acetone (σ = 23 mN/m) as standards. To ensure complete removal of impurities, the platinum plate was rinsed with DI water and acetone, followed by annealing till red hot using a propane burner before each measurement. For each measurement, the solution was kept still for a period of 1 h to ensure an equilibrium surface coverage of surfactant at the air–liquid interface.

2.2.11. Statistical analysis

Linear regression analysis to determine slopes, correlation coefficients and confidence intervals was performed in JMP developed by SAS (Cary, NC). The 95% confidence intervals (CI) were obtained to compare release kinetics.

3. Results and discussion

3.1. Particle size analysis of microemulsions and drug release from microemulsion-laden gels

Fig. 1 plots the particle size distributions for the three microemulsions that were explored in this study. These microemulsions have mean particle sizes ranging from 10 to 13 nm, which is typical for microemulsions. The mean particle size increased with a reduction in surfactant loading, which was expected. The drug release profiles (with PBS change every 24 h) from control p-HEMA gels and gels loaded with microemulsions are compared in Fig. 2. The amount of surfactant in the three systems was 5.6%, 8% and 9.4% (w/dry gel w) for M1, M2 and M3, respectively. The CyA release from p-HEMA gels lasted only about 6–7 days but the microemulsion-laden gels released drug for about 20 days. The results in Fig. 2 clearly demonstrate a significant reduction in delivery rate and a concurrent increase in the duration of release on addition of microemulsions to the gels. We speculate that since CyA is a hydrophobic molecule, it preferentially partitions into the oil phase of the microemulsions, leading to a reduction in the free drug concentration, and thus a reduction in the drug flux. It is also possible that during the gel preparation and subsequent hydration, some surfactant molecules desorb from the oil drops and then aggregate in the gel pores to form micelles. The presence of micelles is also expected to retard drug transport as discussed later in detail.

The short time release from a hydrogel can be described by the following equation (Ritger and Peppas, 1987)

$$\%\text{Drug Release} = 4\sqrt{\frac{Dt}{\pi h^2}} \times 100 \quad (1)$$

This equation is valid for short times when the released drug percentage is less than 60%. Here, '*D*' is the effective diffusivity of the

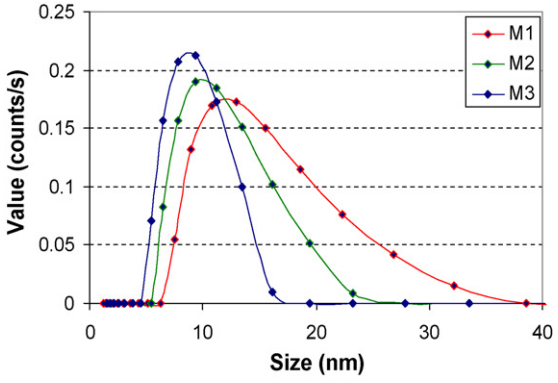

Fig. 1. Size distribution of microemulsions with three different surfactant loadings.

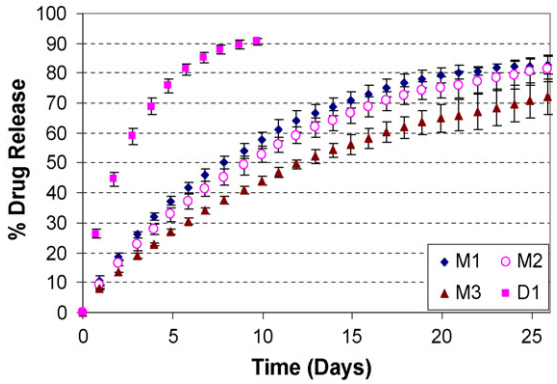

Fig. 2. Cumulative percentage release of drug from microemulsion-laden gels with varying surfactant loading and pure p-HEMA gels. All the gels were 200 μm thick in dry state and gels M1, M2, M3 and D1 contained 48.5, 52.2, 53.4 and 53.3 μg of drug, respectively. Data are plotted as mean ± S.D. for M2, D1 gels ($n = 3$). The error bars for M1 and M3 systems represent half the difference between the data from two repeat runs.

