Manuscript Number: CLAE-D-15-00045R1

Title: Structural design of contact lens-based drug delivery systems; in vitro and in vivo studies of ocular triggering mechanisms.

Article Type: Full Length Paper

Keywords: ocular drug delivery; ophthalmic dyes; trigger release mechanism; ocular environment

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Abstract: This study identifies and investigates the potential use of in-eye trigger mechanisms to supplement the widely available information on release of ophthalmic drugs from contact lenses under passive release conditions. Ophthalmic dyes and surrogates have been successfully employed to investigate how these factors can be drawn together to make a successful system. The storage of a drug-containing lens in a pH lower than that of the ocular environment can be used to establish an equilibrium that favours retention of the drug in the lens prior to ocular insertion. Although release under passive conditions does not result in complete dye elution, the use of mechanical agitation techniques which mimic the eyelid blink action in conjunction with ocular tear chemistry promotes further release. In this way differentiation between passive and triggered in vitro release characteristics can be established. Investigation of the role of individual tear proteins revealed significant differences in their ability to alter the equilibrium between matrix-held and eluate-held dye or drug. These individual experiments were then investigated in vivo using ophthalmic dyes. Complete elution was found to be achievable in-eye; this demonstrated the importance of that fraction of the drug retained under passive conditions and the triggering effect of in-eye conditions on the release process. Understanding both the structure-property relationship between drug and material and in-eye trigger mechanisms, using ophthalmic dyes as a surrogate, provides the basis of knowledge necessary to design ocular drug delivery vehicles for in-eye release in a controllable manner.

1. Introduction

The inefficiency of direct instillation as a delivery method for ophthalmic drugs is well-recognised, as is the potential value of contact lenses for this application [1, 2]. Despite this, there are virtually no commercial examples of this area of technology. One reason for this is the perception, gained from in vitro passive diffusion studies, that the use of contact lenses in this way will lead to rapid and uncontrolled release in which much of the active drug will be lost by premature diffusion into the contact lens packaging solution [3-5]. Whilst these conclusions have some basis in fact, they overlook both the difference between in-eye release and passive diffusion under "sink" conditions into saline, and also the potential for specific design and selection of drug-lens combinations in which specific molecular interactions provide a means of extending in vivo delivery times.

The wide range of lens matrix chemistries and the structural variations found in ophthalmic drugs mean that this is a fruitful area for biomaterials research in this specialised field of ophthalmic biomaterials [6, 7]. The potential range of drugs coupled with the complexities of the ocular environment mean that such studies must be systematically organised and proceed from a sound knowledge of the materials chemistry and the aspects of the anterior eye that are likely to influence drug elution.

The present contact lens market encompasses many materials for a wide range of replacement (disposable, planned and conventional) and wear schedules (daily, extended and continuous wear) [8, 9]. Whereas the primary cosmetic role of contact lenses is vision correction, therapeutic indications for use of bandage lenses include pain relief, corneal protection and enhancement of corneal wound healing [10-12]. Bandage contact lenses play a key role in corneal transplant surgery, and are routinely used in penetrating keratoplasties, pterygia, total superficial keratectomies and corneal ring segment procedures [13, 14]. Patients with chronic epithelial defects or recurrent erosions, bullous keratopathy and dry eye typically stay in lenses for several months and in some cases, years. Commercial considerations have meant that a huge amount of research and product development has been directed to the so-called cosmetic lenses which provide an elective method of vision correction. There is very considerable potential for more detailed studies of structure-effect relationships in the under-

researched area of therapeutic lenses. Although there is less commercial interest here, it is undoubtedly a field of potential social and economic benefit in patient care.

Appropriate use of bandage contact lenses can speed healing, particularly in uncomplicated postoperative cases. In addition to the promotion of healing, bandage lenses provide symptomatic relief of pain, corneal protection, and structural support. Although medication can be distilled onto the eye and absorbed in the presence of the lens this is less effective than the controlled delivery of therapeutic quantities of specific drugs.

The ready availability of drug-loaded contact lenses would be much welcomed by ophthalmologists. Furthermore, common conditions such as contact lens induced dry eye and hay fever which are widely encountered in optometric practice, and can be aggravated by contact lens wear, could ideally be controlled with the use of suitably modified lenses. There are several commonly prescribed ocular drugs that could potentially be released from contact lenses [15-17]. For example, cromolyn sodium, olopatadine and ketotifen fumarate represent a small selection of drugs used to manage ocular allergies that range from seasonal to chronic conditions [18-20]. Given that a small but significant number of allergy sufferers require admission into hospital for eye drop treatment, the administration of the drug using a contact lens is a clear attractive alternative.

Several sophisticated approaches including molecular imprinting and incorporation of discrete nanoparticles have been proposed as strategies for the achievement of zero order release [21-27]. The main disadvantage of these approaches, however, is that purpose-fabricated lenses are necessary to make use of this technology, which has costly manufacturing implications.

Although a number of in vitro studies almost exclusively based on uptake and passive release behaviour of ophthalmic drugs from contact lenses have been carried out [1, 28-30], there has been little attempt to mimic the particular features of the ocular environment. This paper uses the understanding of drug-lens interactions developed from equilibrium passive release studies and examines the potential influence of in-eye trigger effects on the exploitation of this equilibrium

retention in the design of effective in-eye delivery systems. In this respect ophthalmic dyes and dye surrogates enable the use of a simple method to study the quantity retained as the release environment is changed and provide a ready platform for subsequent in vivo studies. This is not readily achieved with conventional ophthalmic drugs for which release monitoring is simple but retained drug extremely difficult to assay accurately. We investigate here the variables that enable maximum retention under passive release conditions from conventional hydrogels and address the effect of the lens material, release media volume and pH, tear proteins and degree of mechanical agitation on the equilibrium of the retained active achieved under passive release conditions.

2. Materials and Methods

2.1 Materials and lens loading

Details of the materials used and the procedures followed for incorporating an active into a lens, passive release and analysis of the release media have been previously published [1]. The range of ophthalmic dyes and structurally related compounds used in this study are based on the same core structure (Figure 1), which is shared by key ophthalmic dyes such as Rose Bengal, Lissamine Green B and sodium fluorescein. The range of substituents, octanol-water partition and distribution coefficients and molecular weights of this family of compounds is shown in Table 1. The shared multi ring core structure is a common feature of many drug systems [1], which have hydrophobicity arising from the aromatic ring systems and hydrophilicity from functional groups. The use of ophthalmic dyes as models indicate the relative influence of the balance of hydrophobicity and hydrophilicity and also relative steric effects which lead to association.

