British Journal of Pharmacy

https://doi.org/10.5920/bjpharm.2017.05

British Journal of Pharmacy

www.bjpharm.hud.ac.uk

Research Article

The preparation of HEMA-MPC films for ocular drug delivery

Athmar Al-Shohani[1,2], Sahar Awwad[1,2*], Peng T. Khaw[2] and Steve Brocchini[1,2*]

[1]UCL School of Pharmacy, London WC1N 1AX, United Kingdom; [2]National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London EC1V 9EL, United Kingdom

ARTICLE INFO

Received: 13/06/2017
Accepted: 21/07/2017
Revised: 25/07/2017
Published: 01/08/2017

*Corresponding author.
Tel.: +44 207 753 5802
Fax: +44 207 753 5942
E-mail: s.awwad@ucl.ac.uk; steve.brocchini@ucl.ac.uk

KEYWORDS: Hydrogels; Polymer; Ocular; Drug delivery

ABSTRACT

There is a need to prolong drug residence time using a biocompatible formulation in the subconjunctival space after surgery to treat glaucoma. Drug releasing discs were prepared with 2-(hydroxyethyl)methacrylate (HEMA) and 2-methacryloyl-oxyethyl phosphorylcholine (MPC). The ratio of bound water (Wb) to free water (Wf) ratio increased from 1:0.3 to 1:6.8 with increasing MPC (0 to 50%, w/w). The optimal balance between water content, SR and mechanical strength were obtained with 10% MPC (w/w) hydrogels. Water-alcohol mixtures were examined to facilitate loading of poorly soluble drugs, and they showed greater hydrogel swelling than either water or alcohol alone. The SR was 1.2 ± 0.02 and 3.3 ± 0.1 for water and water:ethanol (1:1) respectively. HEMA-MPC (10%) discs were loaded with dexamethasone using either water:ethanol (1:1) or methanol alone. Drug release was examined in an outflow rig model that mimics the subconjunctival space in the eye. Dexamethasone loading increased from 0.3 to 1.9 mg/disc when the solvent was changed from water:ethanol (1:1) to methanol with the dexamethasone half-life ($t_{½}$) increasing from 1.9 to 9.7 days respectively. These encouraging results indicate that HEMA-MPC hydrogels have the potential to sustain the residence time of a drug in the subconjunctival space of the eye.

INTRODUCTION

There continues to be a need to develop sustained release formulations for the subconjunctival and periocular spaces (Kuppermann & Loewenstein 2010; Kang-Mieler et al. 2014). Glaucoma is the most common cause of irreversible blindness in the world and the only proven treatment is to decrease the intraocular pressure (IOP) to slow progression of optic nerve damage (Weinreb & Khaw 2004). Glaucoma filtration surgery (GFS) is a procedure to create a pathway to drain aqueous humour from the eye to lower the IOP. Unfortunately, scarring occurs at the site of surgery to block aqueous outflow resulting in a rise of IOP (Kimura et al. 1992). The outcome of GFS can be improved by the modulation of wound healing processes to prevent excessive fibroblast proliferation and scarring (Cui et al. 2008).

The use of cytotoxic agents applied during surgery has revolutionised the outcomes for GFS (Dhingra & Khaw 2009). Localised scarring still remains a challenge because subsequent treatment by direct injection of less toxic agent into the subconjunctiva (Chong et al. 2013) is either not efficacious or practical. Drug residence time in the subconjunctival space is short because aqueous outflow simply takes the drug after injection directly into the conjunctiva and into the systemic circulation. Although eye drops are widely used for a large number of periocular conditions and can be considered for use to treat

https://doi.org/10.5920/bjpharm.2017.05

scarring after GFS (Skuta et al. 1992), we are also developing dosage forms designed for ocular implantation into the subconjunctival space after GFS (Parkinson et al. 2012). Tissue implantable dosage forms can result in prolonged local drug exposure with a lower cumulative, systemic exposure to the drug. One challenge for implantable dosage forms is to avoid a foreign body response, which can readily occur in tissue that is traumatised by surgery.

In GFS, another potential function of an implantable dosage form would be to act as a barrier within the subconjunctival space to separate the conjunctiva from the sclera to help maintain pathways for aqueous outflow. Glaucoma drainage devices (GDDs) are used as an alternative to GFS to shunt aqueous outflow from the eye to lower IOP (Bettin & Di Matteo 2013). Many of these devices have a plate or a spacer that sits in the sub-conjunctival space. Silicone is commonly used in GDDs but a foreign body response around the plate results in scarring that blocks aqueous outflow leading to an increased IOP.

Hydrogels are three dimensional (3D) polymeric crosslinked networks that have been widely investigated for biomedical applications such as tissue engineering, artificial replacement of organs, coating of implantable devices, drug delivery, gene delivery, scaffolding and wound dressings (Du et al. 2013; Vashist et al. 2014; Hoffman 2012). Hydrogels are often considered to be broadly biocompatible and are widely used in the eye (e.g. contact lens and intraocular lens). Hydrogel properties (e.g. swelling) and drug release profiles are frequently related to the water content within the gel (Lee et al. 1975; Jhon & Andrade 1973; Shi et al. 2012). Drug loading into the hydrogel is also an important parameter that can influence by the solvents that are used (Lewis et al. 2008).

In this paper we report the preparation of hydrogel films made from two polymers that are widely used in contact and intraocular lens; 2-(hydroxyethyl)methacrylate (HEMA) and 2-methacryloyloxyethyl phosphorylcholine (MPC) (Tomar et al. 2012; Stirbu et al. 2011; Schlenoff 2014; Ishihara et al. 1990; Lewis 2000). MPC has zwitterionic phosphoryl choline pendent chains that mimic lipid head groups and has been shown to be a biocompatible material (Schlenoff 2014). In addition to contact lens, MPC is used in a wide range of clinical products including coronary stents (Lewis et al. 2008).

A range of HEMA-MPC films in this study were characterised for their water content, water distribution, water permeability and mechanical strength. Steroids such as dexamethasone are often used after GFS to moderate the inflammatory response. The HEMA-MPC films were loaded with dexamethasone and the release profiles were monitored using an *in vitro* model that mimics aqueous outflow of the GFS surgical area (bleb).

