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Zero-order therapeutic release from imprinted hydrogel contact lenses within *in vitro* physiological ocular tear flow

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Abstract

Zero-order or concentration independent release kinetics are highly desirable from drug delivery devices. In this paper we demonstrate experimentally, for the first time, zero-order release of a small molecular weight therapeutic, ketotifen fumarate (MW=425), from molecularly imprinted hydrogels used as therapeutic contact lenses. We performed dynamic, *in vitro* drug release studies from imprinted hydrogel contact lenses within a novel microfluidic device that simulates the volumetric flow rates, tear volume and tear composition of the eye. Imprinted gels with multiple functional monomers and complexation points to the drug demonstrated a significantly delayed release of drug compared to less functionalized systems. There were no statistical differences in experimentally determined equilibrium swollen polymer volume fractions, which correlate with molecular weight between crosslinks and mesh size of the gel. Under infinite sink conditions, imprinted contact lenses demonstrated Fickian (concentration dependent) release kinetics with diffusion coefficients ranging from 4.04×10^{-9} to 5.57×10^{-10} cm^2/s. The highest functionalized gel exhibited a diffusion coefficient averaging ten times smaller than less functionalized gels and released drug for over 5 days with 3 distinct rates of release. Under physiological volumetric flow rates, the release rate was constant for a duration of 3.5 days delivering a therapeutically relevant dosage and was fit to a power law model indicating zero-order release characteristics with $n=0.981\pm0.006$ ($r^2=0.997$). This work demonstrates the potential of micro/nanofluidic devices to determine physiological release rates and stresses the importance of matching local conditions to adequately characterize drug delivery devices. It also demonstrates the enormous potential for molecular imprinting to further tailor therapeutic release kinetics via the imprinting process.

Keywords: Biomimetic; Controlled release; Drug delivery; Microfluidics; Molecular imprinting

1. Introduction: topical administration of ocular therapeutics

The ocular bioavailability of drugs applied to the eye is typically very poor with less than 1–7% of the applied drug being absorbed [1–3], and the rest quickly lost to factors such as ocular protective mechanisms, nasolacrimal drainage, spillage from the eye, lacrimation and tear turnover, metabolic degradation, and non-productive adsorption/absorption. More efficacious ocular delivery rests on enhancing drug bioavailability by extending the residence time or duration of drugs on the eye surface and/or by increasing drug transport through ocular barriers such as the cornea, sclera, and conjunctiva. The low bioavailability of topical drugs can be overcome by administering higher than needed concentrations of drug multiple times daily. However patients typically administer highly variable [4–6] volumes of topical drops leading to a sub-optimal delivery with insufficient or potentially toxic amounts of drug delivered. Thus, there can be a significant variation in the concentration of drug present in the eye. Toxic peak and inadequate valley concentrations [7] could lead to ocular and systemic side effects, and serious complications including loss of vision, depending on the therapeutic window and the underlying pathophysiology.

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Despite the aforementioned issues, traditional ophthalmic delivery vehicles such as solutions, suspensions, and ointments account for 90% of commercially available formulations on the market today [8,1]. We believe there is a strong unmet need for more effective and non-invasive extended delivery of ocular therapeutics. After a brief overview of current non-invasive practices, in this paper we will show how therapeutic contact lenses developed in our lab address the issue and deliver a constant, zero-order dose of drug under *in vitro* ocular flow conditions.

1.1. Enhancing bioavailability by extending residence time

Drug bioavailability can be enhanced by improving retention of drug on the ocular surface thereby limiting loss by lacrimation, tear turnover, and drainage. Under normal lacrimation and blinking rate, the human eye has a tear volume that ranges from 7.0 to 30.0 μL with a tear turnover rate of 1.51 ± 0.58 μL/min normally, and 2.82 ± 1.45 μL/min after 6 h of contact lens wear [1,9], leading to a therapeutically relevant dose residence time of under 5 min with complete exchange of tear volume in approximately 14 min.

The efficacy of topical solutions has been only slightly improved by viscosity enhancers [10,11] and punctum plugs [12]. *In situ* forming polymers [13–15] and mucoadhesive systems [16] can be irritating and difficult to apply, with low capacity and adhesion and anchorage problems [17]. Ocular inserts can achieve a relatively stable or nearly constant extended release of drug [18–20], but have higher costs, occasionally expel from the eye [20], and have the potential for membrane rupture with a burst of drug being released [2].

More convenient, less invasive methods include drug transport from soft, hydrogel contact lenses. This has been a ubiquitous concept since the first patent and paper describing the formation of soft contact lenses [21,22], but a sufficient reservoir of drug for a therapeutically relevant effect is hard to attain and is one of the main reasons, along with poor control of drug release, that these types of systems have not been demonstrated clinically.

Work in the field of contact lens delivery by other researchers has included nanoparticulate [23] and liposomal laden lenses, ion exchange hydrogels [24], long chain molecule eluting hydrogels [25] and molecular imprinting methods [26], but the duration of release has been limited to most of the drug being delivered during *in vitro* and *in vivo* [27] experiments in less than 1 day. Researchers have demonstrated that molecular imprinting leads to under half of loaded drug being released in 3 days [28]. Nanoparticulate-laden lenses have shown promise — *in vitro* studies demonstrate half of loaded drug released in 3 days — but have decreased mechanical stability, reduced optical clarity, and longer production schemes. The above methods suffer from inadequate drug loading for long release times.

For molecularly imprinted systems, in which a macromolecular framework or memory for the drug is produced during polymer synthesis [29,30], it has been shown that the extension of release duration in weakly crosslinked systems has a strong dependence on the monomer to template (M/T) ratio [28] and the diversity and number of interactions at the recognition site [31]. Recently, our group has produced biomimetic hydrogel contact lenses for the enhanced loading and extended release of the anti-histamine, ketotifen fumarate [31,32]. Such lenses could be worn with comfort by allergic conjunctivitis patients [33]. Multiplicity of monomer–template interactions was achieved with four functional monomers chosen from an analysis of histamine ligand-binding pockets leading to significantly enhanced loading and an increased duration of release compared to less functionalized systems at a constant M/T ratio. The drawback of these studies is that release kinetics were accomplished using a conventional "infinite sink" framework, which does not match ocular *in vivo* flow conditions. In this paper, we examine the kinetics of drug release under conditions that match the physiological flow rates of the eye.

