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Composite Bi-layered Erodible Films for Potential Ocular Drug Delivery

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Abstract

Bi-layered hydroxypropylmethylcellulose and Eudragit based films were formulated as potential ocular drug delivery systems using chloramphenicol as a model antibiotic. Films were plasticized with polyethylene glycol 400 present in the Eudragit layer or both Eudragit and hydroxypropylmethylcellulose layers, and loaded with chloramphenicol (0.5% w/v in solution) in the hydroxypropylmethylcellulose layer. The weight, thickness and folding endurance optimized formulations were measured and further characterized for transparency, tensile, mucoadhesive, swelling and *in vitro* drug dissolution properties. The physical form of chloramphenicol within the films was evaluated using differential scanning calorimetry (DSC), and X-ray diffraction (XRD), complimented with scanning electron microscopy and energy dispersive X-ray spectroscopy. Fourier transform infrared spectroscopy was used to assess the interactions between the drug and the film components and confirm chloramphenicol's presence within the sample. Optimum films showed high transparency (≥ 80% transmittance), ease of peeling from Petri dish and folding endurance above 250. Average thickness was lower than contact lenses (0.4 - 1mm), confirming them as thin ocular films. The tensile properties showed a good balance between toughness and flexibility and mucoadhesivity showed that they could potentially adhere to the ocular surface for prolonged periods. The drug loaded films showed swelling capacity which was greater than 300% of their original weight. The physical form of chloramphenicol within the films was amorphous (DSC and XRD) whilst *in vitro* drug dissolution showed sustained drug release from the films for four hours, before complete erosion. The chloramphenicol loaded films represent a potential means of treating common eye infections.

Keywords: Antibacterial, bi-layered films, mucoadhesion, *in vitro* drug release, ocular delivery, plasticizer.

1 Introduction

Vision provides 90% of the information within our surrounding environment, with considerable physiological importance including differentiation between light, shape and colour, spatial orientation, equilibrium and cortical tone [1]. Various conditions can affect the eyes and these are classified as periocular and intraocular, according to their location. Periocular conditions occur around the eye and can cause irritation to different parts of the eye. Common periocular diseases include blepharitis, conjunctivitis and chronic conditions caused by bacteria which can even lead to vision impairment [2]. Effective reduction of bacterial load is very important in the treatment of ocular diseases caused by infection.

The development of ocular drug delivery systems is challenging because of the eyes' complex anatomic structure and protective mechanisms which make it difficult to maintain an effective drug concentration over a prolonged period of time [3-8]. The eye is a very sensitive organ to debris, microorganisms and drugs and therefore, ocular drug delivery systems should be simple, non-invasive (to prevent irritation, inflammation, infection), maintain visual clarity, and enable the drug to penetrate the physiological eye barriers to reach the site of action [9].

Ocular drug delivery for treating conditions affecting the front of the eye depends on the corneal barrier and tear film. Apart from the physiological factors, there are also factors affecting formulation development, of ophthalmic preparations including osmolality, pH, surface tension and viscosity [10].

Topical eye drops represent the most convenient formulation among patients, especially for conditions affecting the anterior segment of the eye. However, only 5% of the instilled dose can penetrate the ocular pre-corneal, dynamic and static barriers, while constant and prolonged drug release cannot be achieved [11]. Though gels and ointments can remain for relatively longer periods, they are quickly diluted by the tear fluid and leak out, therefore reducing bioavailability [12]. Ocular inserts such as films have been developed, which are expected to

maintain the drug on the eye surface for a relatively prolonged period better than drops, gels and ointments. These increase the contact time with the ocular surface and therefore prolong drug delivery, reduce systemic effects, improve patient compliance and increase bioavailability [12].

Erodible films are made of polymers that can be natural, synthetic or semi-synthetic and that provide support for loaded drug. The polymers used need to be bio-compatible, safe, non reactive, stable and mucoadhesive, and release drug appropriately [13]. The drugs contained within the films are usually in the form of a dispersion within the matrix whilst maintaining film clarity [14]. Acyclovir, phenylepherine, diclofenac sodium, and antibiotics are examples of drugs that can be contained within the ocular inserts. All drugs need an appropriate balance between lipid and water solubility for effective corneal permeation. In addition, films generally need a plasticiser to improves their flexibility and reduce the chances of contact irritation due to britleness.

In the present study, bi-layered erodible ocular films were prepared by solvent casting technique from solutions using hydrophobic Eudragit (EUD) and hydrophilic hydroxypropylmethylcellulose (HPMC). These polymers are safe and biocompatible, stable, mucoadhesive and provide sustained drug release *in vitro*, making them suitable for ocular delivery [15]. Polyethylene glycol 400 was used as a plasticiser in either the EUD or both polymeric layers to increase the flexibility of the films.

Chloramphenicol (CHF) was used as model drug and exhibits broad-spectrum antibacterial activity [16] against Gram-positive bacteria (Staphylococcus *aureus* and, Streptococcus *pyogenes*) and Gram-negative bacteria *Haemophilus influenza* and *Neisseria meningitides*) common in ocular infections such as conjunctivitis [17]. Due to its high lipophilicity, CHF can easily penetrate the ocular barriers, and therefore very effective against ocular infections [17] However, its high lipid solubility facilitates easy absorption into the

systemic circulation and side effects such as aplastic anaemia can occur with prolonged exposure. Therefore, CHF is safely and efficiently used in eye drops at of 0.5% w/v (approximately 5000µg/ml) this dose was employed in this study [18]. The MIC values of CHF against the above organisms range from 0.25 - 128µg/ml [19] which is far lower than the dose used in this study. Because the residence time of eye drops on the cornea is poor, the use of the 0.5% CHF dose within a mucoadhesive film which can prolong retention on the cornea and control drug release, is expected to overcome limitation of the former. Further, a more gradual release of CHF which prevents frequent administration, will reduce the incidence of side effects associated with CHF.