2.2 Passive release methodology: parallel measurement of release and retained active

The general procedure for treating loaded lenses of each material, dye and loading combination (e.g. Table 2) involved blotting the loaded lens on filter paper to remove excess dye, placing it in a specified volume of fresh phosphate buffered saline (PBS) release medium at pH 7.4 and stirring constantly (on a shaker at 200 rpm). This regime minimised the formation of a stagnant boundary transfer layer, and maintained optimum sink conditions (receiver concentration 25 times greater than donor

concentration). At the end of each hour the lenses were removed and placed in vials containing the same volume of fresh PBS release media and the process repeated until no further dye was released from the lens. This procedure was used for studies involving a series of discrete receiver volumes ranging from 150 µl to 20 ml (Section 3.4).

The optical density (OD) i.e. absorbance of the release media was measured by UV-Vis spectroscopy using a Molecular Devices SpectraMax M2 spectrophotometer at the maximum absorption wavelength of the released active. The absorbencies were then converted to concentrations using standard calibration curves. Release measurements were carried out in triplicate and averaged.

Non-destructive measurement of quantity of active retained was carried out spectroscopically using a Molecular Devices SpectraMax M2 spectrophotometer at the appropriate maximum absorption wavelength of the active (Figure 4). It is important to note that the extinction coefficients of these actives are stable within the time scales of the spectral assessments [31]. The mass calculations can therefore be reliably linked to absorbencies.

2.3 Mechanical release methodology

2.3.1 Batch triggered release

A healthy human eye has a tear flow rate of circa 1 µl/min, which is conveniently approximated to 100 µl/hr [32]. In order to replicate this in-eye extraction volume as closely as possible, an equivalent volume was used. Thus, a contact lens was inserted into a microtube containing 100 µl of phosphate buffered saline (PBS). Furthermore to mimic the mechanical action of the eye-lid blink, on both the lens and the surrounding tear fluid, the microtube was vortexed at 2400 rpm for 10-15 seconds and placed on the flat bed shaker at 200 rpm for an hour. After an hour the microtube was vortexed again for a further 10-15 seconds and the release media extracted for analysis. 100 µl of fresh release media was placed into the lens containing microtube and this procedure was repeated for the number of hours desired.

2.3.2 Continuous flow triggered release

This method is a modified version of the batch procedure whereby the small reservoir with continuous flow was employed. Thus a contact lens was placed into a microtube which had a 1 mm hole pierced at the bottom. The lens-containing microtube was then inserted into a larger microtube. The microtubes were held in a fixed position on a vortexer and a fluid line passed through the opening of the small microtube. PBS was pumped through the contact lens-containing microtube at a flow rate of 10 µl/min whilst the microtube was vortexed at low speed for an hour. After an hour the larger microtube containing the collected release media was replaced with a fresh larger microtube and the procedure repeated for the duration of the experiment. Here again the aim was to mimic the action of eyelid on both lens and surrounding tear fluid.

2.4 *In vivo release*

The aim of the in vivo experiments described here was not to establish statistically significant rates and ranges on in vivo release, but rather to demonstrate the principle if the triggering action of the ocular environment. PVA (nelfilcon A) and HEMA-MA (etafilcon A) contact lenses were soaked in a low concentration (typically 0.001%) solution of sodium fluorescein in PBS and autoclaved. The lenses remained in the dye solution of PBS for a minimum of 24 hours to ensure uptake equilibrium had been achieved. Untreated and treated lenses were worn contralaterally for three hours by a single subject. To obtain a calibrated quantitation of the lens pre and post wear the in vivo fluorescence of the lenses was observed at specific time intervals with a slit lamp biomicroscope, using white light and a cobalt blue filter, interfaced to a digital camera. The captured images were analysed using NIH Image J software which enabled fluorescence intensity to be expressed as perceived luminance. The images were loaded into the software in jpeg format and the RGB values for an area of 142 x 204 square pixels, from either temporal area of the cornea, were measured. Perceived luminance was subsequently calculated using the formula 0.299×Red + 0.587×Green + 0.114×Blue [33]. The study received prior ethics approval by the Institutional Ethics Committee and was designed to follow the tenets of the Declaration of Helsinki. Written informed consent of each subject was obtained.

2.5 *Determination of distribution coefficients*

Values of logP (octanol-water partition coefficient) and logD (octanol-water distribution coefficient) of both drugs and dyes were determined using the ACD/I-Lab service based on structure.

2.6 *Statistical Analysis*

The experimental data are reported for triplicate samples, unless otherwise stated. Figures show averages and standard error bars where the figure format permits these.

3. Results and Discussion

In initial experiments, passive release under conditions of gentle agitation was studied. Both release and retention were monitored by a combination of colorimetry and UV-Vis spectroscopy in which the optical absorbance of the lens was monitored throughout the release process (Section 2.2). This approach allows dual plots of dye remaining within (i.e. retained) and released from lens matrices to be determined.

3.1 Passive release into PBS: Mass balance between released and retained active

Experiments were carried out with a range of matrix materials and ophthalmic dyes using the methodology described in Section 2.2. Figures 2a and b show results of passive diffusion studies from PVA (nelfilcon A) lenses soaked in 1% Rhodamine B and Bromopyrogallol Red dyes respectively (Section 2.1) and released in PBS. The comparative magnitude of dye uptake and retention as reflected in the optical density of the lenses at passive release equilibrium (i.e. no further release under passive conditions) for PVA lenses is illustrated in Figure 2 inserts.

Figure 3 illustrates the effect of matrix structure on release kinetics of Bromopyrogallol Red under passive release conditions, comparing two non-ionic polymer-matrices of broadly similar water content (65 ± 5 %) but differing in hydrophobicity. The commercial contact lens material nelfilcon A, which is based on polyvinyl alcohol (PVA), is compared here with Filcon 3a, a copolymer of the more hydrophobic 2-hydroxyethyl methacrylate (HEMA) and *N*-vinyl pyrrolidone (NVP). The interaction of Bromopyrogallol Red with HEMA-VP is much greater than that with the PVA matrix, as reflected in the

relative optical densities of the two materials after Bromopyrogallol Red uptake (PVA OD 2.6; HEMA-VP OD 3.7).

Figure 3 shows that the greater interaction of Bromopyrogallol Red with HEMA-NVP results in a higher level of dye uptake coupled with both a greater mass of dye released and a greater quantity of dye retained. Previous experiments [1, 29] have shown marked differences in drug uptake with different polymer matrices, but here we see that polymer structure can additionally influence the mass and proportion of dye retained when passive diffusion has reached equilibrium. This is an extremely important point in relation to the exploitation of contact lenses as delivery reservoirs for ophthalmic drugs. In addition to the baseline passive release process, the potential "triggered" release stimulated by mechanical eyelid interaction, pH shift and compositional change on transferring a lens from packing solution to the ocular environment represents the most interesting and as yet underexploited aspect. This clearly illustrates the advantage of using colorimetry in conjunction with spectroscopy in visualising and understanding these phenomena.