1.2. Extending therapeutic delivery by hydrogel design

Controlled drug release from hydrogels has been extensively studied for the past three decades. Hydrogels are insoluble, crosslinked polymer network structures composed of hydrophilic homo- or hetero-co-polymers, with the ability to absorb significant amounts of water and swell while retaining their shape. They are the material of choice for soft contact lenses for a number of reasons, including their optical quality (i.e., good transmission of visible light), high chemical and mechanical stability, manufacturability at reasonable cost, high oxygen transmissibility and biocompatibility [34]. The value of hydrogels in drug delivery comes from their ability to control the diffusion behavior of molecules through them [35,36].

In a Fickian model of release kinetics, the release rate of a drug from the delivery device is proportional to the concentration gradient between the drug source and the surroundings. In practical terms, this means that as the finite drug source is depleted, the rate of drug release decreases until there is no more drug in the gel, whereupon the rate is zero. A zero-order (i.e., independent of concentration) release rate is preferable because it would deliver medication at a constant rate for an extended time. The challenge is to use a finite drug source to achieve an extended zero-order release, and a number of strategies have been attempted in hydrogel drug delivery systems.

When a dry hydrogel is immersed in a favorable solvent, the hydrogel transitions in a moving front from an unperturbed (glassy) state to a solvated (rubbery) state with an increase in macromolecular mobility due to chain extension, and additional free volume for transport through the gel. For swelling-controlled hydrogels, if there is a constant rate of solvent front penetration which is much smaller than the drug diffusion rate in the swollen gel, a zero-order release arises [37]. Bioerodible and biodegradable hydrogel systems demonstrate zero-order release when the penetration front moves with a velocity similar to the outer eroding front [38]. Other methods that have led to zero-order release within monolithic systems include hydrogels with non-uniform drug distribution [39] or rate controlling-barriers on the surface such as higher crosslinked outer edges [40]. Multi-layers/geometries exploiting drug restrictive diffusion within non-monolithic devices have also been used.

Therapeutic contact lenses are swollen when inserted into the eye, and cannot exploit the solvation–transition rate to control drug delivery. The other strategies also have drawbacks and cannot be easily applied to produce contact lenses. We have

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recently demonstrated with molecular imprinting methods that one can also restrict and delay the transport of drug from the matrix via interaction of numerous functional groups with the template drug [31]. In biomimetic imprinting, monomers chosen to mimic residues in the drug's biological binding molecule are complexed non-covalently to the drug and crosslinked into a hydrogel matrix. The drug's heightened interaction with these residue pockets slows its release from the hydrogel, exploiting a programmable memory within the polymer chains [31] and not the free volume available for drug transport. This type of network formation — with a proper optimization of drug affinity relating to number and strength of functional monomer interactions, crosslinking structure, and mobility of polymer chains — has a strong potential to influence a number of hydrogel systems and add to the variables one can alter to control the release profile.

In efforts to understand the mechanisms behind release kinetics, various mathematical models of solvent penetration and solute release have been developed [37,41]. Typically, in both modeling and experimental work, infinite sink conditions are assumed and accumulation of drug in the solution surrounding the hydrogel is considered to be negligible. This is appropriate for the majority of studied systems but for ocular drug delivery, considering the small tear volume and flow rates encountered *in vivo*, it does not adequately describe drug release kinetics. In these types of physiological situations, it is imperative that microfluidic models be used to characterize the release profiles.

1.3. Microfluidic platforms for evaluating drug delivery devices

Microfluidic platforms, typically dealing with 10^{-9} to 10^{-19} L of small fluid amounts, interface engineering, chemistry, and biology for conducting experiments at very small scales [42,43]. For instance, solid-state silicon microchips can provide controlled release of single or multiple chemical substances on demand [44] or with multi-pulse drug release from resorbable matrices [45]. While there have been an increasing number of cases in the last few years of controlling drug release by the application of micro- and nanotechnology for drug administration, there has been very little to no use of micro- or nanofluidic platforms in the evaluation of drug release devices.

This paper describes the *in vitro* drug release kinetics of imprinted hydrogel contact lenses within physiological volumetric flow rates by developing and implementing a microfluidic chip. This has not been demonstrated to date with any other contact lens drug delivery systems. We hypothesized that the physiological flow model of drug release would show that the therapeutic lens, under flow conditions similar to those in the human eye, would increase the release time and may provide a more linear and sustained release profile.

2. Experimental

2.1. Materials and reagents

Acrylic acid (AA), acrylamide (AM), 2-hydroxyethylmethacrylate (HEMA), *N*-vinyl 2-pyrrolidone (NVP), azobisisobutyronitrile (AIBN) and ketotifen fumarate were purchased from Sigma-Aldrich (Milwaukee, WI). Polyethylene glycol (200) dimethacrylate (PEG200DMA) was purchased from Polysciences, Inc. (Warrington, PA). All chemicals were used as received. Polymer and copolymer networks were made using various mixtures of above monomers (e.g. poly(AA-co-AM-co-HEMA-co-PEG200DMA), poly(AA-co-HEMA-co-PEG200DMA), poly(AM-co-HEMA-co-PEG200DMA), poly (AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA)).

2.2. Synthesis of molecularly imprinted hydrogel networks

Hydrogels of differing compositions were synthesized in a temperature controlled, non-oxidative environment using free-radical UV photopolymerization, according to previously published procedures [31]. Polymer compositions consisted of 5 mol% crosslinking monomer and 95 mol% functional monomer (92 mol% of the backbone functional monomer, HEMA, and the balance 3 mol% as combinations of other functional monomers). The monomer to template ratio was optimized to achieve desired amount of drug loading. Photoinitiation was performed using AIBN and unincorporated initiator and functional monomers were washed out over several days with deionized water until they could no longer be detected by spectroscopic monitoring (UV–Vis/Fluorescence/Luminescence Spectrophotometer, Synergy model, BioTek Instruments, Inc., Vermont, USA). The lenses were then loaded with ketotifen fumarate via equilibrium binding studies as published earlier [31].

2.3. Dynamic therapeutic release studies

Kinetic release studies were conducted in artificial lacrimal 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 [46]). In the infinite sink studies, gels which had been reloaded with drug were placed in 30 mL of fluid which was continuously agitated with a Servodyne mixer (Cole Palmer Instrument Co.) at 120 rpm. Preliminary experiments were conducted to determine the amount of fluid needed to approximate infinite sink analysis by comparing release rates for a fixed amount of fluid versus release rates when refreshing the fluid at specific time intervals. Release of drug was monitored by spectroscopic monitoring at 268 nm by drawing 200 μL of fluid into 96-well Corning Costar UV-transparent microplate and placing in the BioTek spectrophotometer. Absorbances were recorded for three samples and averaged. Solutions were replaced after each reading. In the physiological flow studies, the drug-loaded disk was placed within the chamber of the microfluidic device. A KDS101 Infusion Pump from KD Scientific (Holliston, MA) injected lacrimal fluid into the chamber at 3 μL/min, while an outlet line removed fluid from the chamber at the same rate for collection at regular time intervals. Release of drug for two samples was monitored similarly to the infinite sink case.