2 Experimental

2.1 Materials

Eudragit S100 (EUD) was obtained from Degussa (Germany), hydroxypropylmethylcellulose – HPMC (Methocel™ K100 Premium) was a gift from Colorcon Limited (Dartford, UK). PEG, methanol, absolute ethanol, acetone, isopropyl alcohol, acetonitrile (HPLC grade) and phosphoric acid (HPLC grade) were supplied from Fisher Scientific, (Leicestershire, UK). Chloramphenicol was obtained from Sigma-Aldrich (Gillingham, UK). Sodium chloride, potassium chloride, sodium phosphate dibasic anhydrous (99^+ % extra pure) and potassium phosphate dibasic anhydrous (99^+ % extra pure) were all obtained from Acros Organics Ltd (New Jersey, USA).

2.2 Film formulation development

Different ratios of EUD to HPMC were weighed and dissolved in different mixtures of water with acetone, isopropyl alcohol, ethanol and methanol to obtain the polymeric solutions. However, none of the mixtures resulted in a completely clear film due to the poor water

127 solubility of EUD. The development of a completely clear EUD film was therefore achieved
128 for a 2% w/v solution by dissolving the polymer in a mixture of acetone and isopropyl alcohol
129 (ratio 3:2). HPMC only solution (2% w/v) was obtained by dissolving the required amount of
130 polymer in deionised water. Bi-layered blank (BLK) films were subsequently prepared using
131 the two 2% w/v (HPMC and EUD) solutions.

132 Table 1 Preparation of (a) solutions (2% w/v) required for BLK HPMC-EUD and (b)
133 solutions (2% w/v) required for DL HPMC-EUD; bi-layered film formation.

134 (a)

EUD film				
	Polymer (g)	**PEG (g)**	**Acetone (ml)**	**Isopropyl alcohol (ml)**
EUD 1	2.007	2.001	60	40
EUD 2	2.005	1.001	60	40
HPMC film				
	Polymer (g)	**PEG used (g)**	**CHF (g)**	**Deionized water (ml)**
HPMC 1	2.002	-	-	100
HPMC 2	2.005	1.002	-	100

135
136 (b)
137

EUD film				
	Polymer (g)	**PEG (g)**	**Acetone (ml)**	**Isopropyl alcohol (ml)**
EUD 3	2.007	2.001	60	40
EUD 4	2.001	1.002	60	40
HPMC films				
	Polymer (g)	**PEG (g)**	**CHF (g)**	**Deionised water (ml)**
HPMC 3	2.000	-	0.50	100
HPMC 4	2.004	1.001	0.50	100

138

Subsequently, 10g of the HPMC solution was poured onto the water insoluble EUD films and left to dry at room temperature for a further 24 hours to obtain the final bi-layered film. The drug loaded (DL) bi-layered films were obtained as above but with CHF (0.5% w/v) loaded in the HPMC solution before pouring on the BLK EUD film. For plasticised films, PEG (1.0% w/v) was added into either the EUD only or split between EUD (0.5% w/v) and HPMC (0.5% w/v) solutions. After preparation, 10g of EUD solution was poured into glass a Petri dish (82mm diameter) and left at room temperature for 24 hours to obtain the first film layer. The BLK (HPMC-EUD-PEG & HPMC-PEG-EUD-PEG) and DL (HPMC-CHF-EUD-PEG & HPMC-PEG-CHF-EUD-PEG) films (Table 1) were peeled from the Petri dish, visually examined for any physical defects (e.g. patches, scratches, cracks and tears) and suitable films stored in desiccators over silica until ready for use.

2.3 Physical measurements

2.3.1 Film thickness and weight

Three film samples ($n = 3$) from each formulation were cut into 20 x 20mm^2 strips, individually weighed using a digital balance and average weight per mm^2 calculated. Thickness was measured using a Metric micrometre screw gauge at five different locations i.e. four corners and middle and the average thickness calculated [20].

2.3.2 Transparency test

Light transmittance was measured using a spectrophotometer (Model U-2900) set between the UV and IR range (300-1100nm). A 40 x 10mm^2 strip was cut from each formulation and introduced into the spectrophotometer cell and percentage transmittance measured at a scan speed of 400nm/min.

2.3.3 Folding endurance

A whole film removed from the Petri dish was used. Folding endurance was measured by repeatedly folding the film longitudinally at the same place until it broke [21], but no more than 250 times and recorded.

2.4.1 Texture analysis

2.4.1 Mechanical (tensile) properties

The tensile properties (% elongation at break, tensile strength and elastic modulus) were measured using a TA.HD.*plus* Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 5kg load cell. Films were cut into 50 x 10mm^2 rectangular strips and their thickness measured. Each strip was held between the tensile grips of a texture analyser positioned 30mm apart. The strips were pulled using a trigger force of 0.09N at a crosshead speed of 0.1mms^{-1} until they broke. The tensile properties were calculated using equations 1 - 3.

$$Tensile\ strength = \frac{Force\ at\ failure}{cross\text{-}sectional\ area\ of\ the\ film} \quad \text{Equation (1)}$$

$$Percent\ elongation\ at\ break = \frac{Increase\ in\ length\ at\ break}{Initial\ film\ length} \times 100 \quad \text{Equation (2)}$$

$$Elastic\ modulus = \frac{Slope\ of\ stres\text{-}strain\ curve}{Film\ thickness \times Cross\ head\ speed} \quad \text{Equation (3)}$$

2.4.2 In vitro mucoadhesion

Each formulation was cut into four 10 x 10mm^2 strips and attached onto the adhesive probe of a texture analyser equipped with a 5kg load cell, using double-sided adhesive tape. A model mucosa substrate in a Petri dish (86mm diameter), formed from a 6.67% w/v gelatine solution, allowed to set to form a solid gel, was equilibrated by spreading 20µl PBS solution

(pH 7.4) over its surface to simulate the ocular mucosa. The film attached to the probe was pressed onto the gelatine surface at a force of 0.5N, for 120 seconds (to allow proper contact with the gelatine layer) and detachment initiated [22]. The mucoadhesion strength was measured as the maximum force (F_{max}) required for detaching the sample from the gelatine surface [23].