The release kinetics under passive conditions studied in this way give an indication of the release potential of a contact lens for ocular release and in addition an estimate of the quantity of active that can be retained within the lens matrix. Although these passive release conditions are not representative of in-eye release they provide a fundamental understanding of the retention profile as well as the release profile of various combinations of active and lens matrices. Retention data for an extended range of dyes and matrices is shown in Table 2 and Figure 4.

In addition to the equilibrium release data shown in Table 2, kinetic studies of all dye-lens combinations were studied. It was observed that HEMA, HEMA-VP and HEMA-MA all release a greater quantity of Rhodamine B over a longer period compared than the PVA matrix. Additionally, HEMA-VP releases both Bromopyrogallol Red and Rose Bengal in larger quantities and for a longer duration than the other materials. A range of previous studies, including structural investigations of equilibrium uptake behaviour, show that NVP is capable of conveying additional complexation properties in HEMA copolymers, leading to higher levels of uptake [1, 34, 35]. Unfortunately the extremely high optical

density of the soaked lenses studied here (Figure 4) does not permit differentiation in uptake levels. The most hydrophilic material in this family is nelfilcon A (PVA) which consequently shows rapid diffusional release for a period of 3-4 hours with little ultimate retention. It is important to note, however, that for all materials some dye is retained within the matrix at equilibrium (Figure 4). Although HEMA-MA is the only material to show significant deviation from neutrality, HEMA lenses frequently contain traces of methacrylic acid impurity and HEMA-VP lenses contain a significant proportion of the weakly basic N-vinyl pyrrolidone monomer.

In summary, Figure 4 exemplifies the fact that dye-material interaction ranges from significant dye uptake (e.g. Bromopyrogallol Red-HEMA-VP) to moderate dye uptake (sodium fluorescein-HEMA) and similarly significant release (Rhodamine B-PVA) and moderate release (Bromopyrogallol Red-HEMA-VP). Comparison of the retained intensity of the dyes with different hydrogel compositions, when equilibrium has been achieved under passive release conditions, provides information about the uptake and retention of the dyes by the different lens matrices. This has particular relevance to the equilibrium reached between lens and packing solution after fabrication and before lens insertion (into the eye). Of greater significance for ophthalmic drug delivery, however, is the combination of "equilibrium shift" and triggered release that occurs when the lens is placed into the ocular environment. Sections 3.2 to 3.5 show the effects of such changes.

3.2 Triggered Release: The effect of pH on partition and distribution coefficients

Ophthalmic drugs are frequently packaged in solution below pH 7 and this forms the basis for a potentially important triggering factor. The effect of shifting from an equilibrium packing solution below pH 7 to pH 7 is illustrated here using an exaggerated shift in pH from 4 to 7 (Figure 5) although as subsequently demonstrated a shift of 0.5 to 1 pH unit (Figure 6) can make a significant difference to the partition behaviour of ophthalmic dyes. To demonstrate this effect HEMA and HEMA-VP lenses were soaked in 1% sodium fluorescein and HEMA-MA in 1% Rose Bengal as described previously [1] and then released into buffered saline alternating between pH 4 and pH 7 at hourly intervals (Figure 5).

For both lens material and dye structure the "burst" release during the first hour into pH 4 media is relatively slow, followed by an increase in the quantity released during the second hour into pH 7. The cumulative release into pH 7 is appreciably greater than that into pH 4, which can be explained in terms of the octanol-water distribution coefficients (logD). Although it is true that the effect of pH on net anionicity and water content plays a part here, the magnitude of the changes is far greater than can be explained on the basis of charge and EWC alone. The pKa of drugs and dyes influences the way that the distribution coefficient changes with pH. In general the distribution coefficient becomes more negative as pH moves from a lower pH to pH 7.

It is important to draw attention to the differences between logP, the partition coefficient when the drug is non-ionised (equation 1), and logD which is pH dependent and is influenced by the ionisation behaviour of the active (equation 2). This is an important parameter because the extent of ionisation of individual actives is differently influenced by the pH in which they are placed. The logD of a given drug is therefore influenced by its pKa, is pH-dependent and thus pH-specific and gives an indication of the apparent partition coefficient for all protolytic forms (degrees of dissociation). This fact can be put to good use in release modulation.

$$\log P = \log \frac{[\text{unionised species}]_{\text{octanol}}}{[\text{unionised species}]_{\text{water}}} \qquad \text{Equation 1}$$

$$\log D = \log \frac{[\text{unionised + ionised species}]_{\text{octanol}}}{[\text{unionised + ionised species}]_{\text{water}}} \qquad \text{Equation 2}$$

Figure 6a shows logD as a function of pH for sodium fluorescein. Above pH 7.5 sodium fluorescein tends to favour water more than octanol, this in turn would enhance its release into aqueous. Of equal importance we can see that by reducing the pH to 6 we gain about two logD units on the positive scale. Thus storage of a sodium fluorescein-loaded lens below pH 7 favours greater retention of the dye by the lens. On moving to ocular pH, release of this sequestered dye would be triggered. Thus, not only does the logP of the drug need to be considered but also logD, which takes into account the ionisation

behaviour of the drug. A similar profile is seen with many ocular drugs (e.g. Figure 6b), many of which are formulated at a pH between 4 and 7.

3.3 Triggered Release: The effects of mechanical agitation and tear proteins

Passive diffusion studies demonstrate (e.g. Figure 4) that significant quantities of dye can be retained within the lens when passive release equilibrium has been reached. In-eye release from a lens matrix will present different elution conditions. The mechanical action of the eyelid and the presence of individual tear proteins would be expected to disturb this equilibrium. Triggered in-eye release represents an important possible release route that requires experimental validation. In vitro experimental methodologies described in Section 2.3 were used to investigate the potential significance of mechanical agitation.

Figure 7 shows the onset of release for lenses that were subjected to the two types of mechanical agitation, using batch and continuous flow conditions respectively (Section 2.3). Prior to the release experiments, the lenses were pre-equilibrated in a 1% Rose Bengal dye soak solution and had reached passive release equilibrium at approximately 28 hours.

It is clear that mechanical agitation of both lens and extraction medium causes further release of the dye beyond that achieved at passive equilibrium under both batch and continuous flow conditions. The quantity of dye released increases with agitation intensity, flow rate and duration of mechanical action. As expected there are material-related differences, but as Figure 7 shows there is a clear overriding effect of mechanical agitation irrespective of material. This is clearly consistent with the observations of Mark Byrne's group [36] in which increased agitation of the extraction medium was shown to enhance release.