2.4. Analysis of drug release from hydrogel lenses

We model the transport that arises when a swollen therapeutic lens loaded with ketotifen is placed in an artificial lacrimal fluid environment. Diffusion of drug from a thin

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hydrogel is approximated to a plane sheet. As in this case, for geometries with aspect ratios (exposed surface length/thickness) greater than 10, edge effects can be ignored and the problem approached as a one-dimensional process.

By using Fick's Law, the following partial differential equation, initial conditions, and boundary conditions can adequately describe one-dimensional planar solute release from a hydrogel [47]. A special case of this problem with $C_s=0$ exists with boundary conditions for only one face of a slab predicting fractional release for different proteins over the entire time course of release [48].

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{1}$$

$$t = 0 \quad C(x,t) = C_0 \tag{2}$$

$$t>0 \quad x = 0 \quad \frac{\partial C}{\partial x} = 0 \tag{3}$$

$$t>0 \quad x = \pm L \quad C = C_s \tag{4}$$

where C_0 represents the initial drug concentration (assumed to be uniform in the homogeneous gel), x represents the distance from the center of the sample to the surface, C_s represents the surface concentration, C is the concentration of the drug within the gel, D represents a diffusion coefficient independent of position and concentration, t is time, and 2 L is the thickness of the gel.

Using integral transform techniques, the solution of the PDE is given by,

$$\frac{C - C_0}{C_s - C_0} = 1 - \frac{4}{\pi}\sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} e^{\frac{-(2n+1)^2\pi^2 D}{4L^2}t} \cos\frac{(2n+1)\pi x}{2L} \tag{5}$$

If M_t is the total cumulative mass of therapeutic released at time t, and M_∞ is the total cumulative mass of therapeutic released at infinite time, M_t/M_∞ represents the fractional release of therapeutic with respect to the value at infinite time,

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} e^{\frac{-(2n+1)^2\pi^2 D}{4L^2}t} \tag{6}$$

The above expression is cumbersome to estimate a value of the diffusion coefficient, and hence, a solution is obtained in terms of error functions.

$$\frac{M_t}{M_\infty} = 4\left[\frac{Dt}{L^2}\right]^{\frac{1}{2}}\left[\frac{1}{\pi^{\frac{1}{2}}} + 2\sum_{n=1}^{\infty}(-1)^n ierfc\left(\frac{nL}{2\sqrt{Dt}}\right)\right] \tag{7}$$

The integral complementary error function vanishes for short times (as $t\to 0$), and an expression is obtained for short times of diffusion.

$$\frac{M_t}{M_\infty} = 4\left[\frac{Dt}{\pi L^2}\right]^{\frac{1}{2}} \tag{8}$$

For each polymer network, the fractional release of ketotifen fumarate versus ($t^{0.5}/L$) was plotted and the diffusion coefficient was calculated from the slope. The log of fractional release versus the log of time indicated how well the data matched the Fickian release profile coefficient ($n=0.5$).

For the microfluidic release studies, an empirical power law equation was used to determine the order of release:

$$\frac{M_t}{M_\infty} = kt^n \tag{9}$$

where the variables k and n are constants related to diffusion coefficients and the specific transport mechanism [49]. The equation can be easily linearized by plotting the log of fractional release versus the log of time, where the slope corresponds to the order of release and the y-intercept relates to the structural and diffusional characteristics. It is evident that when n has a value of one, the drug release is independent of concentration and time, corresponding to zero-order release. Eq. (9) is termed the power law model, with n equivalent to the diffusional exponent (e.g. for planar systems, $n=0.5$ describes Fickian behavior indicating diffusion controlled drug release; $n=1.0$ describes zero-order or case II transport; with $n-1$ corresponding to the order of release). In all the hydrogels studied in this work, the diameter (length) over thickness was much greater than 10, indicating slab geometry.

2.5. Microfluidic chip design and fabrication

The microfluidic chips were fabricated by soft lithography [50–52]. Masks defining microfluidic features were designed with AutoCAD 2006 (Auto Desk) and photoplotted on transparencies at the resolution of 4000/5000 dots per inch (DPI) through a commercial printing company (CAD/ART Services, Bandon, Oregon). The transparent masks were used to fabricate microstructures of photoresists on a four-inch silicon wafer with photolithography, an established method for semiconductor production. Two different photoresists, SU-8 2025 (Microchem Co., Newton, MA) for 50-μm thick fluidic channels and SU-8 2100 (Microchem Co.) for a 560-μm thick chamber, were used. For the third step, based on the microstructures on a silicon wafer, the microfluidic layer was made out of transparent silicone polymer, polydimethylsiloxane (PDMS). A drug-loaded lens was placed in the central chamber and the chip was sealed against a glass plate. To ensure reproducible flow rates and to limit non-specific adsorption to the device, each device had a lifetime of three-five runs or less, as determined by monitoring fluid leakage (e.g., the surface free energy decreases with time and the forces holding the chip and plate together weaken) and adsorption in separate experiments.

All the processes for manufacturing the devices, including the chip design, mold fabrication, and chip fabrication were carried out in the Alabama Micro-electronics Science and Technology Center (AMSTC) and Nano/Microfluidics Laboratory (Fig. 1).

The device is designed to mimic the flow rate of tears but does not fully reproduce other ocular conditions. While the device was operated at ambient temperature, ocular physiological temperature is 35 °C and will increase the diffusional transport. In the human eye, the mixing and flow of tears is complicated by the presence of contact lenses. The tear film is thinner and varies in thickness by evaporation between blinks and tear breakup. These

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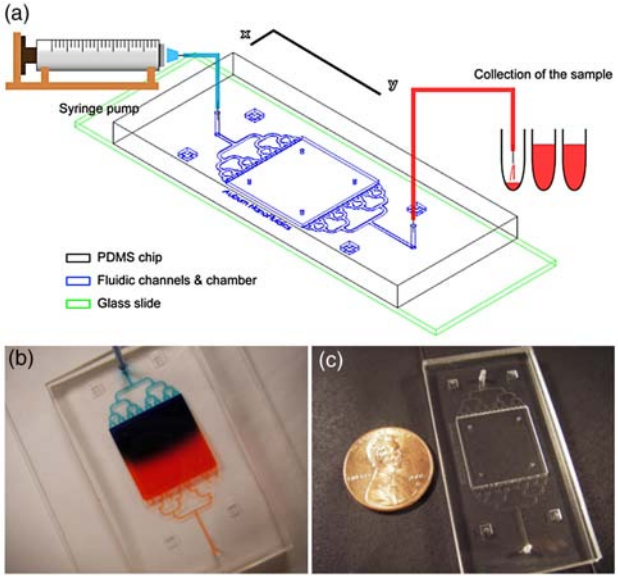