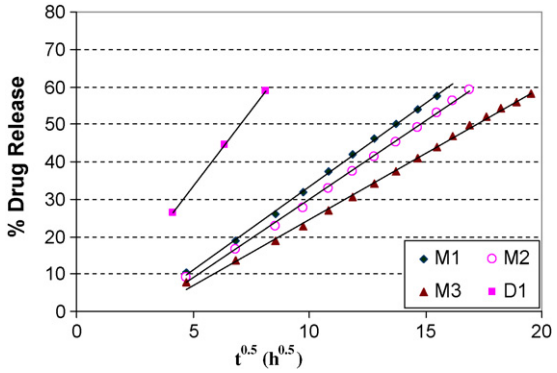

Fig. 3. Linear fits for the short time release data to obtain the effective diffusivity for microemulsion and pure p-HEMA gels.

drug, 'h' is the thickness of the gel, and 't' is the release time. The release data shown in Fig. 2 was fitted to the above equation to determine the effective diffusivity of the drug for the control p-HEMA gel and the microemulsion-laden gels. Based on the above equation, a plot of cumulative release vs. $\sqrt{t}$ must be a straight line. The fit between the data and the model is shown in Fig. 3, and the values of the slopes along with the 95% CI are listed in Table 1. For clarity, only average values for each system are plotted while the fitted line is evaluated by using all the data points for each system. The slopes were then utilized to determine the effective diffusivities, which are also listed in Table 1. We observe that the drug release from the microemulsion-laden systems depends on surfactant loading, and effective diffusivity decreases with increasing surfactant loading. It is noted that the cumulative release profiles for the microemulsion-laden gels are linear at short times but they intercept the x-axis at about $\sqrt{t} = 2.9$, implying $t \sim 8.4$ h. This suggests that at very short times the drug transport rates are much smaller than those predicted by diffusion mechanism, leading to

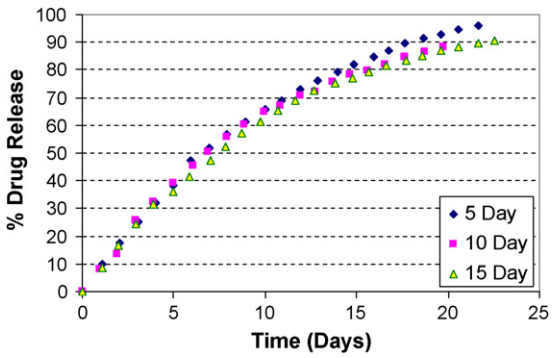

Fig. 4. Cumulative percentage release of drug from microemulsion gels after loading the drug into gels by soaking in a drug solution for 5, 10 and 15 days.

a delay in release. The delay in drug release could potentially be caused due to the time needed to hydrate the interfacial region of the microemulsions. A similar phenomenon is observed with surfactant-laden gels (see Section 3.4) which supports the hypothesis of delay caused by hydration of micelles or microemulsion interface.

3.2. Release of drug after soaking microemulsion-laden gels in drug solution

As explained in Section 2.2.7, in some instances, drug was loaded into the gels after polymerization by soaking them in drug solution. After the soaking phase, the gels were withdrawn and the concentration of drug in the aqueous phase was measured. The mass of drug taken up by the gels was determined by subtracting the mass of drug left in the solution from the initial mass of drug in the soaking solution. The systems explored here had 8% surfactant in the dry gel (w/dry gel w), and Table 2 lists the amounts of drug that was taken up by these gels for the different soaking durations. The results in Table 2 show that the mass of the drug taken up by the gels is relatively similar for all three gels. This shows that 5 days of soaking time is sufficient to establish equilibrium. The drug release profiles shown in Fig. 4 are within 95% CI of each other (CI not shown in the plot), which is expected because each gel adsorbed similar amount of drug. These results also show that the duration of drug release for the systems in which the drug is loaded by soaking in the drug solution is similar to the systems in which the drug is entrapped in the microemulsions before polymerization, which suggests that the surfactant loss during the drug loading step was negligible.

3.3. Effect of packaging conditions on drug release

At the end of the packaging phase described in Section 2.2.8, the gels were withdrawn and the concentration of drug in the liquid was measured to determine the amount of drug that diffused out during this step (Table 3). The amount of drug that diffuses out of the gel during storage is less in salt solutions because CyA is a hydrophobic drug and so increasing ionic strength reduces drug solubility. Also, the amount of drug released into the packaging

Table 1
Diffusion coefficients of the drug for the microemulsion-laden systems

System	Slope	95% CI for slope	$D \times 10^{15}$ m^2/s	R^2
M1	4.47	(4.30, 4.64)	4.36	0.991
M2	4.12	(3.98, 4.26)	3.70	0.982
M3	3.52	(3.41, 3.62)	2.70	0.992
D1	8.12	(7.46, 8.78)	14.4	1.000