MATERIALS AND METHODS

Materials and Instrumentation

Hydroxyethyl methacrylate (HEMA), poly(ethylene glycol dimethacrylate) (PEGDA, Mn 700), 2,2-azo-bis(2-methylpropionitrile) (AIBN), dexamethasone and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich (Gillingham, UK). 2-methacryloyloxyethyl phosphoryl choline (MPC, 295.27 g/moL) was obtained from Vertellus Biomaterials (UK).

UV measurements were performed using a Hitachi U-2800A spectrometer using Quartz cuvettes (Starna Scientific Ltd). Scanning electron microscopy (SEM) was achieved using a QuantaTM 200F instrument (FEI Quanta200 FEGESEM, Eindhoven, The Netherlands). Mechanical properties were measured with an Instron Universal testing instrument (Model 5567, Instron Ltd, Norwood, USA) equipped with Bluehill software 2 (version 6). Freeze-drying was conducted with a VIRTIS-Advantage freeze-dryer. Different scanning calorimetry (DSC) experiments were performed on DSC Q2000 (TA instruments, Waters, LLC) equipped with TA Instruments Universal Analysis 2000 software. High performance liquid chromatography (HPLC) was conducted using an Agilent 1200 series (Agilent, Wokingham, Berkshire, UK) equipped with Chemstation software (Agilent, Wokingham, Berkshire, UK) using a Synergi 4u Hydro-RP 80 A (150 × 4.60 mm, 4 micron) column (Phenomenex Co., California, USA). A 16-channel Ismatec peristaltic pump (Michael Smith Engineers Ltd., Woking, Surrey, UK) was used to generate fluid flow into the flow rigs.

https://doi.org/10.5920/bjpharm.2017.05

Preparation of HEMA-MPC hydrogel films

HEMA-MPC hydrogel films were prepared by thermally induced free radical polymerisation (**Table 1, Fig. 1**). MPC monomer was dissolved in HEMA monomer and mixed till a clear solution was observed. PEGDA (crosslinker) and AIBN (initiator) were separately mixed till a clear mixture was observed. A septum was attached to the lid of the glass tube and an outlet needle 19G was placed at the top of the tube. The mixture was degassed with argon for 5 mins. The reaction mixture was then injected into a polypropylene mould using a 21G needle while avoiding the formation of air bubbles.

Two polypropylene sheets and one silicone sheet were used to prepare the polypropylene mould (**Fig. S1**). The polypropylene and silicone sheets (1.0 mm thickness) were cut in rectangles with 4.0 × 9.0 cm dimensions. The inside of the silicone sheet was further cut into another rectangle, 3.0 × 8.0 cm, leaving a distance of 1.0 cm from the borders. Before assembling the sheets, they were sonicated with isopropanol (10 mins) and dried in the oven at 50°C (30 mins). The silicone sheet was sandwiched between the two polypropylene sheets and the polymer mixture was injected slowly from the edges.

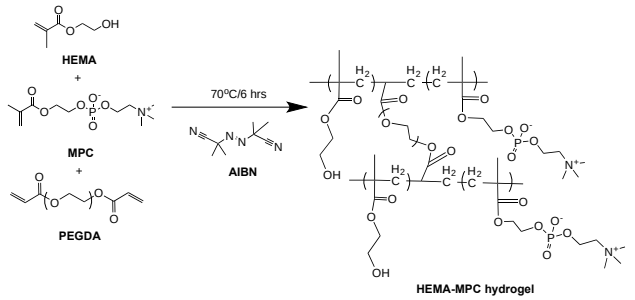


Fig. 1. *Synthesis of HEMA-MPC hydrogel films by free radical polymerisation. The two monomers (HEMA and MPC) were mixed with the crosslinker (PEGDA) to form a clear solution. AIBN (initiator) was added and placed in the oven at 70°C for 6 hours.*

Upon adding the reaction mixture, the mould was then placed flat in the oven at 70°C for 6 hours to conduct the polymerisation and form the xerogel (hydrogel in a completely dried state). The xerogel was gently removed, hydrated in water (50 mL) and the leachables removed by washing. The water was changed twice daily for 4 days and the washed fractions were scanned with UV-Vis spectroscopy (200-400 nm) to detect any unreacted monomers. The hydrogels were considered clean when the washed fractions showed no signal with UV and were stored in plastic containers in water (50 mL) prior to further use. The storage water was changed every week to avoid bacterial growth.

Table 1. *Compositions of different HEMA-MPC hydrogel films prepared by free radical polymerisation*

Code	HEMA (M)	MPC (M)	PEGDA (mM)	AIBN (g)	MPC (%)
S1	8.1	0.0	8.0	0.074	0
S2	7.8	0.1	8.0	0.074	5
S3	7.4	0.3	8.0	0.074	10
S4	7.0	0.5	8.0	0.074	15
S5	6.6	0.7	8.0	0.074	20
S6	5.7	1.0	8.0	0.074	30
S7	4.1	1.7	8.0	0.074	50
S8	0.0	0.3	8.0	0.074	100

Abbreviations: *AIBN:* 2,2-azobis(2-methylpropionitrile, *HEMA:* Hydroxyethyl methacrylate, *MPC:* 2-methacryloyloxyethyl phosphoryl choline and *PEGDA*: polyethylene glycol dimethacrylate.

Characterisation of the hydrogel films

Mechanical testing

Film pieces in a dog bone shape were used to avoid having a break in the area being gripped and they were cut from fully hydrated films. The dimensions of each sample were 15.5 × 3.6 × 1.0 mm (length × width × thickness respectively). Each sample was placed between the clamps of the Instron and pulled apart at a rate of 10.0 mm/min and 100.0 N static load (2 kg). Samples mounted on the grips were sprayed with water to ensure they remained fully hydrated. The cut-off point was when the film was completely

https://doi.org/10.5920/bjpharm.2017.05

separated into two pieces. The tensile modulus of elasticity (represented by Young's modulus) was determined as the slope of the linear part of the stress–strain curve.