In order to study the effect of significant tear proteins (lysozyme, lactoferrin and serum derived albumin [37-39]) on release phenomena, a 1 mg/ml solution of each protein in PBS was prepared and used as the release medium. The actual concentrations of these three proteins in tears are markedly different from each other. The point of this experiment was not to mimic likely in-eye extraction

behaviour at tear-borne concentrations, but to examine structural effects on extraction capability at the same concentration. Lenses were soaked in a 1% dye solution and released, using the trigger-release batch conditions as described in Section 2.3. The effect of these three proteins on the release of Rose Bengal from PVA (nelfilcon A) and HEMA-MA-PVP (vifilcon A) is outlined in Table 3.

It is clear, and unsurprising that mechanical agitation in the presence of tear proteins produces an enhanced rate of release relative to that obtained with PBS alone. Further studies of dye-protein combinations would be necessary to make any definitive comments relating to specific mechanisms, but there is an obvious variation as the structure and size of the protein are changed. The fact that lysozyme and lactoferrin, both positively charged, represent the extremes of observed behaviour suggest that charge-related effects do not play a major part. Increasing molecular weight of the protein seems to favour extraction and that also supports the view that hydrophobic interaction may be influential. Of the three proteins, albumin is the best known for strong interaction with long chain fatty acids [40]. There is little structural similarity between Rose Bengal and fatty acids, except that they both possess significant regions of hydrophobicity. The great difference in the molecular conformations of the two species suggest that steric factors may be influential in the interactions with these proteins.

There is strong evidence from these observations that the ocular environment, as reflected in both the mechanical action of the eyelid and the protein content of tear aqueous, would be expected to produce very different release characteristics when compared to passive release in PBS. These are in principle experiments and it is logical to test their implications by moving directly to the ocular environment – a possibility facilitated by the use of ophthalmic dyes in the in vitro experiments.

3.4 Volume of extraction media

There are obvious advantages in carrying out comparative passive release studies under sink conditions using volumes saline (ca. 5ml) that are much greater than the volume of tear surrounding the lens in eye. The desirability of designing release devices that more closely replicate in-eye conditions is now well recognised. Byrne and his co-workers [21, 41] have made excellent progress in

this regard. The important effect of extraction volume in release studies is demonstrated in figure 8. PVA (nelfilcon A) lenses were soaked in a 1% Rhodamine B solution (Section 2.1) and released in 150 μl, 0.5 ml, 1 ml, 5 ml and 20 ml (Section 2.2). The amount of Rhodamine B retained by PVA (nelfilcon A) at end of the first hour of release is shown as a function of extraction volume (figure 8), which shows that the quantity retained after a given extraction period within the lens reduces with increasing extraction. Thus reducing the extraction volume slows down the rate of the release effectively releasing the same payload over a longer time period. The use of ophthalmic dyes as described herein enables the principles established by *in vitro* studies to be investigated *in vivo* (section 3.5).

3.5 *In vivo release studies: preliminary demonstration of ocular triggering phenomena*

To examine the combined influence of the in-eye trigger release mechanism, PVA (nelfilcon A) and HEMA-MA (etafilcon A) daily wear lenses were soaked in 0.001 % sodium fluorescein and worn for three hours as discussed in Section 2.4. This concentration was chosen in order to mimic the equilibrium reached with in vitro passive release. Sodium fluorescein enables the use of digital photography to obtain valuable visual monitoring of in-eye release behaviour from the lens over time. Changes in the observed rate of reduction in fluorescence with wear time (figure 9a) enabled a comparison of the in vivo release behaviour of both materials in a single subject. Analysis of the images obtained (Figure 9b) indicate that the equilibrated nelfilcon A lens shows a more prominent in-eye fluorescein “burst” than etafilcon A. However, both treated lenses subsequently attained similar fluorescence intensity to the untreated lens after three hours of wear.

The images and their subsequent analysis (figure 9) indicate that the equilibrated nelfilcon A lens shows a more prominent in eye fluorescein “burst” than etafilcon A. However, both treated lenses subsequently attained similar fluorescence intensity to the untreated lens after 2 hours of wear. The in vivo release of ophthalmic dyes has clearly demonstrated that to reasonably represent the effects of agitation, pH shift and protein-mediated extraction, in vitro models need to give a more accurate representation of ocular conditions than is possible with models that rely on passive release into sink volumes of PBS. Such experiments are extremely valuable where the rate determining step involves diffusion of an active species from a polymer matrix but do not represent the complex situation

encountered by the contact lens in the ocular environment. An additional but extremely important aspect of in vivo extraction that needs to be reflected in vitro models of ocular release is the effect of tear volume and tear turn over. The work of Byrne [21, 41] has produced considerable advances in reduced volume *in vitro* devices. These parallel studies using ophthalmic dyes have aimed to link *in vitro* studies of three specific physic chemical properties to observed in-eye behaviour. The studies do, however, highlight the importance of the design of a continuous small volume flow cell for on-going studies.

The limited in vivo data presented here cannot in anyway establish mean and range rate data for in eye elution, that is not the purpose of this experiment. In vitro extraction release is extremely consistent and always leaves, at the point of equilibrium under the particular extraction conditions, residual dye/ drug within the lens. The aim of these experiments was not to determine statistical reliability of elution rate in eye but merely to demonstrate that when in vitro elution had reached a consistent equilibrium point, the in vivo ocular environment produced an eluent – and a significant eluent – of triggered release.

4.0 Conclusions

The results presented and discussed in this paper indicate that triggered release in the ocular environment, as distinct from passive release into excess volumes of saline, can form an effective platform for daylong delivery of drugs from daily disposable contact lenses. The work described here involving the use of ophthalmic dyes has enabled both release phenomena and retention of active compound within the lens matrix to be directly predicted. It is clear that to design a vehicle with an effective payload for daylong delivery the specific interactions of drug structure and matrix and the contributory factors affecting in-eye release need to be studied and understood.

It is important to note that silicone hydrogels share similarities, but exhibit significant structural and behavioural differences, from conventional hydrogels. In summary, functional group chemistry has a much stronger influence on the uptake and release behaviour of conventional hydrogels, whereas silicone hydrogels provide additional interactive sites for strongly hydrophobic drugs [1]. Furthermore

silicone hydrogels as a group exhibit a degree of phase separation which allows access to these domains through aqueous channels. Silicone hydrogel chemistry can only be understood if the functional group-based interactions of drugs with the structurally homogenous conventional hydrogels described in this paper are first elaborated.

The growing need for controlled delivery of ophthalmic drugs will undoubtedly require the exploitation of effects of ocular triggering on the release characteristics of specific drug-matrix combinations. The platform of understanding described here can thus potentially contribute to the selection of successful contact-lens based delivery systems for clinical applications.