2.5 Scanning electron microscopy (SEM)

SEM was used to analyse the surface morphology of the BLK and DL bi-layered films. Two small strips (0.5 x 0.5cm^2) were cut from each film, attaching to a sample holder and coating with chromium to make them conductive and protect from heat. Both EUD and HPMC layers were analysed, to observe differences in morphology. The images were acquired using a Hitachi SU8030 scanning electron microscope with electronic beam voltage of 1.0kV, working distance of 8mm at different magnifications (x 250, 1000, 5000 and 10000).

2.6 Analytical characterisation

2.6.1 Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy

FTIR spectroscopy was used to analyse the presence of CHF in the DL films as well as the chemical interactions between the drug and the polymers. Testing was performed on a Perkin Elmer ATR-FTIR machine (Model Spectrum Two), at a wavelength between 450 and 4000cm^{-1}. The BLK and DL films, and starting materials were analysed. Prior to sample analyses, a background scan was performed and subtracted from all sample spectra.

211 *2.6.2 Differential scanning calorimetry (DSC)*

212 DSC was used to assess the thermal behaviour of the BLK and DL films, and pure CHF.
213 A small sample (2-5mg) was placed into a 40µl aluminium pan and sealed hermetically. The
214 samples were heated between 25°C and 250°C at a rate of 10°C/min.

215

216 *2.6.3 X-ray diffraction (XRD)*

217 XRD was used to determine the physical form of BLK and DL films and CHF. The films
218 were cut into six pieces and placed on top of each other in the transmitter holder. The samples
219 were analyzed at an angular range of 4-45° 2θ, rotation of 15rpm and 0.6mm exit slit at
220 increments of 0.02° 2θ [24].

221

222 *2.6.4 Energy dispersive x-ray* (*EDX*) *spectroscopy*

223 A Hitachi SU8030 SEM equipped with a Thermo Fisher Scientific EDX was used to
224 analyse the energy spectrum of the samples. The analysis was performed at an accelerating
225 voltage of 8kV, working distance of 8mm and magnification of x9020, in order to determine
226 the chlorine atoms present on the surface of the DL films and therefore demonstrate the
227 distribution of CHF.

228

229 *2.7 Swelling capacity*

230 Swelling test was carried out to measure the swelling capacity of the optimum BLK and
231 DL films. The films were cut into 20 x 20mm^2 square strips and weighed accurately. Each strip
232 was immersed into a Petri dish containing 5ml PBS solution at pH 7.4 and incubated at
233 37±0.5°C [25]. At specific time intervals (10 minutes), the films were removed, blotted
234 carefully with tissue paper to remove the excess PBS solution and accurately weighed. Then
235 5ml of PBS solution was added to the previously swollen film and the procedure repeated till

no more increase in weight was observed. The swelling capacity (Q_S) was calculated for each time point using equation 4.

$$Q_s = \frac{W_t - W_0}{W_0} \times 100 \qquad \text{Equation (4)}$$

Where, W_t is the weight of the swollen sample and W_0 is the weight of the dried film.

2.8 In vitro drug dissolution studies

Prior to drug dissolution studies, HPLC was performed on an Agilent 1200 instrument, equipped with an auto sampler, using a 150mm × 4.6mm × 5µm column. The mobile phase comprised acetonitrile, deionised water and phosphoric acid – in volume ratio of 65: 35: 0.1 with flow rate of 1.0ml/min and detection wavelength set at 280nm. Initially, standard solutions 0.05, 0.10, 0.15, 0.20 and 0.25mg/ml of CHF in PBS solution (pH 7.4) were used to plot a calibration graph (R^2 = 0.997) and used to calculate % drug release. For the drug dissolution studies, each DL film was cut into 20 × 20mm^2 strips, weighed and placed in 20ml PBS, in a Petri dish. At pre-determined time intervals (0, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 minutes), 1.0ml aliquots were removed from each Petri dish and replaced with 1.0ml of fresh medium. The sampled solutions were analysed by HPLC as above.

3 Results and discussion

Ocular drug delivery systems need to possess functional characteristics such as being simple, non-invasive, maintaining visual clarity, allowing prolonged residence of the drug on the eye, to penetrate the physiological eye barriers and reach the site of action [9].

3.1 Formulation development and optimisation

Bi-layered, erodible HPMC and EUD films containing CHF were successfully developed and tested. These two polymers are well characterised for safety and

biocompatibility and known to be stable under normal processing and storage conditions. The 2% w/v EUD and HPMC solutions used to prepare films were easy to handle and no heat was required during their formation. EUD only films were too thin, and difficult to remove from the Petri dish, but the addition of an HPMC solution layer on top of the EUD film led to the formation of a bi-layered film that was easy to peel from the Petri dish. Unplasticised films were brittle and addition of PEG made them more flexible and transparent. Figure 1 shows digital images of the four optimum bi-layered (two BLK and two DL) formulations selected for further testing.

3.2 Physical measurements

3.2.1 Thickness and film weight

The thickness and weight of the optimized BLK and DL HPMC-EUD-PEG- and HPMC-PEG-EUD-PEG were measured. The average thickness of the films was between 12 and 17μm, which classifies them as thin ocular films, and expected to be comfortable to the eyes **Error! Reference source not found.**. Further, the weight/mm^2 increased as more components (PEG and CHF) were added to the HPMC solution layer. The starting weight/mm^2 for the BLK HPMC-EUD-PEG film was 14.5, which increased by 15.51% after the addition of PEG within the HPMC layer, 6.89% after the addition of drug and by 29.31% after the addition of both plasticiser and drug.