Distribution of water inside the hydrogel, free to bound water ratio

DSC can measure the free to bound water ratio of hydrogels. Only free water (W_f) and moderately bound water (W_b) are frozen so the endotherm obtained from DSC represents the amount of frozen water only. **Eq. 1** assumes that the heat of fusion of frozen hydrogel water is the same as ice. The amount of W_b is the difference between the total water content and frozen water. The melting enthalpies achieved from DSC are used to calculate the W_b to W_f ratio (**Eq. 1**):

$$Wb\ (\%) = EWC\% - (Wf + Wfb) \times 100$$

$$Wb(\%) = EWC\% - (\frac{Qendo}{Qf}) \times 100$$

Eq. 1

Where, W_f is the amount of free water, W_{fb} is the amount of lightly bound water, Q_{endo} is the melting enthalpies derived from the DSC chart and Q_f is the melting enthalpies of free water, which similar to ice (79.9 cal/g) (Rohindra et al. 2004). Experiments were performed at a heating rate of 3.0°C/min from -40 to 20°C. Calibration with indium (T_m = 156.6, ΔH_f =28.71 J/g) was performed according to the manufacturer instructions. Nitrogen was purged gas at 50 mL/min. TA zero aluminium hermetic pans and lids were used.

Scanning electron microscopy (SEM)

The dried samples were cut and adhered onto aluminium SEM stubs using carbon-coated double-sided tape. They were sputter coated with gold prior to imaging to make them electrically conductive

Equilibrium water content (EWC%)

Equilibrium water content percentage (EWC%) is the percentage of water absorbed by a xerogel at full hydration. Hydrogel discs (1.0 cm in diameter) were cut from fully hydrated hydrogel films and weighed, to give hydrated equilibrium weight (W_e). The discs were dried in a vacuum oven at 70°C until they reached constant weight (W_d). EWC% was calculated using **Eq. 2**.

$$EWC\% = \left(\frac{We - Wd}{We}\right) \times 100$$

Eq. 2

Swelling ratio (SR)

Swelling ratio (SR) is the ratio between the weight of solvent absorbed by the hydrogel and the dry weight of the hydrogel. It gives an indication to the increase in size of the dry xerogel when fully hydrated. Hydrogel discs (1.0 cm in diameter) were cut at ambient temperature from a fully hydrated hydrogel film, weighed and dried in a vacuum oven at 70°C until reaching a constant weight (W_d). Different water miscible alcohols were investigated to see the difference in SR. The dry discs were incubated in a solvent (5.0 mL) i.e. water, methanol, ethanol, water: methanol (1:1) or water: ethanol (1:1) for 48 hours (25°C) to become hydrated. The discs were removed; carefully wiped and weighed at equilibrium (W_e). The SR was calculated using **Eq. 3**.

$$SR = \left(\frac{We - Wd}{Wd}\right)$$

Eq. 3

Drug loading and release from 10% MPC hydrogels

Dexamethasone is a poorly water-soluble drug (~0.1 mg/mL). Water:ethanol (1:1) was used to improve the loading efficiency of dexamethasone in the 10% HEMA-MPC hydrogel with a solubility increase to 1.0 mg/mL. Two methods were used to estimate the loading efficiency and the amount of dexamethasone loaded in each disc. One method was based on the difference in UV reading between the starting solution and the solution left after incubation. This approach assumes that the difference between the readings represents the amount loaded in the disc. The other method is based on complete extraction of dexamethasone from the loaded discs using methanol. Methanol was used as extraction solvent because the discs swell to a higher extent in methanol compared to water or PBS.

Discs of 1.0 mm × 1.0 cm (thickness × diameter) were dried at 70˚C in vacuum for 24 hours. The discs were then soaked in drug solution for 24 hours. Water: ethanol (1:1) and methanol were used to load 1.0 and 15.0 mg/mL of dexamethasone respectively. With water:ethanol system, the discs were carefully removed from drug solution after incubation and

https://doi.org/10.5920/bjpharm.2017.05

placed in deionised water (5.0 mL) for 30 sec to remove unbound drug. The discs were dried at ambient temperature (~25°C) under vacuum for 24 hours. With methanol system, the discs were removed from the incubation solution and placed in ethanol (4.0 mL) for 1 min to remove unbound drug. They were placed in deionised water (2.0 mL) for 4 hours and dried under vacuum for 24 hours. The amount of drug loaded for each disc was calculated as the difference in UV absorbance reading between the starting solution and the solution left after loading. The loading efficiency was also calculated using **Eq. 4**.

$$Load.eff. = \left(\frac{amount\ loaded}{actual\ amount\ in\ loading\ sol.}\right) \times 100$$

Eq. 4

HPLC method for dexamethasone

The mobile phase composed of acetonitrile and aqueous trifluoroacetic acid (TFA) solution (0.1% v/v) at 40:60 volumetric ratio. The flow rate was 1.0 mL/min with an injection volume of 10.0 µL, a UV detection wavelength of 240 nm and a temperature of 40°C. The retention time for dexamethasone was 4.8 min. A calibration curve with HPLC and UV were plotted with a drug concentration range of 3.0-100.0 µg/mL (R^2=1) and 1.5-25.0 µg/mL (R^2: 0.999) in water respectively.

Hydrogel release of dexamethasone

Release studies were performed in an in house flow rig model (**Fig. 2**) that mimics the bleb formed after GFS. There is no reported eye model to study the release of formulations in the anterior segment. Our rig models have been previously characterised and extensively used during the evaluation of formulations targeted to the anterior segment of the eye. The rigs were rinsed, cleaned and dried prior to each experiment. The model was disassembled by removing the screws. The rigs were assembled again after placing the drug loaded discs. All rigs were placed in a pre-heated oil bath at 37°C and temperature was monitored with a thermometer. The ports had a small inner diameter (0.92 mm) to ensure the size did not significantly enlarge the volume of the chamber. An inlet port allows a flow rate similar to that in the subconjunctival space (2.0 µL/min) (Brubaker 1982; Toris et al. 1999; Maurice 2001; Siggers & Ethier 2012; Ethier et al. 2004). Constant flow of PBS (pH 7.4, at 37°C) supplemented with sodium azide (0.02%) was provided. The volume (50-400 µL) and shape of blebs varies among individuals, therefore, to obtain consistent results the rigs were round with a capacity of 400 µL. An outlet port was present for sample collection and samples were quantified with HPLC (240 nm).