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Table 1

Table 1 Variations in the nature of ring substituents (Figure 1) together with consequent logP (octanol-water partition coefficient) and logD (octanol-water distribution coefficient) values calculated using the ACD/I-Lab service and molecular weight (M_w).

	Bromopyrogallol Red	**Rhodamine B**	**Rose Bengal**	**Lissamine Green B**	**Sodium fluorescein**
substiutents R1	O, 3 x OH, Br	COOH	Cl, COONa	$N(CH_3)_2$	COOH
substiutents R2	3 x OH, Br	$N(CH_2CH_3)_2$	2 x I, NaO	$N(CH_3)_2$	NaO
substiutents R3	SO_3H	$N^+(CH_2CH_3)_2$	2 x I, O	OH, SO_3^-, $C_4H_3SO_3H$	O
linking group X	H, H	O	O	H, H	O
logP	-1.57±0.55	2.21±1.09	10.14±1.32	-3.95±1.42	4.8±0.84
logD at pH 7	-6.1 ±1.0	2.8 ± 1.0	6.1 ±1.0	-4.4 ±1.0	2.2 ±1.0
M_w	558	479	1107	577	376

Table 2

Table 2 Total cumulative release of Rhodamine B, Bromopyrogallol Red, Rose Bengal and sodium fluorescein from HEMA-based and PVA lens materials, measured at point of passive release equilibrium after soaking in 1% dye solutions for 24 hr.

	μg released (±5%)			
	Rhodamine B	**Bromopyrogallol Red**	**Rose Bengal**	**Sodium fluorescein**
HEMA (Filcon 1A)	4199	531	2103	549
HEMA-VP (Filcon 3A)	3283	1366	7018	960
HEMA-MA (etafilcon A)	2345	407	4206	388
PVA (nelfilcon A)	239	291	4408	348

HEMA = 2-hydroxyethyl methacrylate, MA = methacrylic acid, NVP = N-vinylpyrrolidone, PVA = poly(vinyl alcohol)

Table 3

Table 3 Cumulative mechanically agitated 6 hour release (μg per lens) of Rose Bengal from PVA (nelfilcon A) and HEMA-MA-PVP (vifilcon A) into 600 μl saline reservoirs each containing one of three main tear proteins differing in molecular weight (Mw) and isoelectric point (pI) values.

	μg released (±5%)		**Protein**	
	PVA (nelfilcon A)	**HEMA-MA-PVP (vifilcon A)**	**M_W kDa**	**pI**
PBS	36	218	-	-
lysozyme	84	262	14.3	11.0
albumin	140	-	66.4	4.7
lactoferrin	284	395	82.4	8.7

Figure Legends

Figure 1 Ring structure common to the chosen ophthalmic and related dyes.

Figure 2 Passive diffusion of a) Rhodamine B, and b) Bromopyrogallol Red from PVA (nelfilcon A) lens showing data points for both retention and release. Cumulative increase in dye release and drug retained were measured by optical density at the respective maximum absorption wavelengths (shown in Figure 4). Embedded images show lenses at initial and equilibrium stages of the passive release process with the corresponding relative optical densities.

Figure 3 Passive diffusion of Bromopyrogallol Red from HEMA-NVP (Filcon 3a) and PVA (nelfilcon A) lenses of similar water contents monitored by UV-Vis. Inserts show both the quantity of dye retained and the optical density of the lenses at initial and final stages of passive release monitored by colorimetry.

Figure 4 Visual level of retained dye, together with optical density at λmax, for HEMA-based and PVA lenses presoaked in Bromopyrogallol Red, Rhodamine B, Rose Bengal and sodium fluorescein at initial and equilibrium stages of the passive release process. The λmax determined for each dye, together with EWC, ionicity and the octanol water partition (LogP) and distribution (LogD) coefficients of the repeat unit of the lens backbone repeat unit are detailed.

Figure 5 Effect of hourly alternating pH (pH 4 and pH 7) on the release of active (μg per lens) into 5ml release media for different active-lens combinations. Sodium fluorescein from a) HEMA-VP (Filcon 3a) and b) HEMA (Filcon 1a); Rose Bengal from c) HEMA-MA (etafilcon A).

Figure 6 LogD versus pH profile of a) sodium fluorescein and b) sodium cromoglycate, calculated using the SPARC online calculator.

Figure 7 Triggered release of Rose Bengal retained at passive release equilibrium under a) batch and b) continuous flow release conditions with mechanical agitation.

Figure 8 Comparison of amount of Rhodamine B dye retained from PVA (nelfilcon A) lenses soaked in varying volumes of extraction media at the end of the first hour of extraction. The hatched area a) indicates volume equivalent to that in the eye and b) the volume used in most of the published literature.

Figure 9a) Digital images captured using a slit lamp which enable a comparison between the non-fluorescent untreated lens and the reduction in fluorescence intensity of i) PVA (nelfilcon A) and ii) HEMA-MA (etafilcon A) fluorescein treated lenses with in-eye wear time for a single subject. b) Perceived Luminance values of images in a) were determined using NIH ImageJ software; untreated lens baselines shown for comparison.

Figure 1

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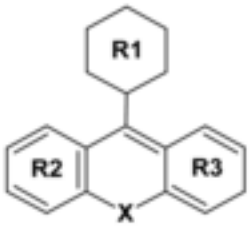

Figure 2

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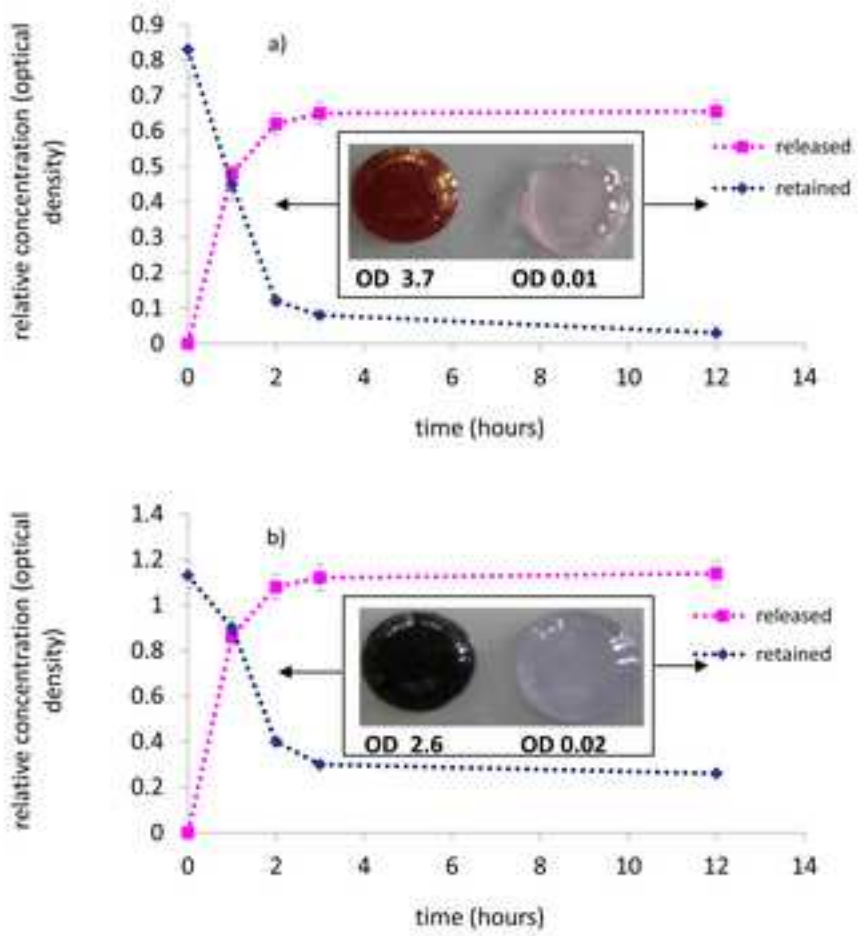