Table 2
Drug uptake by microemulsion-laden gels M2 after soaking in drug solution

X days	Amount of CyA initially in solution (μg)	Amount of CyA left in solution after *X* days (μg)	Amount of CyA loaded in the gel (μg)
5	46	16.2	29.8
10	46	15.1	30.9
15	46	15.2	30.8

Table 3
Drug released in the packaging medium from microemulsion-laden gels M2

	X Days	Drug inside the gel before packaging (μg)	Drug released in packaging (μg)	Drug left in the gel after packaging (μg)
Solution I (DI water)	10	44.2	7.1	37.1
	30	49.8	9.7	40.1
	100	46.9	17.6	29.3
Solution II (0.85%, w/w salt solution)	10	49.8	6.7	43.1
	30	47.7	10.3	37.4
	100	47.9	16.6	31.3
Solution III (4.25%, w/w salt solution)	10	52.3	3.3	48
	30	47.9	5.6	42.3
	100	52.6	9.6	43

solution is largest for the 100 days soak period which shows that the equilibration time for drug release from these gels is at least more than 30 days.

After the end of the storage phase, the gels were withdrawn and drug release experiments were conducted as described earlier. At short times, all the drug release profiles in Fig. 5 are within 95% CI except the 100 days soaking in 0.85% salt solution. Also the release duration from these systems is comparable to that from gels that were not subjected to packaging (Fig. 4). These results demonstrate that the drug release profiles are relatively unaffected by soaking in packaging solution, and also by the composition of the packaging solution.

The results shown above are encouraging but the microemulsion-laden gels also have several drawbacks. Firstly, preparation of microemulsion-laden gels is a two-step process, which renders it cumbersome. Secondly, although the oil phase of the microemulsion is only slightly soluble in tears, it will likely elude at a slow rate, and thus could potentially cause ocular toxicity. Ethyl butyrate is food grade oil, found suitable for *in vivo* applications (Warisnoicharoen et al., 2000; Morey et al., 2004), but to our knowledge ocular toxicity of ethyl butyrate has not been investigated. To avoid potential ocular toxicity due to oil and to simplify the gel preparation, it was desirable to replace the microemulsions with micelles which may also impede drug release leading to extended drug delivery. To test this hypothesis, surfactant-laden gels were fabricated, and drug release studies from these systems are described below.

3.4. Drug release from micelle laden hydrogels

Fig. 6 shows the drug release profile for S3 gels, i.e., gels loaded with 8% surfactant (w/dry gel w) with daily PBS change. The drug release profiles from microemulsion-laden gels (M2) with similar surfactant loading are included in the figure for comparison. The results show that surfactant-laden gels also provide extended drug release lasting more than 20 days. The release rates of the microemulsion-laden gels are less than that for the surfactant-laden gels with 8% suractant loading suggesting that the presence of oil further slows down drug transport. The effective diffusivity of the drug from the S3 gels was obtained by fitting the short time data to Eq. (1) (Fig. 7). The fitted diffusivity is 4.34×10^{-15} m^2/s, which is more than that for the M2 gels within 95% CI. We speculate that the surfactant-laden gels contain micelles and the drug preferentially partitions into the hydrophobic core of these micelles. The reported values of the critical micelle concentration (CMC) of Brij 97 range from 0.217 mM (Klammt et al., 2005) to 0.94 mM (Hait and Moulik, 2001). The hydrated p-HEMA gels absorb about 40%

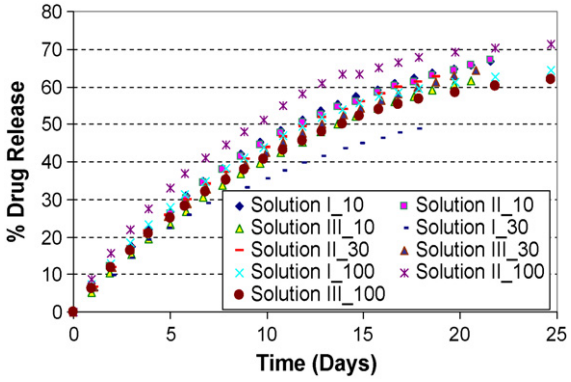

Fig. 5. Cumulative percentage release of drug from microemulsion-laden gels after packaging in three different solutions for different durations of time. Solution I ≡ DI water; Solution II ≡ 0.85% salt solution; Solution III ≡ 4.25% salt solution. All the gels were 200 μm thick in dry state.