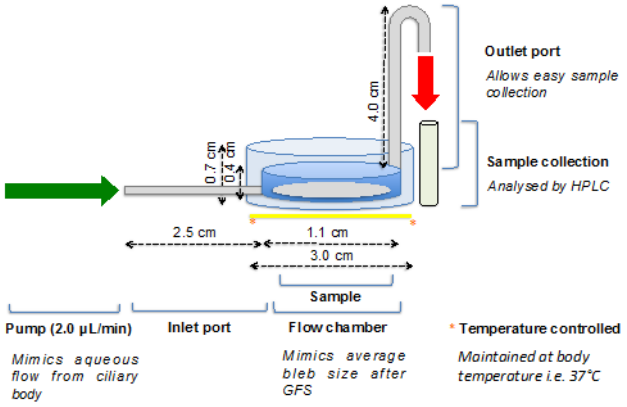


Fig. 2. *Schematic representation of the flow rig used for the release studies. Each hydrogel was placed into a rig, which was continuously supplied with phosphate buffered saline (PBS, pH 7.4, 37°C) at 2.0 µL/min using a peristaltic pump to mimic subconjunctival aqueous outflow. Aliquots were collected at predetermined time points from the outlet tube and analysed by HPLC.*

Data analysis

All results are presented as the mean and standard deviation (± STD) from triplicate (n=3) unless stated otherwise. Data was plotted using OriginPro 9.1 (software, Origin lab cooperation, USA) and the half-life ($t_{½}$) was calculated according to the best fitting model in OriginPro. First-order kinetic rate constants (k) were derived from the mono-exponential curve and $t_{½}$ was calculated with $t_{½}=0.693/k$. The analysis of variance (one-way and repeated measure ANOVA) with Tukey's post hoc test was conducted to evaluate differences between the experimental data (mean values) using OriginPro 9.1 and IBM SPSS statistics 23. Probability values less than 0.05 ($p<0.05$) were considered as indicative of statistically significant differences.

RESULTS AND DISCUSSION

Hydrogels derived from MPC and HEMA monomers that are crosslinked with PEGDA are hydrophilic. Total water content and distribution within a hydrogel are important properties needed to optimise drug loading and release properties. Variation of the

https://doi.org/10.5920/bjpharm.2017.05

structure and stoichiometry of either monomer or the crosslinker can influence hydrogel properties. Hydrogels were prepared via free radical polymerisation using AIBN and the relative MPC to HEMA monomer ratio was varied (**Fig. 1**).

Hydrogels were prepared using moulds to give flat sheets and hydrogel films were obtained after hydration. The relative monomer proportion of MPC was varied from 0 to 100%. Hydrogels prepared using 0 to 50% MPC (labelled S1 to S7, **Table 1**) were hard and glassy prior to hydration. One hydrogel (S8) prepared with 100% MPC was jelly-like in consistency and was not a hard film as a xerogel. The thickness of all of the xerogels (S1 to S7) was 1.1 ± 0.1 mm. When fully hydrated, formulations S1 to S6 remained intact as films, however the 50% MPC film (S7) was fragile and easily breakable. As an example of hydration time, the fully dried 10% MPC hydrogel requires at least 6 hours to reach maximum hydration and 30 minutes to reach around 50% of hydration when placed in PBS at 25 °C. The thickness of the hydrogel films increased with increasing MPC content upon hydration (**Table 1**) indicating that there was more water associated with MPC compared to HEMA.

The number and size of pores increased with increasing MPC (**Fig. 3A**). Larger pores would allow more water to pass through with a higher percentage of free water being entrapped within the films. The water content and mechanical strength are both important when considering an implantable hydrogel that must also release drug. As crosslinked materials, hydrogels can be susceptible to tearing, so we also characterised Young's modulus of the gels. Water content and knowledge of the bulk (free) and bound water are also important when considering the use of a hydrogel in drug delivery.

Mechanical properties results

A decrease in Young's modulus in the prepared hydrogels was seen with increasing relative amounts of MPC (**Fig. 3B**). These results are consistent with the previously reported data for contact lens (Hamilton & Pye 2008; Monti & Simonib 1992). The reported Young's modulus values were 0.8 and 0.5 MPa for HEMA and Proclear® contact lenses with 15% MPC respectively (Young et al. 2010; Tranoudis & Efron 2004). High MPC containing hydrogels (50%, S7) easily broke apart upon hydration. The zwitterionic charged MPC phosphoryl choline pendent chains readily associate with water and may not sufficiently interact with the hydroxyethyl pendent chains from HEMA. The presence of PEGDA derived crosslinks also inhibits interactions between polymer chains. Increased internal water reduces polymer-polymer chain association resulting in reduced mechanical strength of the formed gels (Monti & Simonib 1992).

Water distribution measurements

The effects of MPC on the bound to bulk water ratio were determined by DSC (**Fig. 3C** and **Table 2**). As the relative percentage of MPC increased there was an increase in the bulk free water ratio compared to bound water. The increased amount of zwitterionic MPC pendent chain results in an increase in bulk water (Shi et al. 2012; Chen et al. 2010). The primary hydroxy group in the HEMA pendent chain can also form hydrogen bonds with water. As the hydrogel becomes more hydrated due to increased bound water due to MPC, there may also be increased solubilisation of the HEMA pendent chain to further increase the bulk water content of the hydrogel.