Fig. 1. Microfluidic chip design with physiological ocular flow. (a) Schematic of experimental set-up for contact lens drug delivery evaluation. The hydrogel is placed in the microfluidic chamber (chamber height — 560 μm, and width — 1600 μm) between the four posts and drug release is measured within artificial lacrimal fluid flow rates. (b) Example of microliter/nanoliter flow control and near-plug flow profile in the microfluidic chip (two different food dyes were used). (c) Length of scale.

factors may also affect drug release, but relative to tear flow rate these effects are small [1,53]. In future versions of the device, we plan to reproduce more of these conditions *in vitro*.

2.6. Dynamic weight/volume swelling studies and distribution coefficients

Imprinted and control gels were dried at room temperature for 24 h, followed by vacuum drying (T=30 °C, 28 in. Hg vacuum) until no change in dry weight was observed (i.e., less than 0.1 wt.% difference). Dry samples manufactured with and without drug (n=3) were placed in a constant volume of deionized water at 25 °C. The gels were weighed by removing the gels from the swelling media at specific time points and blotting with absorbent, lint-free tissue to remove excess surface solvent. When the samples reached equilibrium water uptake the weight swelling ratio at equilibrium (q_∞) (the weight of the swollen polymer divided by the weight of the dry polymer at equilibrium) was calculated.

The equilibrium volume swelling ratio, Q, was calculated as the ratio of the swollen gel volume at equilibrium to the volume of the dry polymer. The volume of the gel in the swollen or dry state was obtained by determining its weight in air and in *n*-heptane, a non-solvent for the polymer, and calculated using Archimedes buoyancy principle.

The distribution coefficients of the gels (calculated as the ratio of the drug concentration in the gel to the equilibrium drug concentration in solution) were obtained by immersing gels in ketotifen fumarate solution and obtaining the concentrations via mass balances.

Aqueous solubility was measured by saturating ketotifen fumarate in deionized water and stirring overnight. The solution was adjusted to pH 7 and filtered. The concentration was measured by absorbance at 268 nm against a series of ketotifen standards.

Log $P_{\text{octanol/water}}$ was calculated as the logarithm of the ratio of the equilibrium concentrations of ketotifen in octanol to ketotifen in water. A volume of 4 mL of a known concentration of aqueous ketotifen solution was shaken with 4 mL of octanol for 24 h and then let rest for 24 h. Concentration of ketotifen in octanol was obtained by mass balance.

3. Results and discussion

The release profiles of gels of various compositions were compared by plotting fractional drug released (M_t/M_∞) against time normalized to the square of the thicknesses of the gels (t/L^2) (Fig. 2(a)). Among the infinite sink dynamic release studies, the most structurally functional network, poly(AA-co-AM-co-NVP-co-HEMA-PEG200DMA), exhibited an extended release profile for a duration of over 5 days within artificial lacrimal solution (80% of drug was released in approximately 4 days) (Fig. 2(b)). This system also demonstrated the highest loading. Other less functionalized systems demonstrated controlled release for approximately 1 day. The distribution coefficients are 5.65, 7.13,

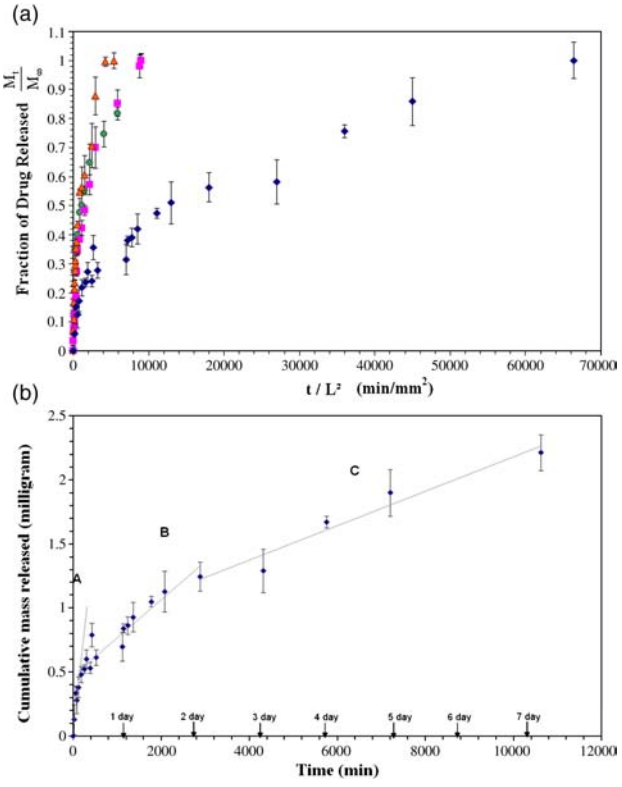

Fig. 2. Conventional infinite sink drug release from imprinted contact lenses. (a) Fractional release profiles of therapeutic contact lenses for poly(*n*-co-HEMA-co-PEG200DMA) networks in artificial lacrimal fluid at 25 °C, where *n* is AA (●), AM (■), AA-co-AM (▲) or AA-co-AM-co-NVP (◆) imprinted networks. The abscissa is time normalized to the square of thickness, as the thicknesses differed; 700 μm (■, ▲, ●) and 400 μm (◆) [31]. (n=3) (b) Cumulative mass released from the poly(AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA) lens with three phases of release. A: 0–120 min, B: 120 min to 2 days, and C: 2 to 7.3 days.