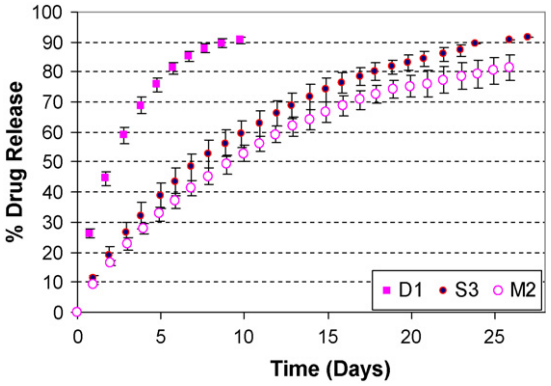

Fig. 6. Cumulative percentage release of drug from Brij 97 surfactant laden, microemulsion-laden and pure p-HEMA gels. All the gels were 200 μm thick in dry state and gels S3, M2 and D1 contained 49.2, 52.2 and 53.3 μg of drug, respectively. Data are plotted as mean ± S.D. (n = 3).

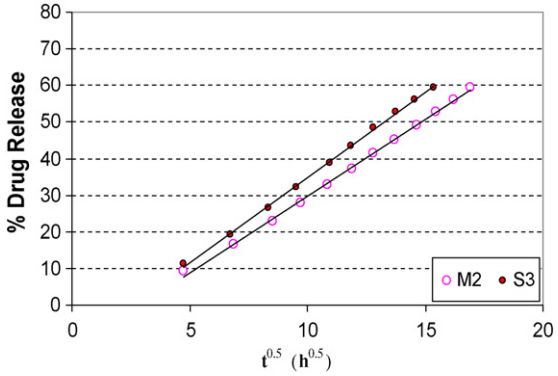

Fig. 7. Linear fits for the short time release data to obtain the effective diffusivity for surfactant-laden gels.

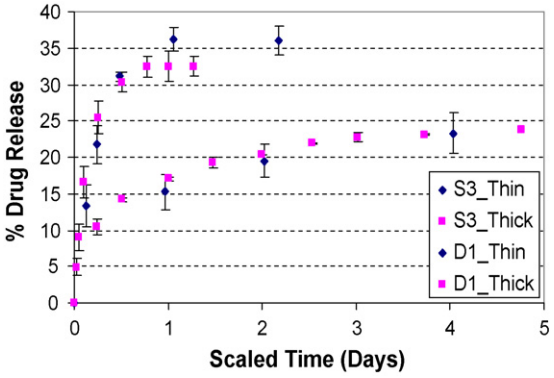

Fig. 8. Effect of thickness on percentage release for p-HEMA gels and surfactant-laden gels for equilibrium experiments. S3_Thin, S3_Thick, D1_Thin and D1_Thick contained 49.2, 52.4, 56.2 and 57.7 μg of drug, respectively. Data are plotted as mean ± S.D. ($n = 3$).

water (w/w). Based on these values, if the surfactant loading in a gel exceeds 0.061–0.27% (w/dry gel w), its concentration in the hydrated state is expected to be above the CMC. It is noted that this estimation neglects binding of surfactant to the gel, which is likely because the gel has some hydrophobic sites to which the surfactants are expected to adsorb. Also, the shapes of these micellar aggregates may be complex due to the confining effects of the gel. The cumulative release profile for surfactant system intercepts the x-axis at $\sqrt{t} = 2.5$, which lies within 95% CI of the intercepts for the microemulsion-laden gels (2.9 ± 0.6), supporting the hypothesis that the initial delay in the drug release is caused due to hydration of surfactant aggregates.

3.5. Mechanism of drug release

The drug release from the surfactant-laden gels could be controlled by two potential mechanisms: transport of the drug from inside the micelles trapped in the gel to the bulk gel, or diffusion through the gel. The linear relationship between cumulative release and $\sqrt{t}$ suggests that the transport is controlled by diffusion through the gel. If the release is controlled by diffusion, the release time scales as (thickness)^2, and if the release is controlled by transport from inside the micelles to outside, the release time should be independent of thickness.