3.2.2 Transparency test

An effective ocular formulation is expected not to interfere with sight and vision of the patient to avoid non-compliance [27], therefore in addition to visual observation, transmittance was recorded to determine the transparency of the BLK and DL films. Within the visible spectrum (400 and 700nm), the light transmittance of all samples was above 80% and no major peak was

present within this range. The BLK and DL films with only the EUD layer plasticised (HPMC-EUD-PEG and HPMC-CHF-EUD-PEG respectively) had approximately 80% light transmission, while the BLK and DL films with both layers plasticised (HPMC-PEG-EUD-PEG and HPMC-PEG-CHF-EUD-PEG) was higher at 87.5%. Therefore, drug loading did not appear to significantly affect the clarity of the samples, whilst the addition of PEG in the formulation of both HPMC and EUD solutions led to a higher transparency, making plasticised films more suitable for ocular drug delivery. Though the films were clear when observed visually, further improvement of transparency to 96%, to match that of contact lenses, will be ideal **Error! Reference source not found.**. This may be achieved by the addition of more plasticiser in both polymeric layers.

3.2.3 Folding endurance

Optimized BLK and DL films were longitudinally folded at the same place to assess their resistance to folding during repeated handling. None of the films broke after being folded 250 times, implying good resistance to handling, which is an essential functional characteristic for any ocular drug delivery system which must be safe to the patient if it needs to be removed and reinserted [29] while remaining intact. The folding endurance is also an indication of mechanical resistance to deformation, however, to more accurately determine the mechanical properties of the films, tensile properties were measured using a texture analyser.

3.3 Texture analysis

3.3.1 Mechanical (tensile) properties

The tensile properties (tensile strength, elastic modulus and % elongation at break) of the BLK and DL EUD-HPMC films are shown in Figure 2a. All the films had % elongation values within the reported ideal range of 30 – 50% [30] where films are neither too brittle nor too

sticky, which is important to avoid breaking or difficulty in handling. The highest value for elastic modulus was achieved by the BLK HPMC-EUD-PEG films. The values of elastic modulus showed that addition of PEG in both EUD and HPMC layers made the films more flexible, which was also confirmed by the higher values of % elongation at break for these films. Addition of CHF made them more flexible with HPMC-PEG-CHF-EUD-PEG having better tensile properties compared to HPMC-CHF-EUD-PEG film due to the addition of PEG in both polymeric layers of the former. This resulted in better elasticity, decreased stiffness and brittleness, making it more suitable for ocular drug delivery as its flexibility makes it less likely to cause contact irritation, therefore potentially providing better patient compliance [31]. Plasticisers generally act by interrupting the intermolecular interactions between polymer chains and increasing the specific volume, thus increasing their molecular mobility and therefore making them more flexible [32].

3.3.2 In vitro mucoadhesion studies

Mucoadhesion strength was measured using the gelatine layer, to simulate bioadhesion of the films to the ocular surface. As shown in Figure 2b, the highest detachment force necessary to remove the film from the gelatine layer was observed for BLK and DL films with only the EUD layer plasticised (HPMC-EUD-PEG and HPMC-CHF-EUD-PEG). This is interesting and appears to confirm the swelling capacity results below. This is because the initial stages of mucoadhesion involve polymer hydration and swelling which enhance the inter-diffusion process, allowing physical entanglement and enhanced surface availability for hydrogen bonding and electrostatic interaction between the polymeric chains and the model mucosal substrate.

Overall, the average detachment force required for all samples was higher than the force (0.2N) required by the eyelids to blink, implying that the formulated films will not be easily

dislodged by blinking. In addition, due to their thin nature, the hydrated ocular films are expected to be comfortable to patients though this will need to be confirmed in an *in vivo* study.

3.4 Scanning electron microscopy (SEM)

The SEM images for CHF confirmed its crystalline structure, as shown in Figure 3a. The images obtained from the surface of both sides of the BLK films, shown in the Figure 3 (b & c) revealed the presence of flat and irregular three-dimensional crystals on the surface of the HPMC layer. These may be due to the incomplete dissolution of HPMC in water, or the slow drying process at room temperature, which allowed some of the dissolved polymer to recrystallize on the surface as HPMC is known to be semi-crystalline. The EUD layer was smooth, possibly due to the complete dissolution of the polymer in organic solvents during formulation. The SEM images obtained for the surface of the DL HPMC-EUD films shown in Figure A1 (d – g) of the appendix revealed the presence of needle shaped structures, which may be CHF crystals. The EUD layer remained smooth, confirming the possibility of complete dissolution of EUD within the acetone and isopropyl alcohol.

SEM of the HPMC layer of DL films appears to contradict the results from DSC and XRD testing, which showed that the drug was possibly changed to its amorphous form or molecularly dispersed during the formulation process. Further analysis of the needle-shaped crystals was therefore necessary in order to determine whether they represent another crystalline form of CHF, and this was done using EDX analysis.

3.5 Analytical characterisation

3.5.1 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra obtained for the BLK and DL films are shown in Figure A2 of the appendix, showing similar peak patterns, due to similar formulation components (HPMC and EUD). The peak present around 3500cm^{-1} is attributed to the presence of the OH group of

HPMC. The C=O group of EUD was observed around 1750cm^{-1} and the peaks present around 1000cm^{-1} is due to the presence of the C-O bond, from HPMC and EUD. The addition of PEG in both polymeric layers and of CHF resulted in additional sharp peaks. A peak was observed at 1522cm^{-1} for only DL films, and attributed to the aromatic ring present in CHF, suggesting little or no interaction between the drug and the polymer within the HPMC layer.

3.5.2 Differential scanning calorimetry (DSC)

Figure 4 shows the DSC thermograms obtained for CHF and BLK and DL bi-layered HPMC-EUD films. The sharp peak for pure CHF (Figure 4a) revealed its crystalline nature and its melting point matched that previously reported at 159.06°C [33]. The thermograms for the BLK and DL films (Figure 4b) showed no obvious sharp peaks expected for crystalline materials, suggesting that CHF was converted to the amorphous form or molecularly dispersed within the polymeric matrix. It is also possible that the amount of CHF present in the small 2-5mg sample analysed was too low to allow detection. The BLK films showed a broad endothermic peak beyond 210°C which was not found in the corresponding DL films. This might relate to the presence of free PEG which was not available in the DL films due to the presence of CHF.