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Table 1
Varying release rates from AA–AM–NVP lenses under infinite sink conditions

Phase	Release rate (mg/min)	Duration of phase
A	3.04×10^{-3}	120 min
B	2.97×10^{-4}	120–2280 min (2 h to 2 days)
C	1.34×10^{-4}	2880–10,620 min (2 days to 7.3 days)

18.06 and 45.05 for poly(AA-co-HEMA-PEG200DMA), poly (AM-co-HEMA-PEG200DMA), poly(AA-co-AM-co-HEMA-PEG200DMA) networks and poly(AA-co-AM-co-NVP-HEMA-PEG200DMA) networks, respectively when the gels were placed in solutions of ketotifen fumarate in deionized water of concentration 0.4 mg/mL. Additionally, the bound concentrations of ketotifen within the gels are 5.1×10^{-3} mmol/g, 7.4×10^{-3} mmol/g, 1.7×10^{-2} mmol/g, and 4.9×10^{-2} mmol/g for poly(AA-co-HEMA-PEG200DMA), poly(AM-co-HEMA-PEG200DMA), poly(AA-co-AM-co-HEMA-PEG200DMA) networks and poly(AA-co-AM-co-NVP-HEMA-PEG200DMA) networks, respectively. Ketotifen fumarate is hydrophilic with $\log_{10}$ octanol–water distribution coefficient of −0.3, and an aqueous solubility of 3.4 mg/mL at pH 7±0.2 and 20 °C. This indicates that molecular imprinting and multiplicity of interactions have a greater influence on binding than general hydrophobic interactions. Fig. 2 (b) and Table 1 highlight the cumulative mass released from the poly(AA-co-AM-co-NVP-co-HEMA-PEG200DMA) lenses indicating 3 release rates of varying durations.

Within a Fickian diffusion process, the fractional mass released (M_t/M_∞) depends linearly on $t^{0.5}/L$ at short times or fractional release less than 0.67 with a slope directly proportional to the diffusion coefficient. Poly(AA-co-AM-co-NVP-co-HEMA-PEG200DMA) networks exhibited a ketotifen fumarate diffusion coefficient of 5.57×10^{-10} cm^2/s, which was a factor of 9, 7.2, and 13.8 less than poly(AA-co-HEMA-PEG200DMA), poly(AM-co-HEMA-PEG200DMA), and poly (AA-co-AM-co-HEMA-PEG200DMA) networks, respectively (Fig. 3, Table 2). These results show that therapeutic release can be tailored via the memory within the polymer chains, through the arrangement, type, and amount of functionality. This is significant considering all hydrogels exhibited equilibrium volume swelling ratios that were in close agreement with one another suggesting similar structures available for free volume transport (Table 2). Also, the monomers PEG200DMA and HEMA make up 97% of the feed monomers in each gel, and reaction analysis indicated that most of the double bonds within the systems reacted [31].

Because the hydrogels were produced without solvent, the Flory–Rehner equation [54] can be used with the experimentally derived equilibrium swollen polymer volume fraction to determine network structural parameters such as the molecular weight between adjacent crosslinks, and also the correlation length or network mesh size. Therefore the polymer volume fraction can be used as an indicator that correlates with structural parameters. Fig. 4 highlights the significant difference in the diffusion coefficient of the different gels as compared to the equilibrium polymer volume fraction in the swollen state. Once again, the equilibrium polymer volume fraction in the swollen state was not statistically different between all the gels.

In order to gauge the appropriateness of the fit to a Fickian mechanism, the log of the fractional drug release was plotted against the log of time. The exponents of all gels in the infinite sink release indicated that they were in agreement with a Fickian diffusion mechanism, where the values of n are approximately equal to 0.5 (Table 2).

The fractional release at physiological flow rates for the poly (AA-co-AM-co-NVP-co-HEMA-PEG200DMA) networks shows that under physiological flow, drug is released in a linear manner and at much lower concentrations than conventional infinite sink release studies suggested, indicating that such hydrogel lenses have the capacity to deliver sustained amounts of drug in a constant manner over an extended time period. Results demonstrate a slower release of drug with a constant, zero-order rate of release for approximately 3 1/2 days (i.e., independent of concentration or time). Fig. 5 shows the log of the fractional drug release plotted against the log of time for the infinite sink and physiological flow cases. For the physiological release case, using the empirical power law equation indicates the slope is equal to n, which is 0.981±0.006. Therefore, zero-order release is achieved by reducing the concentration gradient through the accumulation of ketotifen in the slow moving fluid at simulated physiological tear turnover rates. The importance of matching physiological flow is crucial to the characterization of this delivery system.

Compared to the infinite sink release profile, the cumulative mass released under physiological conditions is reduced by a large amount and is dependent on the volumetric flow rates. In 3.5 days, 45 μg is released at a constant rate of 12.9 μg/day compared to approximately 1200 μg in the infinite sink release study which shows decreasing rates of release. This is a decrease of a factor of 27 (Fig. 6). These lenses are about 3–4 times thicker than conventional contact lenses and normalizing for the difference in thickness, with fractional mass released being proportional to the inverse of the square of the thickness, yields 0.8 to 1.4 μg/day release. Conventional ketotifen topical drops deliver approximately 1–1.5 μg/day based on the recommended dosage regimen (assuming the maximum 7% bioavailability) within a typical topical peak and valley profile (i.e., Zaditor™ ophthalmic solution

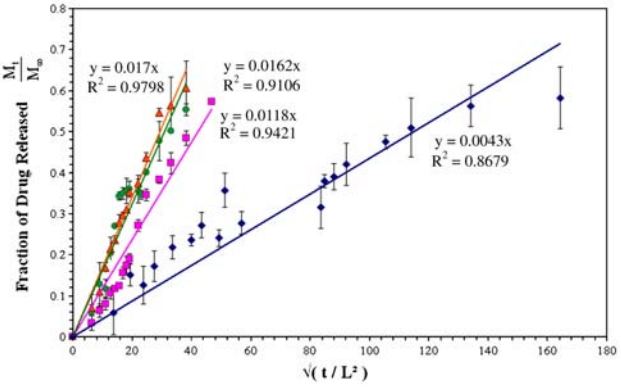

Fig. 3. Ketotifen fumarate diffusion coefficients from imprinted contact lenses. The diffusion coefficient of the drug from poly(*n*-co-HEMA-co-PEG200DMA) networks in artificial lacrimal fluid at 25 °C, where *n* is AA (●), AM (■), AA-co-AM (▲) or AA-co-AM-co-NVP (◆).