Figure 3

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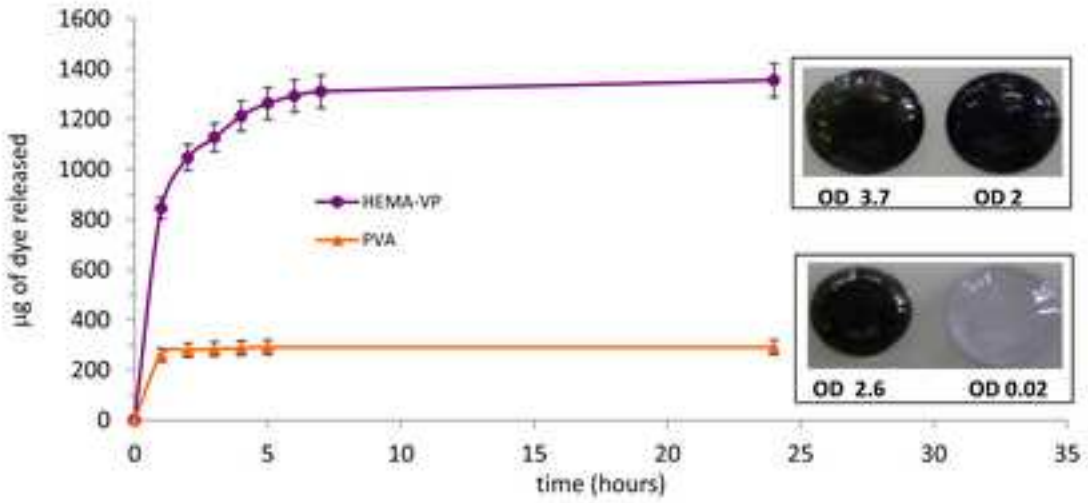

Figure 4

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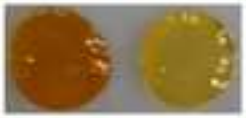
	HEMA	HEMA-VP	HEMA-MA	PVA
Bromopyrogallol Red λmax = 552nm OD 0.001% = 0.38				
	OD 3.7 0.19	**OD 3.7 2**	**OD 3.7 0.05**	**OD 2.6 0.1**
Rhodamine B λmax = 543 nm OD 0.001% = 0.84				
	OD 3.7 0.7	**OD 3.7 0.3**	**OD 3.7 0.1**	**OD 3.7 0.01**
Rose Bengal λmax = 549 nm OD 0.001% = 0.18				
	OD 3.7 0.1	**OD 3.7 1.8**	**OD 3.7 0.1**	**OD 3.7 0.07**
Sodium fluorescein λmax = 496 nm OD 0.001% = 0.59				
	OD 3.2 0.4	**OD 3.6 1.0**	**OD 2.8 0.2**	**OD 3.3 0.1**
EWC %	38	60	58	69
Ionicity	neutral (mildly anionic)	neutral (mildly basic)	anionic	neutral
repeat unit hydrophobicity (LogD at pH 7)	0.3	0.2	0.24	-0.2

Figure 5

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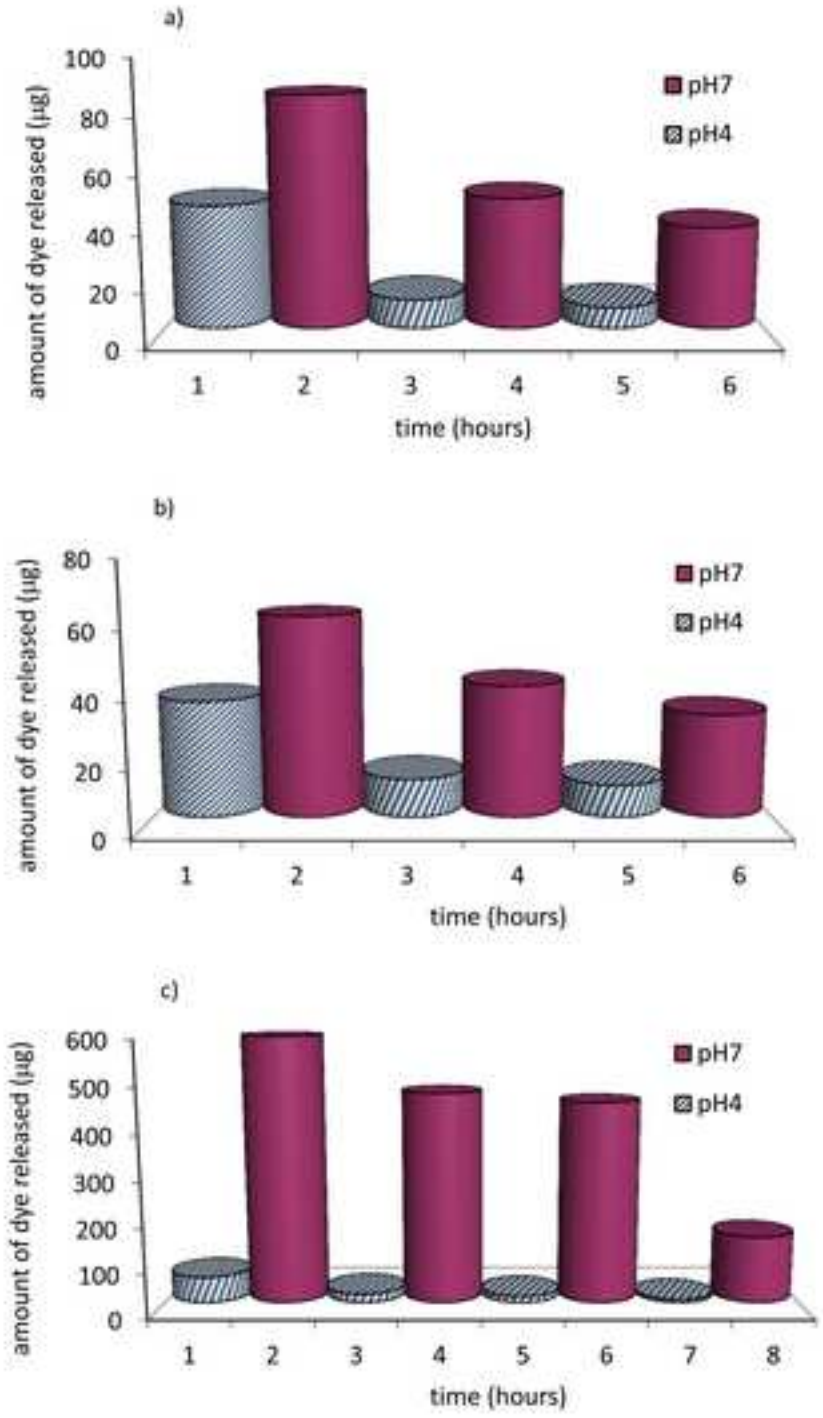