3.5.3 X-ray diffraction (XRD)

XRD diffractograms for the BLK and DL films are shown in Figure 5a. The diffractogram obtained for CHF (data not shown) clearly showed its crystalline nature with sharp peaks comparable with reference peaks in the instrument database. For the BLK HPMC-EUD film samples, the diffractograms showed a typically amorphous pattern with a broad peak. The analysis of the DL HPMC-EUD films gave similar results, confirming the DSC results that

385 the drug was possibly changed to its amorphous form or molecularly dispersed during film
386 formulation.
387

3.5.4 Energy dispersive x-ray spectroscopy (EDX)

EDX spectroscopy confirmed the presence of chlorine and nitrogen atoms within pure CHF. However, it did not confirm the presence of the drug in the DL film, as no chlorine or nitrogen atoms were detectable (Figure 5b). This may be due to the low amount of CHF within the small section of the films cut for analysis, which was therefore not detectable, or possibly due to the fact that the molecular dispersion of CHF within the polymer matrix caused the chlorine and nitrogen atoms to be shielded by significantly high amounts of polymer chains surrounding them.

3.6 Swelling capacity

The initial hydration and swelling of a film are important functional characteristics as they significantly affect other important properties such as mucoadhesion and drug release [34]. The results (Figure 6a) showed that HPMC-CHF-EUD-PEG film hydrated and swelled most rapidly, showing a maximum swelling of 557%. The swelling capacity for the DL films (HPMC-PEG-CHF-EUD-PEG and HPMC-CHF-EUD-PEG) were higher compared to the corresponding BLK films. The higher swelling capacity of DL films can be attributed to the release of CHF from the HPMC layer which allowed further ingress of more fluid into the swollen matrix. Comparing the two DL films, the formulation with both layers plasticised remained intact for a longer period of time, representing an optimum ocular drug delivery system. In addition, all films completely disintegrated after 270 minutes, meaning that they are erodible and will be able to provide sustained drug release for at least 4 hours.

3.7 In vitro drug dissolution studies

Drug dissolution profiles of both DL HPMC-EUD films loaded with CHF was monitored using PBS (pH 7.4), to simulate the ocular tear fluid. Both DL films had similar

drug dissolution profiles (Figure 6b), showing a biphasic release, with an initial phase where approximately 60% of the drug was released from the matrix within the first two hours, followed by a second sustained release phase. The initial 60% released corresponds to 2273µg of CHF which is significantly higher than the MIC of the drug required to kill bacteria which cause common eye infections such as conjunctivitis. However, this will require confirmation in a future *in vitro* antibacterial assay. Further, up to 70% of the drug will be released from the polymeric matrix before the film completely erodes. This bi-phasic release make the films suitable ocular drug delivery systems offering sustained release up to 4 hours, until they completely erode, and therefore more efficient against bacteria in comparison with conventional eye drops and ointments. The plasticiser in both polymeric layers was associated with a slightly reduced drug release, due mainly to the interaction between PEG and the polymers suggesting that it might be better to have the PEG present in just one layer.

Other studies [35, 36] have reported the use of drug-soaked soft contact lenses to deliver appropriate amounts of drug, however, though these offer an improvement over eye drops, they have limitations. Firstly, drug loading capacity is dependent on equilibrium drug solubility within the lens. Drug diffusion from the stock solution does not happen instantly and requires several hours for complete saturation. Further, drug concentration in the original solution must be higher than the final soaked lens to maintain effective diffusion gradient, resulting in drug being wasted and therefore uneconomical [37]. Finally, the lens will require re-soaking in drug solution after a short period. On the other hand, the erodible films degrade slowly and therefore can remain on the eye for four hours but without the need for removal. More advanced options include imprinted and liposome loaded lenses or films for sustained drug delivery, however, these are more complex to formulate and more likely to be significantly more expensive than eye drops. The bi-layered films are are simple and cost effective and therefore an ideal alternative to eye drops.

4 Conclusion

Bi-layered ocular films were successfully prepared using hydrophobic EUD and hydrophilic HPMC. The HPMC-CHF-EUD-PEG film was the most suitable for ocular drug delivery, having good physicochemical properties, providing sustained drug release, expected to maintain the drug on the eye surface for a relatively prolonged period and also expected to completely erode. The DL films have great potential as ocular drug delivery system for treating common eye infections as they are expected to be easy to use, comfortable, and efficient and their removal would not be necessary. However, an antibacterial study of these films is essential to determine the efficiency of CHF against bacteria that affect the eyes.

5 References

[1] Cristescu, D, Salavastru, C., Voiculescu, B., Niculescu, C. and Carmaciu, R. (2008). *Biologie*. 1st ed. Bucuresti: Corint, 44-48.

[2] Pflugfelder, S., Karpecki, P. and Perez, V. (2014). Treatment of Blepharitis: Recent Clinical Trials. *The Ocular Surface*, 12(4), 273-284.

[3] Hermans, K., Van den Plas, D., Kerimova, S., Carleer, R., Adriaensens, P., Weyenberg, W. and Ludwig, A. (2014). Development and characterization of mucoadhesive chitosan films for ophthalmic delivery of cyclosporine A. *International Journal of Pharmaceutics*, 472(1-2), 10-19.

[4] Pahuja, P., Arora, S., Pawar, P. (2012). Ocular drug delivery system: a reference to natural polymers. *Expert Opinion on Drug Delivery*, 9(7), 837–861.

[5] Nisha, S., Deepak, K. An insight to ophthalmic drug delivery system. (2012). *International Journal of Pharmaceutical Studies Research*, 3(2), 9–13.

[6] Gaudana, R., Jwala, J., Boddu S.H.S., Mitra, A.K. (2009). Recent perspectives in ocular drug delivery. *Pharmaceutical Research*, 26(5), 1197–1216.

[7] Rajasekaran, A., Kumaran, K.S.G.A., Preetha, J.P., Karthika, K. (2010). A comparative review on conventional and advanced ocular drug delivery formulations, *International Journal of PharmTech Research,* 2(1), 668–674.

[8] Tangri, P., Khurana, S. (2011). Basics of ocular drug delivery systems. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 2(4), 1541–1552.

[9] Lai, S., Wang, Y., Hanes, J. (2009). Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Advanced Drug Delivery Reviews*, 61(2), 158-171.