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Table 2
Summary of diffusion coefficients, power law exponents and swelling data

Network type (n)	Ketotifen diffusion coefficient ($\times 10^{10}$ cm^2/s)	Correlation coefficient of diffusion coefficient	Power law exponent (n)	Correlation coefficient of exponent	Q (Equilibrium volume swelling ratio)	υ2,s (Polymer volume fraction in the swollen state)
AA[1]	50.2±4.8	0.911	0.459±0.041	0.833	1.547±0.027	0.647±0.011
AM[1]	40.4±1.9	0.942	0.620±0.028	0.966	1.544±0.051	0.648±0.021
AA-co-AM[1]	77.3±3.5	0.980	0.521±0.025	0.965	1.513±0.094	0.661±0.041
AA-co-AM-co-NVP[1]	5.57±0.31	0.868	0.406±0.022	0.946	1.646±0.136	0.608±0.050
AA-co-AM-co-NVP[2]	N/A	N/A	0.981±0.006	0.997	1.646±0.136	0.608±0.050

Caption: Superscript [1] = infinite sink. [2] = microfluidic physiological flow.

concentration is 0.345 mg ketotifen fumarate/mL; assuming a typical eye drop volume of 20 μL per drop at the recommended frequency of administration of 2–3 drops per day yields 1–1.5 μg/day at 7% bioavailability). Therefore, there is strong potential to release therapeutically relevant concentrations of drug from a contact lens platform. However, it is important to note that these calculations should be taken as estimates since the lens release studies were conducted at a temperature of 25 °C and not 32 °C, the temperature of the eye. At physiological temperature, the release of drug will be faster from the lens [55].

This long, constant duration of release of a therapeutic dosage from a contact lens has not been demonstrated previously and is inherently linked to finite tear flow rates. Considering we know the total amount that is delivered from these lenses with the infinite sink study, we have delivered less than 5% of the loaded drug in 3.5 days in the physiological flow case. Thus the potential to deliver for extended time periods much greater than a week could be possible with a contact lens platform, depending on the tolerability of the lenses in the eye. A near zero-order release profile may be able to be maintained for a much greater amount of time, for at least a week.

These studies demonstrate that it is imperative to evaluate hydrogel release kinetics within finite turnover conditions if they are similar to the *in vivo* environment. The results clearly demonstrate that release from imprinted lenses is further delayed in an *in vitro* environment by matching the ocular volumetric flow rates. This effect may be due to two reasons — finite tear turnover rates which lead to significant boundary layers compared to the infinite sink case, and molecular imprinting strategies which lead to delayed release kinetics despite equivalent effective volume of transport through the polymer chains. Within the device, both lens surfaces are in contact with the flowing fluid and the lens rests on the bottom surface of the device. The average velocity in the device is 5.5×10^{-4} m/s considering a cross sectional area of 8.96×10^{5} μm^2 (560 μm height by 1600 μm width) and a volumetric flow rate of 3 μL/min. This translates to a Reynolds number of approximately 2–8 along the length of the lens (using physical properties of water at 25 °C), indicating laminar flow. The Peclet number (which is the Reynolds number multiplied by 7, the Prandtl number of water at 25 °C) ranges from approximately 14 to 56. The Peclet number indicates convection relative to molecular transport. Thus, it can be interpreted as the ratio of the characteristic velocity for convection to a characteristic velocity for diffusion. In our case, the convective time scale is approximately one order of magnitude higher than the molecular diffusion time scale indicating that neither is controlling and both are leading to the observed mass transfer. In the infinite sink case, the velocity is much higher across the lens (indicating a higher Peclet number) and boundary layers will be reduced in comparison to the microfluidic case.

The microfluidic release rate is lower and more constant compared to infinite sink conditions. Within infinite sink conditions there is sufficient fluid volumes producing a maximum concentration driving force and stirring which disrupts boundary layers. It is clear that boundary layer effects are important to the differences in release comparing the microfluidic and the infinite sink cases. It is premature to ascertain the effect of imprinting on these results, but the infinite sink case and polymer volume

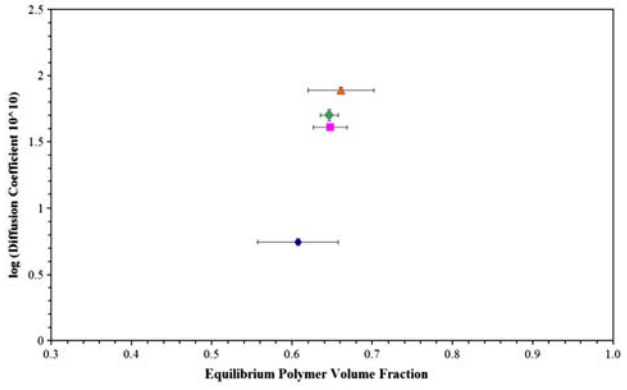

Fig. 4. Ketotifen fumarate diffusion coefficient versus the polymer volume fraction in the swollen state. Poly(n-co-HEMA-co-PEG200DMA) networks where n is AA (●), AM (■), AA-co-AM (▲) or AA-co-AM-co-NVP (◆).

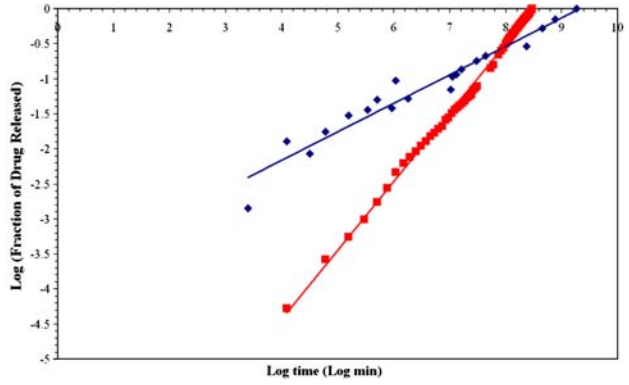

Fig. 5. Zero-order of release for imprinted lenses in physiological flow. The slope yields n, where $n-1$ is the order of release, for the infinite sink method (◆, $n=0.406\pm0.022$) and the physiological flow case utilizing the microfluidic device (■, $n=0.981\pm0.006$, zero-order) for poly(AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA) networks.

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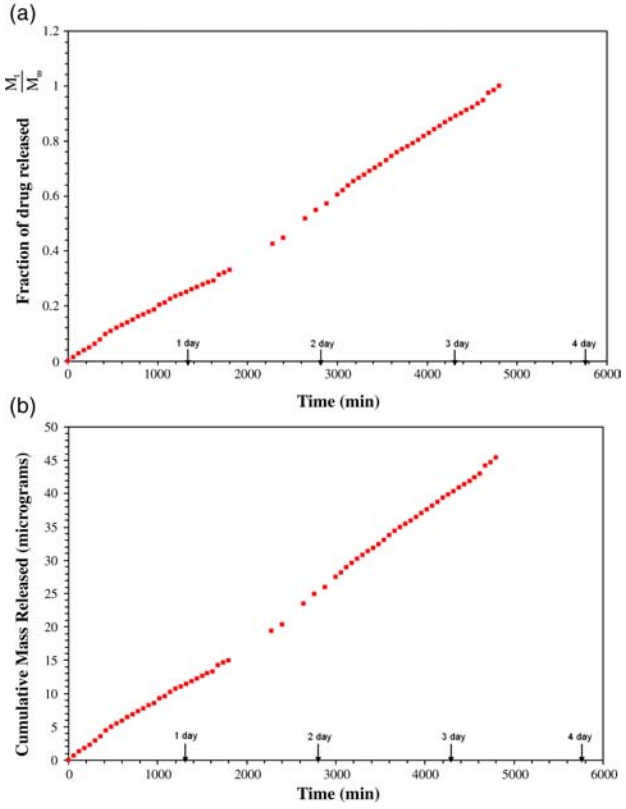

Fig. 6. Zero-order fractional and cumulative drug release from imprinted lenses in a physiological flow. (a) The fraction of ketotifen fumarate and (b) cumulative mass released from poly(AA-co-AM-co-NVP-co-HEMA-co-PEG200DMA) lenses in artificial lacrimal fluid at 25 °C via steady *in vitro* physiological flow rate of 3 μL/min using the microfluidic device (n=2).

fraction analysis highlights a potential mechanism for delayed transport via imprinting.