Equilibrium water content (EWC) and swelling ratio (SR) measurements

To better quantitate water content, the EWC (left panel) and the SR (right panel) of the prepared hydrogels (S1-S7) were determined (**Fig. 3D**). There was significant increase ($p<0.05$) in both EWC and SR with increasing MPC. In other MPC co-polymers, a dramatic increase in water content was observed when MPC was copolymerised with hydrophobic monomers such as *n*-butyl methacrylate (Ishihara et al. 1990). The zwitterionic phosphoryl choline pendent chains of MPC results in bound water associations and this bound water forms hydrogen bonds with other water molecules. There is essentially a continuum of bound water to less bound and then to bulk water. It is thought that most water is in the free (bulk) form (Morisaku et al. 2008) in MPC rich polymers. The hydroxyethyl pendent chain on HEMA once incorporated into a polymer is also hydrophilic, but less so than the phosphoryl choline MPC pendent chain. The amount of MPC dominates the changes in overall water content in these HEMA-MPC hydrogels.

https://doi.org/10.5920/bjpharm.2017.05

Mixed aqueous solvents on the swelling ratio (SR)

Some drugs are more soluble in water miscible solvents such as methanol and ethanol than in water alone. Polymer pendent chain solubilisation and solvent association properties in the hydrogels would be expected to differ in these alcoholic solvents. The SR as a function of the relative amount of MPC was also determined in methanol, ethanol, water: methanol (1:1) and water:ethanol (1:1) (**Fig. 4** and **Table S1**).

Table 2. *The ratio of bound to free water in a hydrogel films based on MPC%*

MPC (%)	0	5	10	15	20	30	50
Wb:Wf	1:0.3	1:1.2	1:1.8	1:2.5	1:3.2	1:4.1	1:6.8

Abbreviation: *Wb: bound water and Wf: free water*

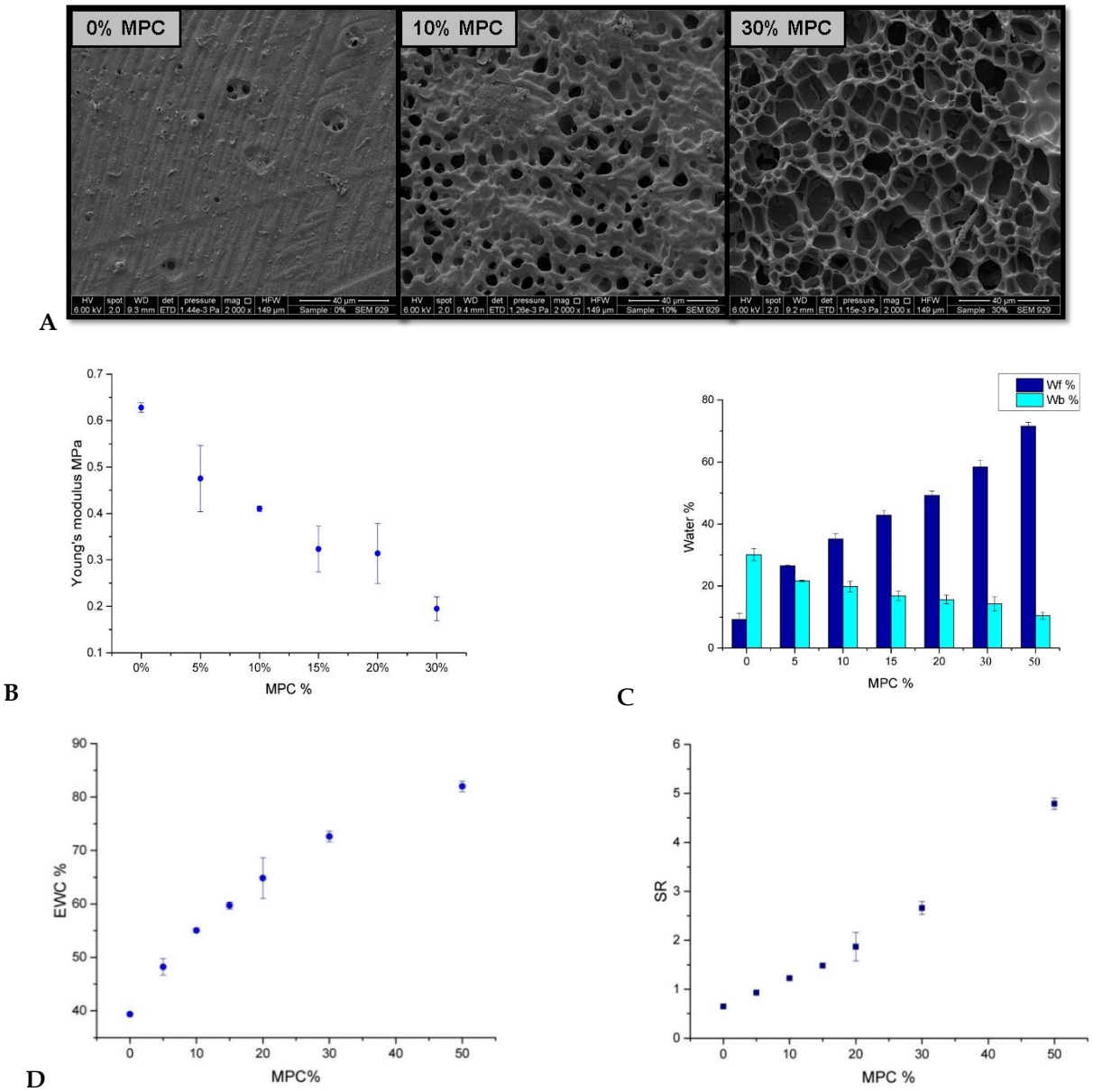


Fig. 3. *The effect of MPC% on (A) pore size analysed by SEM (scale bar is 40 µm), (B) Young's modulus values, (C) free bulk water (Wf) and bound water (Wb) and (D) EWC % as measured using eq. 2 and (right panel) on SR as measured using eq. 3. All results are displayed as the average of the triplicate (n=3) and standard deviation (±STD).*