[10] Aulton, M. (2013). P*harmaceutics: The Science of Dosage Form Design*. 4th ed. Edinburgh: Churchill Livingstone.

[11] Patel, A. (2013). Ocular drug delivery systems: An overview. *World Journal of Pharmacology* 2(2), 47.

[12] Boateng, JS, Okeke, O., Khan, S. (2015). Polysaccharide Based Formulations for Mucosal Drug Delivery: A Review. *Current Pharmaceutical Design*. 21(33), 4798-4821.

[13] Kesarwani, R., Harikumar, S., Rana, A., Kashyap, C., Kaur, A. and Seth, N. (2011) Ocular Implants: a novel approach to ocular drug delivery review. *International Journal of Drug Formulation and Research*, 2(5), 93-119.

[14] Gupta, S., Songara, R. and Lokwani, P. (2011). Ocular Inserts: An Overview. *International Journal of Pharmaceutical Research and Development*, 3(6), 141-148.

[15] Ludwig, A. (2005). The use of mucoadhesive polymers in ocular drug delivery. *Advanced Drug Delivery Reviews*, 57, 1595–1639.

[16] Fraunfelder, F., Fraunfelder, F. (2013). Restricting Topical Ocular Chloramphenicol Eye Drop Use in the United States. Did We Overreact? *American Journal of Ophthalmology* 156(3), 420-422.

[17] Lam, R.F., Lai, J.S.M., Ng, J.S.K., Rao, S.K., Law, R.W.K., and Lam, D.S.C. (2002). Topical chloramphenicol for eye infections. *Hong Kong Medical Journal*, 8(1), 44-47.

[18] Titcomb, C.L. (2000). Topical ocular antibiotics: part 1. *The Pharmaceutical Journal*, 264(7084), 298-301.

[19] Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48, Suppl. S1, 5-16.

[20] Ghosal, K., Ranjan, A. and Bhowmik, B. (2014). A novel vaginal drug delivery system: anti-HIV bioadhesive film containing abacavir. *Journal of Material Science: Material Medicine*, 25(7), 1679-1689.

[21] Boateng, J.S., Mani, J., Kianfar, F. (2013). Improving drug loading of mucosal solvent cast films using combination of hydrophilic polymers with amoxicillin and paracetamol as model drugs. *BioMed Research International*, vol. 2013, Article ID 198137, 8 pages, 2013. doi:10.1155/2013/198137.

[22] Morales, J., McConville, J. (2011). Manufacture and characterization of mucoadhesive buccal films. *European Journal of Pharmaceutics and Biopharmaceutics*, 77(2), pp.187-199.

[23] Carvalho, F., Bruschi, M., Evangelista, R. and Gremiao, M. (2010). Mucoadhesive drug delivery systems. *Brazilian Journal of Pharmaceutical Sciences*, 46(1), pp.1-19.

[24] Kumar, L., Kumar, P., Narayan, A. and Kar, M. (2013). Rietveld analysis of XRD patterns of different sizes of nanocrystalline cobalt ferrite. *International Nano Letters*, 3(1), 8.

[25] Amar, A. (2012). Formulation and evaluation of controlled release ocular inserts of betaxolol hydrochloride. *IOSR Journal of Pharmacy (IOSRPHR)*, 2(5), 34-38.

[26] Jethava, J. and Jethava, G. (2014). Design, formulation, and evaluation of novel sustain release bioadhesive in-situ gelling ocular inserts of ketorolac tromethamine. *International Journal of Pharmaceutical Investigation*, 4(4), 226.

[27] Jung, H.J., Abou-Jaoude, M., Carbia, B.E., Plummer, C., Chauhan, A. (2013). Glaucoma therapy by extended release of timolol from nanoparticle loaded silicone-hydrogel contact lenses, *Journal of Controlled Release*, 165(1), 82–89.

[28] Harris, M. and Chamberlain, M. (1978). Light Transmission of Hydrogel Contact Lenses. *Optometry and Vision Science*, 55(2), 93-96.

[29] Rathod, K., Shah, C., and Shah, H. (2014). To optimize the effect of polymers on matrix type transdermal patch of Trandolapril. *International Journal of Pharmaceutical Research and Bioscience* 3(2), 540-554.

[30] Boateng, J.S., Stevens, H.N.E., Eccleston, G.M., Auffret, A.D., Humphrey, M.J., Matthews, K.H. (2009). Development and mechanical characterization of solvent-cast polymeric films as potential drug delivery systems to mucosal surfaces. *Drug Development and Industrial Pharmacy*. 35(8), 986-996.

[31] Kianfar, F., Antonijevic, M., Chowdhry, B. and Boateng, J.S. (2011). Formulation Development of a Carrageenan Based Delivery System for Buccal Drug Delivery Using Ibuprofen as a Model Drug. *Journal of Biomaterials and Nanobiotechnology*, 2, 582-595.

[32] Momoh, F., Boateng, J.S., Richardson, S., Mitchell, J., Chowdhry, B. (2015). Development and functional characterization of alginate dressing as potential protein delivery system for wound healing, *International Journal of Biological Macromolecules*, 81, 137–150.

[33] Anshu, S., Jain, C. (2011). Solid dispersion: A promising technique to enhance solubility of poorly water-soluble drug. *International Journal of Drug Delivery*, 3, 149-170.

[34] Aburahma, M.H., Mahmoud A.A. (2011). Biodegradable ocular inserts for sustained delivery of brimonidine tartarate: preparation and in vitro/in vivo evaluation, *AAPS PharmSciTech*, 12(4), 1335–1347.

[35] Hehl E.M., Beck R., Luthard K., Guthoff R. (1999). Improved penetration of aminoglycosides and fluoroquinolones into aqueous humour of patients by means of Acuvue contact lenses. *European Journal of Clinical Pharmacology*, 55, 317-323.

[36] Schultz CL, Mint JM. 2002. Drug Delivery system for antiglaucomatous medication. US Patent 6 410 045, 25 June.