The mechanism of delayed transport due to imprinting is hypothesized to consist of multiple on–off binding interactions between ketotifen and the memory 'sites' consisting of multiple functionality within the network. Thus as ketotifen moves through the network, its transport is slowed down due to interactions with the polymer chains. Also, molecular imprinting strategies pull the technology into a clinically significant reality by enhancing the therapeutic loading, which is crucial for achieving therapeutically relevant delivery levels [31]. Further control of the tear flow rate with a partially non-wetted surface, mixing similar to the flow profiles induced by the lid blinking process, appropriate tear film, and addition of protein and lipid to the artificial lacrimal fluid are warranted for further study in the near future, and will ultimately confirm the high potential for drug releasing contact lenses. However, the results from the device, as presented within this paper, are a much better approximation to the actual ocular conditions than the infinite sink model.

4. Conclusion

Molecularly imprinted hydrogel contact lenses consisting of poly(n-co-HEMA-PEG200DMA) where n is a copolymer combination of AA, AM and/or NVP, were placed in infinite sink and physiological flow conditions to compare the ketotifen fumarate release kinetics from both protocols. The conventionally used infinite sink protocol indicated Fickian concentration dependent kinetics, but such a protocol is inadequate for modeling the small volume and flow conditions found in the eye. We demonstrated that under physiological ocular volumetric flow rates the release kinetics is zero-order, or concentration independent, for an extended time of at least 3.5 days. The imprinting process extends further control over the release kinetics independent of polymer swelling behavior, resulting in dramatic variations in diffusion coefficients.

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References

[1] R.D. Schoenwald, in: T.J. Zimmerman, K.S. Kooner, M. Sharir, R.D. Fechtner (Eds.), Textbook of Ocular Pharmacology, Lippincott-Raven, Philadelphia, 1997, pp. 119–138.
[2] M.F. Saettone, Progress and problems in ophthalmic drug delivery, Business Briefing: Pharmatech (2002) 1–6.
[3] D.H. Geroski, H.F. Edelhauser, Drug delivery for posterior segment eye disease, Invest. Ophthalmol. Vis. Sci. 41 (5) (2000) 961–964.
[4] E.J. German, M.A. Hurst, D. Wood, Eye drop container delivery: a source of response variation? Ophthalmic. Physiol. Opt. 17 (3) (1997) 196–204.
[5] Z. Sklubalova, Z. Zatloukal, Systematic study of factors affecting eye drop size and dosing variability, Pharmazie. 60 (12) (2005) 917–921.
[6] Z. Sklubalova, Z. Zatloukal, Study of eye drops dispensing and dose variability by using plastic dropper tips, Drug Dev. Ind. Pharm. 32 (2) (2006) 197–205.
[7] R. Langer, Drug delivery and targeting, Nature. 392 (6679 supp.) (1998) 5–10.
[8] J.C. Lang, Ocular drug delivery conventional ocular formulations, Adv. Drug Delivery Rev. 16 (1) (1995) 39–43.
[9] M.J. Glasson, F. Stapleton, L. Keay, M.D. Willcox, The effect of short term contact lens wear on the tear film and ocular surface characteristics of tolerant and intolerant wearers, Cont. Lens Anterior Eye. 29 (1) (2006) 41–47.
[10] O. Olejnik, Conventional systems in ophthalmic drug delivery, in: A.K. Mitra, M. Dekker (Eds.), Ophthalmic Drug Delivery Systems, Informa Health Care, New York, 1993, pp. 177–198.
[11] V.H.L. Lee, Mechanisms and facilitation of corneal drug penetration, J. Control. Release. 11 (1-3) (1990) 79–90.
[12] J.D. Bartlett, K. Boan, D. Corliss, I.B. Gaddie, Efficacy of silicone punctal plugs as adjuncts to topical pharmacotherapy of glaucoma — a pilot study. Punctal Plugs in Glaucoma Study Group, J. Am. Optom. Assoc. 67 (11) (1996) 664–668.
[13] H.R. Lin, K.C. Sung, Carbopol/pluronic phase change solutions for ophthalmic drug delivery, J. Control. Release. 69 (3) (2000) 379–388.
[14] G. Wei, H. Xu, P.T. Ding, S.M. Li, J.M. Zheng, Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies, J. Control. Release. 83 (1) (2002) 65–74.
[15] A. Rozier, C. Mazuel, J. Grove, B. Plazonnet, Gelrite: a novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol, Int. J. Pharm. 57 (2) (1989) 163–168.
[16] J.L. Greaves, C.G. Wilson, Treatment of diseases of the eye with mucoadhesive delivery systems, Adv. Drug Deliv. Rev. 11 (3) (1993) 349–383.