https://doi.org/10.5920/bjpharm.2017.05

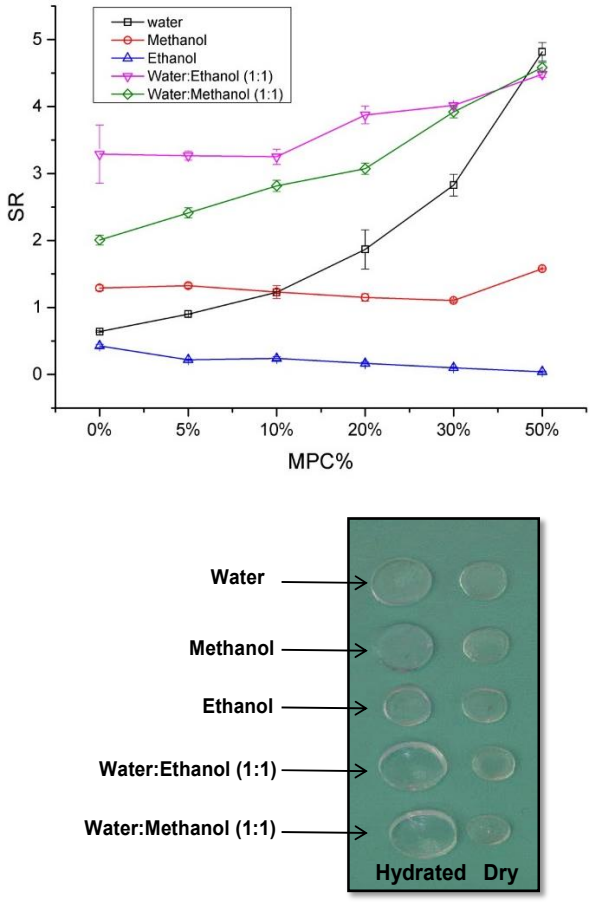


Fig. 4. *The effect of solvent used on SR of films (n=3 ±SD) with different percentages of MPC (top) and example SRs of 10% MPC in the different solvents (bottom).*

The methanol SR was greater than the ethanol SR. There was little variation in the methanol SR with increasing MPC monomer content while the ethanol SR slightly decreased with increasing MPC monomer content. The methanol and ethanol SRs contrasts with the SR of water, which increased with increasing MPC. The water SR became greater than the methanol SR at relative MPC monomer contents above 10%. In 100% MPC hydrogels both methanol and ethanol were also taken up less than water alone (Kiritoshi & Ishihara, 2003).

The 1:1 mixed alcohol-water SRs were higher than water up to 50% MPC and also displayed increasing SR with increasing MPC content. Bulk alcohol association around the zwitterionic MPC pendent chain is thought to be less than water association due to decreased hydrogen bonding of the alcohol. It is possible that the ethyl-hydroxy HEMA pendent chains are better solubilised by methanol and ethanol than water. The increased solubilisation of the ethyl-hydroxy HEMA pendent chains by alcohol allowed the influx of miscible water resulting in greater SRs up to the threshold SR at 50% MPC monomer incorporation. The implication is that water is better associated with the MPC pendent chain and that alcohol is better associated with the HEMA pendent chain.

Dexamethasone loading and *in vitro* release of hydrogel films

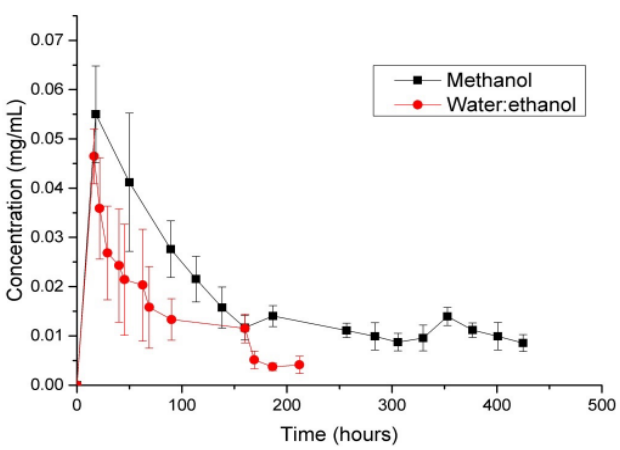
High water content and a low mechanical strength were observed for the 20 and 30% MPC films. Suboptimal mechanical strength is not desired for an implantable device. Although there was no significant difference in the EWC% and SR of 10 and 15% MPC films, the 15% MPC films displayed more free water compared to bound water, which is thought to decrease the mechanical strength of the hydrogel. The 10% MPC film was thus selected for further study to determine the release profile of dexamethasone.

In an effort to increase the drug loading and to prolong the release of dexamethasone, the discs were incubated in a dexamethasone-methanol solution (15 mg/mL, volume: 1.0 mL). The dexamethasone saturated HEMA-MPC discs were rinsed with water (1.0 mL for 4 hours) to remove methanol. Removal of methanol resulted in the precipitation of dexamethasone inside the disc. The precipitated dexamethasone within the HEMA-MPC hydrogel disc created a depot. The initial amount of dexamethasone loaded in each disc before washing with water was 2.1 mg. Dexamethasone lost during washing was 0.2 mg (10% of the loaded drug). The final loading after washing was 1.9 mg which is 6 times higher compared to using water:ethanol as loading solution. The release of dexamethasone was also sustained and the $t_{½}$ was 9.7 days. The depot created with dexamethasone helped to prolong dexamethasone release in a more controlled manner.

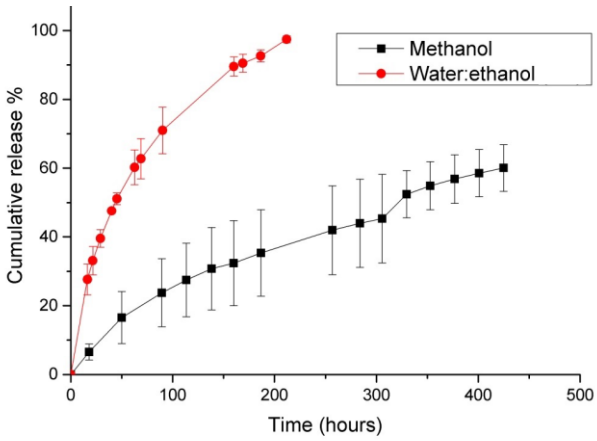
Storage and delivery of hydrogel films

One of the problems associated with hydrogels is their delivery in the clinic and storage stability prior to surgery. A dry 10% MPC hydrogel requires 30 mins to reach approximately 50% hydration at least 6 hours to reach maximum hydration when placed in PBS at 25°C (**Fig. S2**). Although placing a hydrogel in a storage solution will be more convenient for use in the

https://doi.org/10.5920/bjpharm.2017.05



Concentration (mg/mL)



Cumulative release (%)