[37] Gulsen, D., Chauhan, A. (2004). Ophthalmic drug delivery through contact lenses. *Investigative Ophthalmology and Visual Science*, 45(7), 2342-2347.

Figure legends

Figure 1: Digital images of optimum films formulated (a) BLK HPMC-EUD-PEG (b) BLK HPMC-PEG-EUD-PEG (c) DL HPMC-CHF-EUD-PEG (d) DL HPMC-PEG-CHF-EUD-PEG.

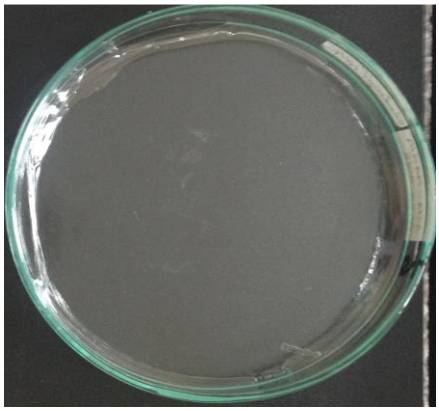
Figure 2: (a) Tensile profiles showing average (± SD, *n* = 3) % elongation at break, tensile strength and elastic modulus for BLK (HPMC-EUD-PEG & HPMC-PEG-EUD-PEG) and DL (HPMC-CHF-EUD-PEG & HPMC-PEG-CHF-EUD-PEG) films and (b) Mucoadhesion profiles (± SD, *n* = 3) showing average detachment force for both BLK and DL films.

Figure 3: SEM images for (a) pure CHF, (b) HPMC layer of BLK HPMC-EUD-PEG films and EUD layer of BLK HPMC-EUD-PEG; bi-layer films [(the SEM images of DL films containing CHF are shown in supplementary data section (Figure A1)]

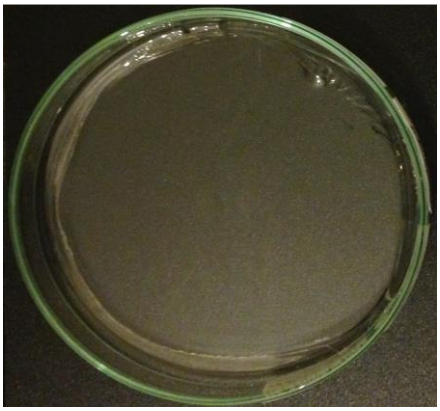
Figure 4: DSC thermograms obtained for (a) pure CHF and (b) BLK (HPMC-EUD-PEG & HPMC-PEG-EUD-PEG) and DL (HPMC-CHF-EUD-PEG & HPMC-PEG-CHF-EUD-PEG) films.

Figure 5: (a) XRD diffractograms for BLK (HPMC-EUD-PEG & HPMC-PEG-EUD-PEG) and DL (HPMC-CHF-EUD-PEG) films and (b) EDX results for CHF and DL HPMC-PEG-CHF-EUD-PEG film.

Figure 6: (a) Swelling profiles comparing swelling capacity for BLK (HPMC-EUD-PEG & HPMC-PEG-EUD-PEG) and DL (HPMC-CHF-EUD-PEG & HPMC-PEG-CHF-EUD-PEG) bi-layered films and (b) *In vitro* drug dissolution profiles (± SD, *n* = 3) of (a) DL HPMC-CHF-EUD-PEG and DL HPMC-PEG-CHF-EUD-PEG films.