Figure 6

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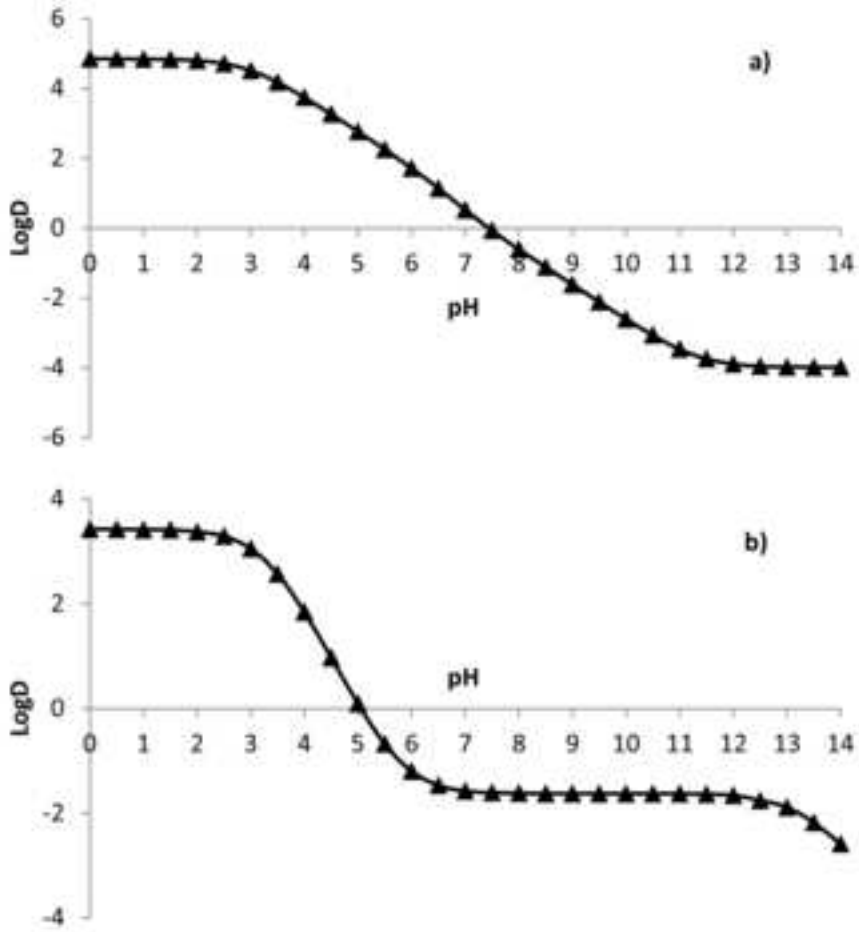

Figure 7

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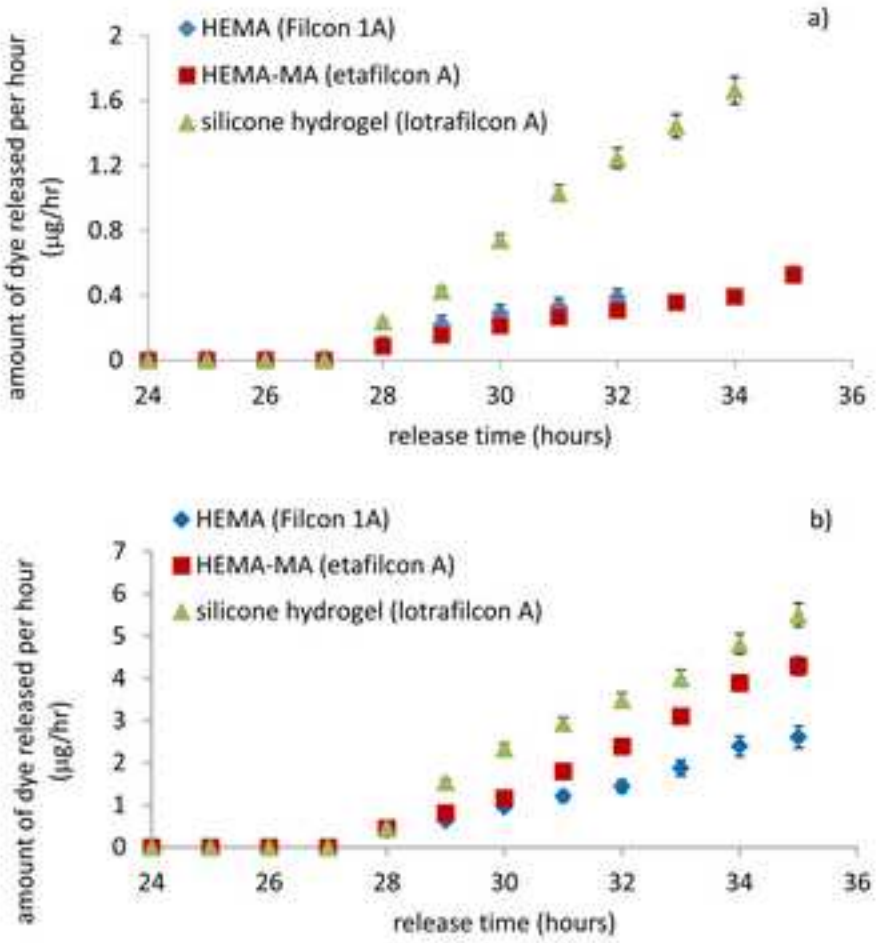