To determine the rate limiting step, drug release profiles from 100 μm thick gels were compared with those from the 200 μm thick gels. These were equilibrium studies in which PBS was not replaced and the system was allowed to equilibrate. It is noted that the weights of both the thick and thin gels were about same because the cross sectional area of the thin gel was double that of the thick gel. The results from these studies are shown in Fig. 8, where the percentage release of drug is plotted as a function of scaled time, where

$$\text{Scaled Time} = \text{Time} \times \left(\frac{100}{h\,(\text{in}\,\mu\text{m})}\right)^2 \quad (2)$$

Here, 'h' represents the thickness of the hydrogel. To compare the profiles shown in Fig. 8, we computed the relative error defined as the ratio of the difference in cumulative percentage release between the thin and the thick gels and the cumulative release for the thick gels. We observe that the percentage release vs. scaled time profiles for the thin and the thick pure p-HEMA gels and also for the thick and the thin S3 gels are similar with root mean square of the relative error being 13.5% and 5.7% for the p-HEMA and the S3 gels, proving that diffusion through the gel matrix is the rate controlling step.

3.6. Processing conditions

S1, S2, S3, M2 and D1 gels were prepared by following procedures described earlier, except that the drug was not loaded in these gels. These gels were about 100 μm thick and weighed about 40 mg. After monomer extraction described earlier, drug was loaded in these gels by soaking in drug solution and the amount of drug loaded into the gel was determined by calculating the difference between the initial and the final concentrations in the drug solution. The results for the amount of drug loaded into these gels are shown in Table 4.

After autoclaving and 10 days storage in 1.5 ml DI water, the concentration in the aqueous phase was measured to determine the amount of drug that was released from the gel during the autoclaving and storage period. By subtracting this amount from the amount of drug taken up by the gel, amount of drug retained by the gel was determined. These results are also shown in Table 4. Each gel released about 25% of the entrapped drug into the solution during autoclaving and packaging.

The drug release profiles for the cumulative release as a function of time are plotted in Fig. 9 and the drug amounts released are noted in Table 4. We observe that each gel releases almost 100% of the entrapped drug. A 100% release along with the fact that the elusion spectra of the drug from the HPLC column (absorbance vs. time) is not altered by autoclaving (data not shown) suggests that the drug does not degrade during processing. The duration of

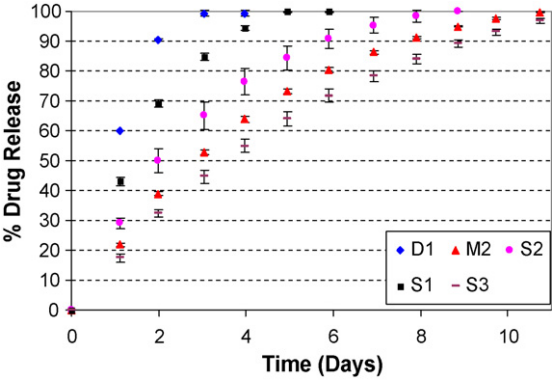

Fig. 9. Effect of surfactant loading and processing conditions on cumulative percentage release from pure p-HEMA, the microemulsion-laden and surfactant-laden gels. All the gels were 100 μm in thickness. The error bars represent half the difference between the data from two repeat runs.

Table 4
Drug loading and release from surfactant-laden and microemulsion-laden gels subjected to processing conditions. The error bars represent half the difference between the data from two repeat runs

System	Drug in solution initially (μg)	Drug remaining in the solution after 12 days of soaking (μg)	Amount of drug inside the gel (μg)	Amount of drug released during storage (μg)	Amount of drug retained in the gel (μg)	Amount of drug released during experiments (μg)
S3	48	18.20 ± 1.1	29.80 ± 1.1	5.45 ± 0.35	24.35 ± 1.50	22.10 ± 0.02
S2	48	25.00 ± 2.9	23.00 ± 2.9	4.45 ± 0.07	18.55 ± 2.90	20.00 ± 1.13
S1	48	23.15 ± 0.6	24.85 ± 0.6	6.95 ± 0.07	17.90 ± 0.56	14.60 ± 0.99
M2	48	22.45 ± 0.6	25.55 ± 0.6	6.50 ± 0.14	19.05 ± 0.50	19.45 ± 0.07

drug release from the surfactant and microemulsion-laden gels is much longer than that for the pure p-HEMA gels, which shows that the processing steps, particularly autoclaving do not cause significant changes in the gel structure. We also believe that a significant amount of surfactant diffuses out from these systems during processing and hence the release duration decreases due to processing conditions. Nevertheless, the systems with the higher surfactant loading release drug at the slower rate compared to p-HEMA gels, which suggests that even after processing steps there is enough surfactant in these systems to attenuate the drug release rates.