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[17] A. Ludwick, The use of mucoadhesive polymers in ocular drug delivery, Adv. Drug Deliv. Rev. 57 (11) (2005) 1595–1639.
[18] J. Urquhart, Development of the OCUSERT pilocarpine ocular therapeutic systems — a case history, in: J.R. Robinson (Ed.), Ophthalmic Delivery Systems, American Pharmaceutical Association, Academy of Pharmaceutical Science, Washington, D.C, 1980, pp. 105–118.
[19] M.F. Armaly, K.R. Rao, The effect of pilocarpine ocusert with different release rates on ocular pressure, Invest. Ophthalmol. 12 (7) (1973) 491–496.
[20] J.U. Prause, Treatment of keratoconjunctivitis sicca with Lacrisert, Scand. J. Rheumatol. Suppl. 61 (1986) 261–263.
[21] O. Wichterle, D. Lim, Hydrophilic gels for biological use, Nature. 185 (4706) (1960) 117–118.
[22] O. Wichterle, Cross-linked hydrophilic polymers and articles made therefrom, U.S. Patent 3,220,960, November 30, 1965.
[23] D. Gulsen, A. Chauhan, Dispersion of microemulsion drops in HEMA hydrogel: a potential ophthalmic drug delivery vehicle, Int. J. Pharm. 292 (1-2) (2005) 95–117.
[24] R. Uchida, T. Sato, H. Tanigawa, K. Uno, Azulene incorporation and release by hydrogel containing methacrylamide propyltrimenthylammonium chloride, and its application to soft contact lens, J. Control. Release. 92 (3) (2003) 259–264.
[25] L.C. Winterton, J.M. Lally, K.B. Sentell, L.L. Chapoy, The elution of poly (vinyl alcohol) from a contact lens: the realization of a time release moisturizing agent/artificial tear, J. Biomed. Mater. Res. B Appl. Biomater. 80 (2) (2007) 424–432.
[26] H. Hiratani, C. Alvarez-Lorenzo, The nature of backbone monomers determines the performance of imprinted soft contact lenses as timolol drug delivery systems, Biomat. 25 (6) (2003) 1105–1113.
[27] H. Hiratani, A. Fujiwara, Y. Tamiya, Y. Mizutani, C. Alvarez-Lorenzo, Ocular release of timolol from molecularly imprinted soft contact lenses, Biomat. 26 (11) (2005) 1293–1298.
[28] H. Hiratani, Y. Mizutani, C. Alvarez-Lorenzo, Controlling drug release from imprinted hydrogels by modifying the characteristics of the imprinted cavities, Macromol. Biosci. 5 (8) (2005) 728–733.
[29] G. Wulff, Molecular imprinting in cross-linked materials with the aid of molecular templates — a way towards artificial antibodies, Angew. Chem. Int. Ed. Engl. 34 (17) (1995) 1812–1832.
[30] J.Z. Hilt, M.E. Byrne, Configurational biomimesis in drug delivery: molecular imprinting of biologically significant molecules, Adv. Drug Delivery Rev. 56 (11) (2004) 1599–1620.
[31] S. Venkatesh, S.P. Sizemore, M.E. Byrne, Biomimetic hydrogels for enhanced loading and extended release of ocular therapeutics, Biomat. 28 (4) (2007) 717–724.
[32] S. Venkatesh, S.P. Sizemore, M.E. Byrne, Therapeutic contact lenses: a biomimetic approach towards tailored ophthalmic extended delivery, ACS PMSE Preprints 94 (2006) 766–767.
[33] V.Y. Hayesa, C.M. Schnidera, J. Veys, An evaluation of 1-day disposable contact lens wear in a population of allergy sufferers, Cont. Lens Anterior Eye. 26 (2) (2003) 85–89.
[34] M.F. Refojo, Ophthalmologic applications, in: B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons (Eds.), Biomaterials Science, Academic Press, San Diego, 1996, pp. 328–335.
[35] N.A. Peppas, Hydrogels in Medicine and Pharmacy, CRC Press, Boca Raton, FL, 1987.
[36] N.A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, Hydrogels in pharmaceutical formulations, Eur. J. Pharm. Biopharm. 50 (1) (2000) 27–46.
[37] C.S. Brazel, N.A. Peppas, Modeling of drug release from swellable polymers, Eur. J. Pharm. Biopharm. 49 (1) (2000) 47–58.
[38] K. Tahara, K. Yamamto, T. Nishihata, Overall mechanism behind matrix sustained release tablets prepared with hydroxypropyl methylcellulose 2910, J. Control. Release. 35 (1) (1995) 59–66.
[39] P.I. Lee, Novel approach to zero-order drug delivery via immobilized non-uniform drug distribution in glassy hydrogels, J. Pharm. Sci. 73 (10) (1984) 1344–1347.
[40] E.S. Lee, S.W. Kim, J.R. Cardinal, H. Jacobs, Drug release from hydrogel devices with rate controlling barriers, J. Membr. Sci. 7 (3) (1980) 293–303.
[41] C.C. Lin, A.T. Metters, Hydrogels in controlled release formulations: network design and mathematical modeling, Adv. Drug Deliv. Rev. 58 (12-13) (2006) 1379–1408.
[42] G.M. Whitesides, The origins and the future of microfluidics, Nature. 442 (27) (2006) 365–373.
[43] A. Manz, et al., Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip, J. Chromatogr. 593 (1-2) (1992) 253–258.
[44] J. Santini Jr., A. Richards, R. Scheidt, M. Cima, R. Langer, Microchips as controlled drug-delivery devices, Angew. Chem. Int. Ed. 39 (14) (2000) 2396–2407.
[45] A.C. Grayson, I.S. Choi, B.M. Tyler, P.P. Wang, H. Brem, M.J. Cima, R. Langer, Multi-pulse drug delivery from a resorbable polymeric microchip device, Nat. Mater. 2 (11) (2003) 767–772.
[46] N.J. Van Haeringen, Clinical biochemistry of tears, Surv. Ophthalmol. 26 (2) (1981) 84–96.
[47] J. Crank, The Mathematics of Diffusion, Oxford University Press, New York, 1975.
[48] R. Bawa, R.A. Siegel, B. Marasca, M. Karel, R. Langer, An explanation for the controlled release of macromolecules from polymers, J. Control. Release 1 (4) (1985) 259–267.
[49] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs, J. Control. Release, 5 (1) (1987) 23–36.
[50] J.W. Hong, S.R. Quake, Integrated nanoliter systems, Nature Biotechnol. 21 (10) (2003) 1179–1183.
[51] J.W. Hong, Y. Chen, N.F. Anderson, S.R. Quake, Molecular biology on a microfluidic chip, J. Phys. Condens. Matter. 18 (18) (2006) S691–S701.
[52] J.W. Hong, V. Studer, G. Hang, N.F. Anderson, S.R. Quake, A nanoliter scale nucleic acid processor with parallel architecture, Nature Biotechnol. 22 (4) (2004) 435–439.
[53] S.P. Srinavas, In situ measurement of fluorescein release by collagen shields in human eyes, Curr Eye Res. 13 (4) (1995) 281–288.
[54] N.A. Peppas, E.W. Merrill, Crosslinked poly(vinyl alcohol) hydrogels as swollen elastic networks, J. Appl. Polym. Sci. 21 (7) (1977) 1763–1770.
[55] U. Brohede, G. Frenning, M. Stromme, Characterization of the drug release process by investigation of its temperature dependence, J. Pharm. Sci. 93 (7) (2004) 1796–1803.

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