Fig. 5: *HPLC results of in vitro release of dexamethasone from 10% MPC films using methanol versus water:ethanol. All results are displayed as the average of the triplicate (n=3) and standard deviation (±STD).*

Table 3*. The amount loaded and loading efficiency of DEX in 10% MPC discs calculated using two different methods (UV and extraction).*

Method	**Amount loaded (mg)**	**Loading efficiency** (%)
UV	0.3 ± 0.03	30.0
Extraction with methanol	0.4 ± 0.10	39.0

Note: *No significant difference (p>0.05) was observed in the amount loaded and loading efficiency between the two methods.*

clinic, the stability of loaded drugs inside a hydrated hydrogel would be a problem. Some of the drug loaded will diffuse from the hydrogel matrix to the storage solution during storage and reduce the amount loaded. The best method is to deliver the hydrogel spacer in dry form with instructions to rehydrate them in 0.5 mL sterilised water for 1 hour prior to use to avoid drug hydrolysis. The hard glassy nature of dry hydrogels may cause irritation and discomfort if placed in dry form so rehydration for 1 hour prior to use would ensure at least 50% of swelling is achieved without drug loss.

CONCLUSION

Hydrogels derived from HEMA-MPC crosslinked with PEDGA were made by free radical polymerisation using varying amounts of MPC ranging from 0 to 100%. The pore size of the hydrogel films increased with increasing MPC, and hydrated films were difficult to fabricate with hydrogels made with greater than 30% MPC. Increased MPC also resulted in an increased SR and EWC%, but with lower water permeability. It was found that 10% MPC was optimal for further evaluation with dexamethasone using two different solvents to maximise drug loading. It is known that the use of this amount of MPC is enough to impart biocompatibility properties, which can be beneficial during GFS. The loaded dexamethasone increased from 0.3 to 1.9 mg/disc when the solvent changed from water:ethanol (1:1) to methanol, with an *in vitro* $t_{½}$ increased from 1.9 to 9.7 days respectively. The results obtained in this study demonstrate the possibility of extending the duration of action of a hydrogel formulation of dexamethasone by increasing the drug loading within the hydrogel.

ACKNOWLEDGMENTS

A.S. is grateful for funding from HCED Iraq, S.A. gratefully acknowledges funding from the UCL Overseas Research Student Fund. We are also grateful for funding from the National Institute of Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, Moorfields Special Trustees, the Helen Hamlyn Trust (in memory of Paul Hamlyn), Medical Research Council, Fight for Sight and Michael and Ilse Katz Foundation.

https://doi.org/10.5920/bjpharm.2017.05

REFERENCES

Bettin, P. & Di Matteo, F., 2013. Glaucoma: Present challenges and future trends. *Ophthalmic Research*, 50(4), pp.197–208.

Brubaker, R.F., 1982. The flow of aqueous humor in the human eye. *Transactions of the American Ophthalmological Society*, 80, pp.391–474.

Chen, S. et al., 2010. Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials. *Polymer*, 51(23), pp.5283–5293.

Chong, R.S. et al., 2013. Patient Acceptance and Attitude Toward an Alternative Method of Subconjunctival Injection for the Medical Treatment of Glaucoma. *Journal of Glaucoma*, 22(3), pp.190–194.

Cui, L. et al., 2008. Subconjunctival sustained release 5-fluorouracil for glaucoma filtration surgery [1]. *Acta Pharmacologica Sinica*, 29(9), pp.1021–1028.

Dhingra, S. & Khaw, P., 2009. The Moorfields Safer Surgery System. *Middle East African Journal of Ophthalmology*, 16(3), p.112.

Du, W. et al., 2013. The Effect of Ocular Pigmentation on Transscleral Delivery of Triamcinolone Acetonide. *Journal of Ocular Pharmacology and Therapeutics*, 29(7), pp.633–638.

Ethier, C.R., Johnson, M. & Ruberti, J., 2004. Ocular biomechanics and biotransport. *Annual review of biomedical engineering*, 6, pp.249–73.

Hamilton, K.E. & Pye, D.C., 2008. Young's modulus in normal corneas and the effect on applanation tonometry. *Optometry and vision science : official publication of the American Academy of Optometry*, 85(6), pp.445–50.

Hoffman, A.S., 2012. Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, 64, pp.18–23.

Ishihara, K., Ueda, T. & Nakabayashi, N., 1990. Preparation of Phospholipid Polylners and Their Properties as Polymer Hydrogel Membranes. *Polymer Journal*, 22(5), pp.355–360.

Jhon, M.S. & Andrade, J.D., 1973. Water and hydrogels. *Journal of biomedical materials research*, 7(6), pp.509–22.

Kang-Mieler, J.J., Osswald, C.R. & Mieler, W.F., 2014. Advances in ocular drug delivery: emphasis on the posterior segment. *Expert opinion on drug delivery*, 11(10), pp.1647–60.

Kimura, H. et al., 1992. Injectable microspheres with controlled drug release for glaucoma filtering surgery. *Investigative Ophthalmology and Visual Science*, 33(12), pp.3436–3441.

Kiritoshi, Y. & Ishihara, K., 2003. Molecular recognition of alcohol by volume phase transition of cross-linked poly(2-methacryloyloxyethyl phosphorylcholine) gel. *Science and Technology of Advanced Materials*, 4(2), pp.93–98.

Kuppermann, B.D. & Loewenstein, A., 2010. Drug Delivery to the Posterior Segment of the Eye. In *Macular Edema*. Basel: KARGER, pp. 59–72.

Lee, H., Jhon, M. & Andrade, J., 1975. Nature of water in synthetic hydrogels. I. Dilatometry, specific conductivity, and differential scanning calorimetry of polyhydroxyethyl methacrylate. *Journal of colloid and interface science*, 51(2), pp.225–231.

Lewis, A. et al., 2008. Poly(2-methacryloyloxyethyl phosphorylcholine) for protein conjugation. *Bioconjugate Chemistry*, 19(11), pp.2144–2155.

Lewis, A.L., 2000. Phosphorylcholine-based polymers and their use in the prevention of biofouling. *Colloids and Surfaces B: Biointerfaces*, 18(3–4), pp.261–275.