3.7. Surfactant release from p-HEMA hydrogels

Surfactant is likely to diffuse into the tear film after a surfactant-laden contact lens is placed on the eye. It was thus important to measure the rate of surfactant release from the gels. The rate of surfactant released was measured from surfactant-laden gels which contained 8% surfactant by weight in dry gel (S3). By performing control experiments, it was verified that other components in the gels were not surface active. During the surface tension measurement, the surface area created was small and so the change in bulk concentration due to surfactant adsorption at the surface was negligible. Firstly, relationship between the surface tension and the bulk concentration of Brij 97 was measured, and this was used as a calibration curve to later relate the measured surface tension to the bulk concentration of the surfactant in the release experiments (data not shown). To maximize the sensitivity of the measurements, the 3.5 ml solution was diluted by trial and error to surface tensions above 40 mN/m at which the surface tension is most sensitive to concentration (data not shown). The percentage releases of the surfactant from both thick and thin gels are plotted against $\theta \equiv \sqrt{t(100^2/h^2)}$ in Fig. 10 where '*h*' is the thickness of the hydrogel and 't' is time in hours. These curves when fitted with a straight line had a slope of 1.47 ± 0.08 (R^2 = 0.99) for the thick gels and 1.4 ± 0.23 (R^2 = 0.98) for thin gels, matching within a 95% CI. Effective diffusivity of the surfactant from these systems could then be calculated from Eq. (1) to be 11.8×10^{-17} m^2/s. The thin gels, which were about the same thickness as typical contact lenses, released about 48% of the surfactant in a period of 65 days. This corresponds to around 1500 μg of surfactant released in 65 days, or equivalently an average of 23 μg/day. Brij 97 surfactant has been previously explored as oral delivery vehicle (Warisnoicharoen et al., 2000; Agatonovic-Kustrin et al., 2003; Warisnoicharoen et al., 2003) but ocular toxicity of this surfactant has not been evaluated in literature. However, similar surfactants from the series of Brij surfactants (Brij 35, Brij 78, Brij 98) have been shown to be non-toxic at high concentrations on the corneal surface and have also been shown to be useful as cornea permeability enhancers. (Saettone et al., 1996; Lee et al., 1997; Furrer et al., 2002) Thus a slow release of surfactants from the lens could have the beneficial effect of increase in corneal permeability of the drug leading to increased bioavailability. Ocular toxicity of Brij 97 surfactant will be explored in future animal studies.

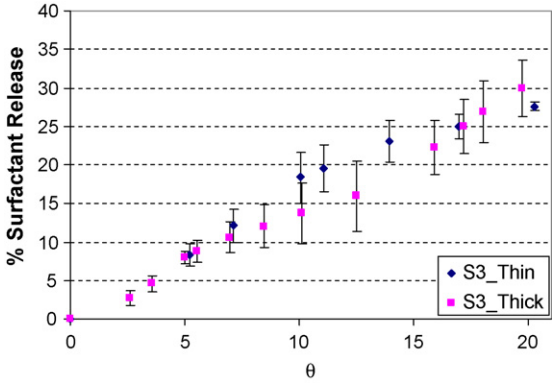

Fig. 10. Cumulative percentage release of surfactant from surfactant-laden gels. Thin gels contained 3245 μg of surfactant and thick gels contained 3344 μg of surfactant. Data are plotted as mean ± S.D. (n = 3).

4. Conclusion

This paper focused on exploring microemulsion and surfactant-laden hydrogels for extended delivery of CyA. We show that by using Brij 97 surfactant, both surfactant and microemulsion-laden gels exhibit slow and extended drug release lasting for about 20 days. This is a significant improvement compared to the control (pure p-HEMA gels), which releases drug for less than 7 days. The duration of drug release depends on the surfactant loading, and the rates of drug release are slightly smaller for microemulsion-laden gels compared to surfactant-laden gels with same surfactant loading.

The transport of CyA in the surfactant-laden gels is controlled by diffusion. The hydrated gels are expected to contain surfactant aggregates and CyA, which is a hydrophobic drug, partitions into the hydrophobic domains of these aggregates leading to an increase in partition coefficient resulting in slower transport rates from the gel.

These results are very encouraging and it seems that surfactant or microemulsion-laden gels may be suitable for delivering CyA to eyes. While these systems are promising, it is noted that p-HEMA lenses cannot be used for extended wear because of low oxygen permeability. Thus the surfactant-laden p-HEMA contact lenses will need to be taken off at night and cleaned to remove the protein and lipid deposits. The impact of these steps on CyA release needs to be assessed. Furthermore, the toxicity of these systems needs to be evaluated. These issues will be investigated in future studies.

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