Figure 8

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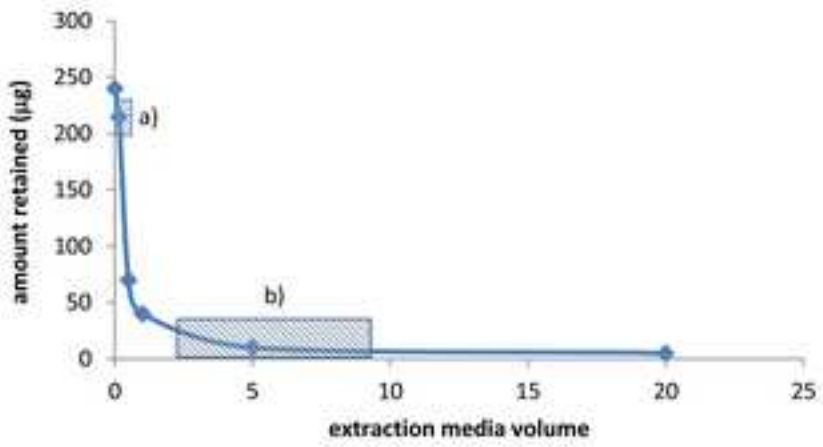

Figure 9a

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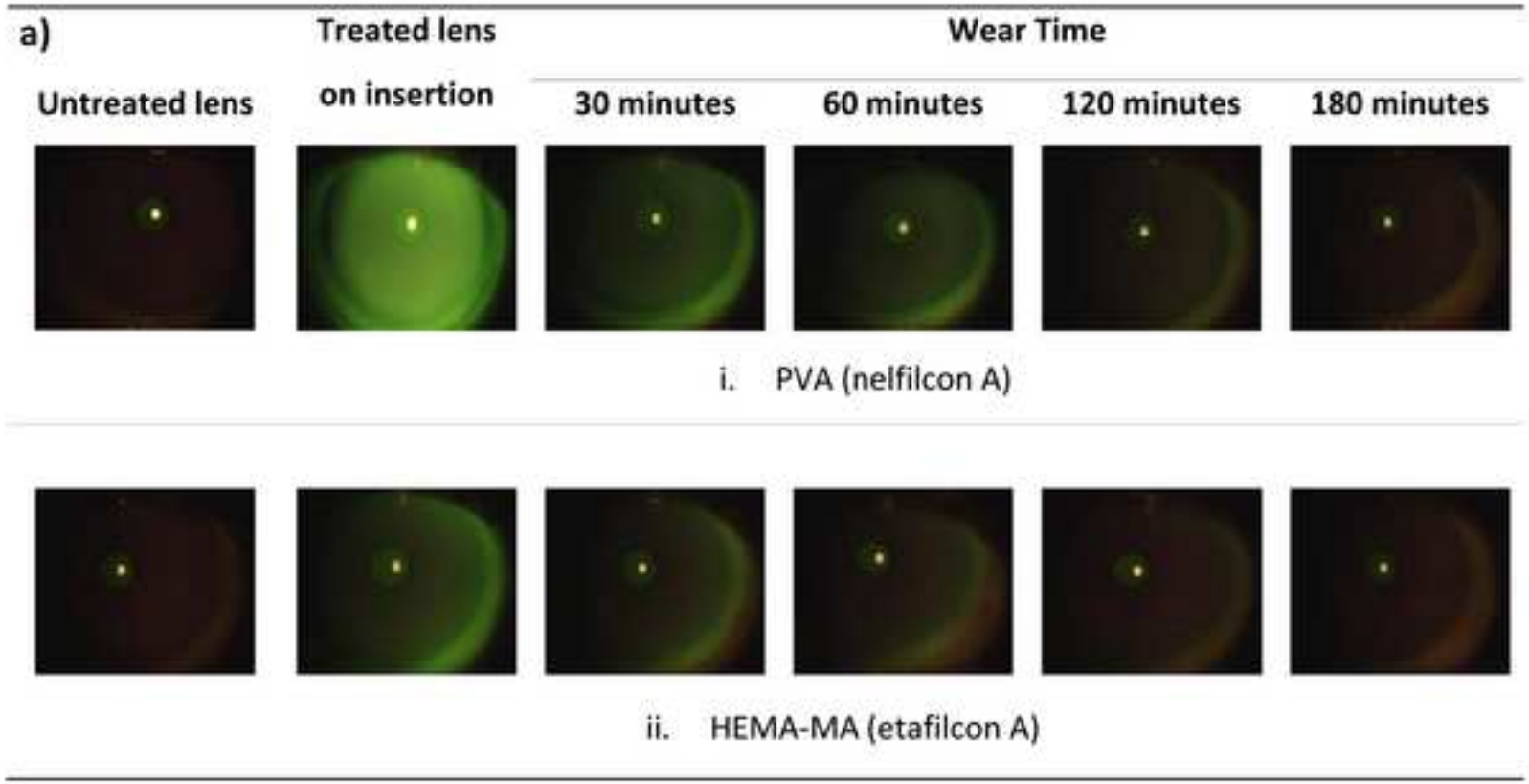

Figure 9b

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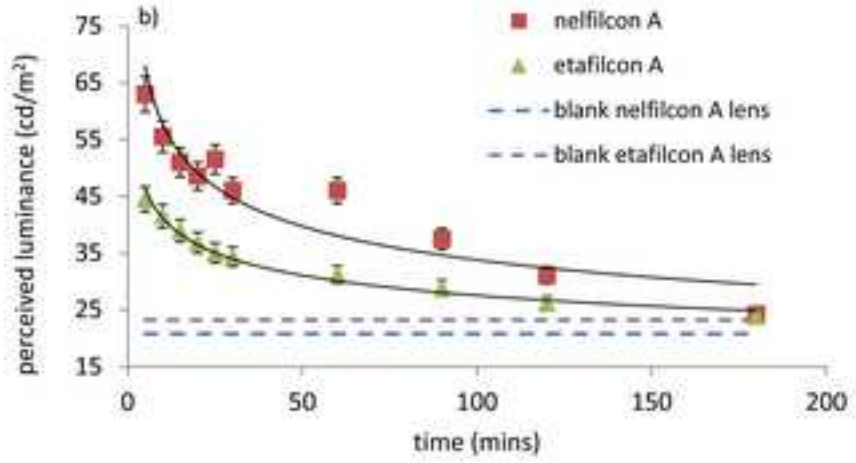

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Structural design of contact lens-based drug delivery systems; in vitro and in vivo studies of ocular triggering mechanisms.

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