Maurice, D., 2001. Review : Practical Issues in Intravitreal Drug Delivery. *Journal of ocular pharmacology and therapeutics*, 17(4), pp.393–401.

Monti, P. & Simonib, R., 1992. The role of water in the molecular structure and properties of soft contact lenses and surface interactions. *Journal of Molecular Structure*, 269, pp.243–255.

Morisaku, T. et al., 2008. Hydration of phosphorylcholine groups attached to highly swollen polymer hydrogels studied by thermal analysis. *Polymer*, 49(21), pp.4652–4657.

Parkinson, G. et al., 2012. Characterisation of Ilomastat for Prolonged Ocular Drug Release. *AAPS PharmSciTech*, 13(4), pp.1063–1072.

Rohindra, D., Nand, A. & Khurma, J., 2004. Swelling properties of chitosan hydrogels. *The South Pacific Journal of Natural and Applied Sciences*, 22(1), pp.32–35.

Schlenoff, J.B., 2014. Zwitteration: Coating surfaces with zwitterionic functionality to reduce nonspecific adsorption. *Langmuir*, 30(32), pp.9625–9636.

Shi, D. et al., 2012. Synthesis and biocompatibility of phosphoryl polymer and relationship between biocompatibility and water structure. *Polymer Science Series B*, 54(5–6), pp.335–341.

Siggers, J.H. & Ethier, C.R., 2012. Fluid Mechanics of the Eye. *Annual Review of Fluid Mechanics*, 44, pp.347–372.

Skuta, G.L. et al., 1992. Intraoperative Mitomycin versus Postoperative 5-Fluorouracil in High-risk Glaucoma Filtering Surgery. *Ophthalmology*, 99(3), pp.438–444..

Stirbu, O. et al., 2011. A new implant for deep sclerectomy: Esnoper®. *Journal of Current Glaucoma Practice*, 5(3), pp.40–43.

Tomar, N. et al., 2012. pHEMA hydrogels: Devices for ocular drug delivery. *International Journal of Health & Allied Sciences*, 1(4), p.224.

Toris, C.B. et al., 1999. Aqueous humor dynamics in the aging human eye. *American journal of ophthalmology*, 127(4), pp.407–12.

Tranoudis, I. & Efron, N., 2004. Tensile properties of soft contact lens materials. *Contact Lens and Anterior Eye*, 27(4), pp.177–191.

Vashist, A. et al., 2014. Recent advances in hydrogel based drug delivery systems for the human body. *J. Mater. Chem. B*, 2(2), pp.147–166.

Weinreb, R.N. & Khaw, P.T., 2004. Primary open-angle glaucoma. *Lancet (London, England)*, 363(9422), pp.1711–20.

Young, G. et al., 2010. The effect of soft contact lens care products on lens modulus. *Contact lens & anterior eye : the journal of the British Contact Lens Association*, 33(5), pp.210–4.

https://doi.org/10.5920/bjpharm.2017.05

SUPPLEMENTARY MATERIAL

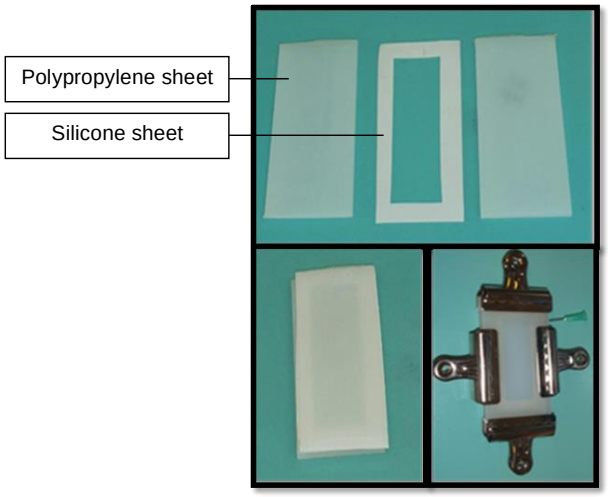


Fig. S1*. The mould used for the hydrogel fabrication before and after assembly. The mould consists of two polypropylene sheets and one silicone sheet sandwiched between them.*

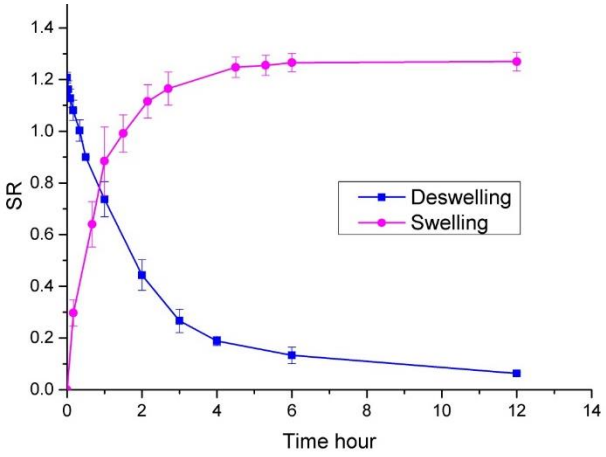


Fig. S2. The swelling and deswelling behaviour of 10% MPC hydrogel. All results are displayed as the average of the triplicate (n=3) and standard deviation (±STD).

Table S1*. The swelling ratio (SR) of S1 to S6 films in different solvent and solvent systems*

Solvent system	MPC (%)					
	0	**5**	**10**	**20**	**30**	**50**
Water	0.6 ± 0.01	0.9 ± 0.003	1.2 ± 0.02	1.9 ± 0.3	2.8 ± 0.2	4.8 ± 0.1
Methanol	1.3 ± 0.04	1.3 ± 0.03	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.02	1.6 ± 0.01
Water: Methanol (1:1)	2.0 ± 0.07	2.4 ± 0.1	2.8 ± 0.1	3.1 ± 0.1	3.9 ± 0.1	4.6 ± 0.1
Water: Ethanol (1:1)	3.3 ± 0.4	3.3 ± 0.1	3.3 ± 0.1	3.9 ± 0.1	4.0 ± 0.03	4.5 ± 0.1
Ethanol	0.4 ± 0.03	0.2 ± 0.01	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.01	0.04 ± 0.01