(a) HPMC-EUD-PEG



(b) HPMC-PEG-EUD-PEG

(c) HPMC-CHF-EUD-PEG

(d) HPMC-PEG-CHF-EUD-PEG

Figure 1

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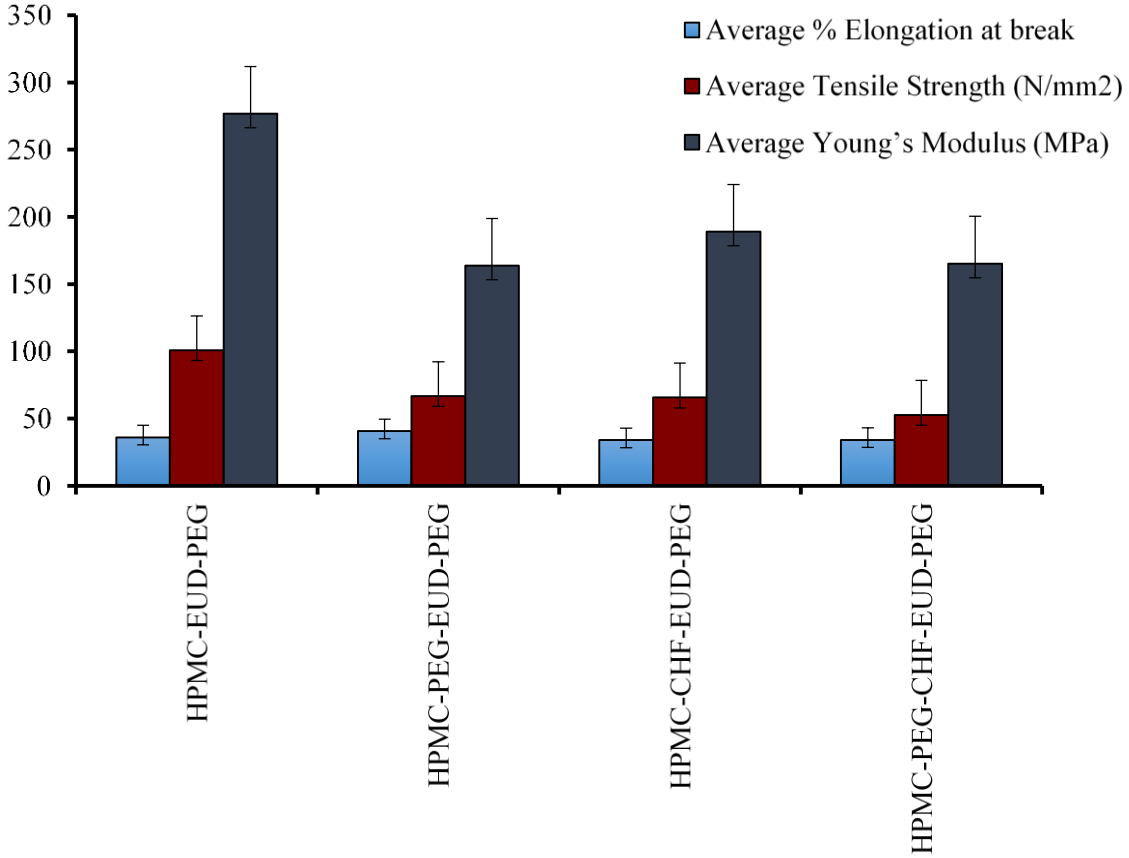
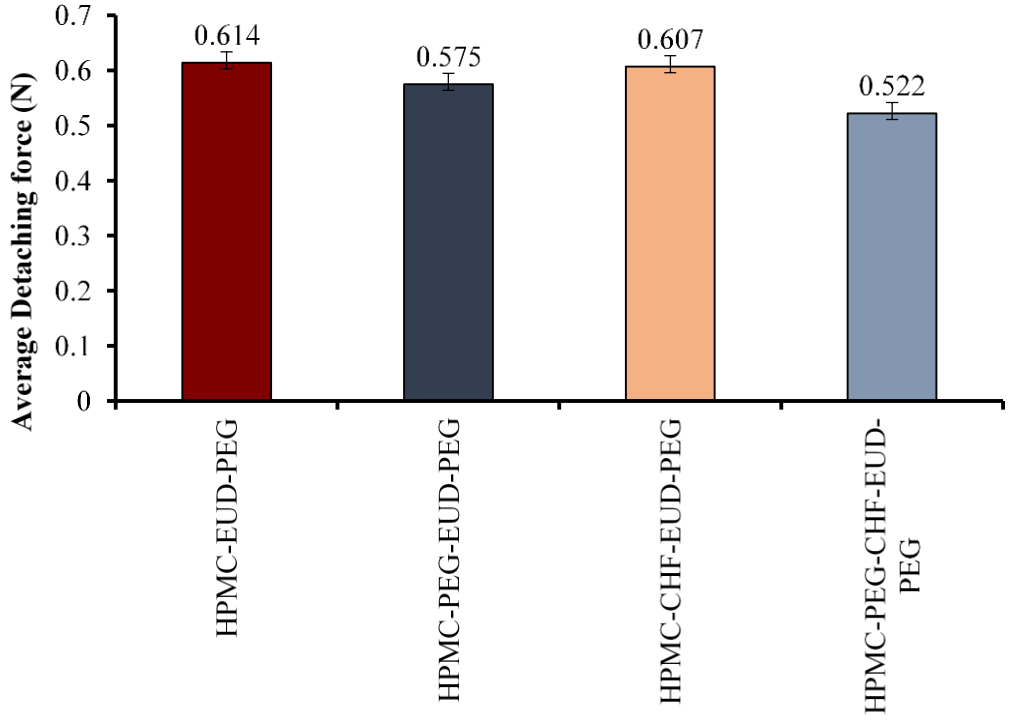


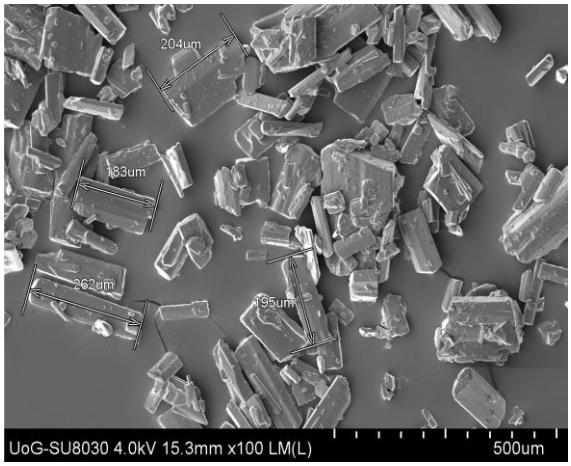
(a)



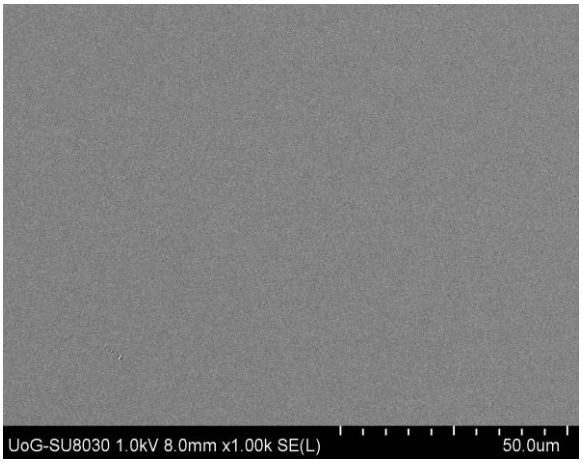
(b)

Figure 2

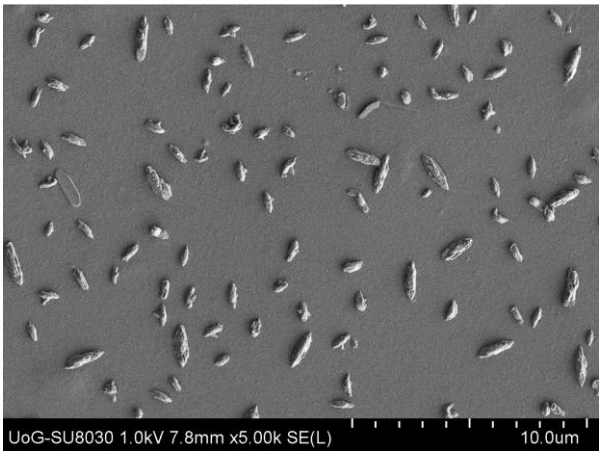
580



(a) CHF



(b) HPMC-EUD-PEG film: EUD layer



(c) HPMC-EUD-PEG film: HPMC layer

Figure 3

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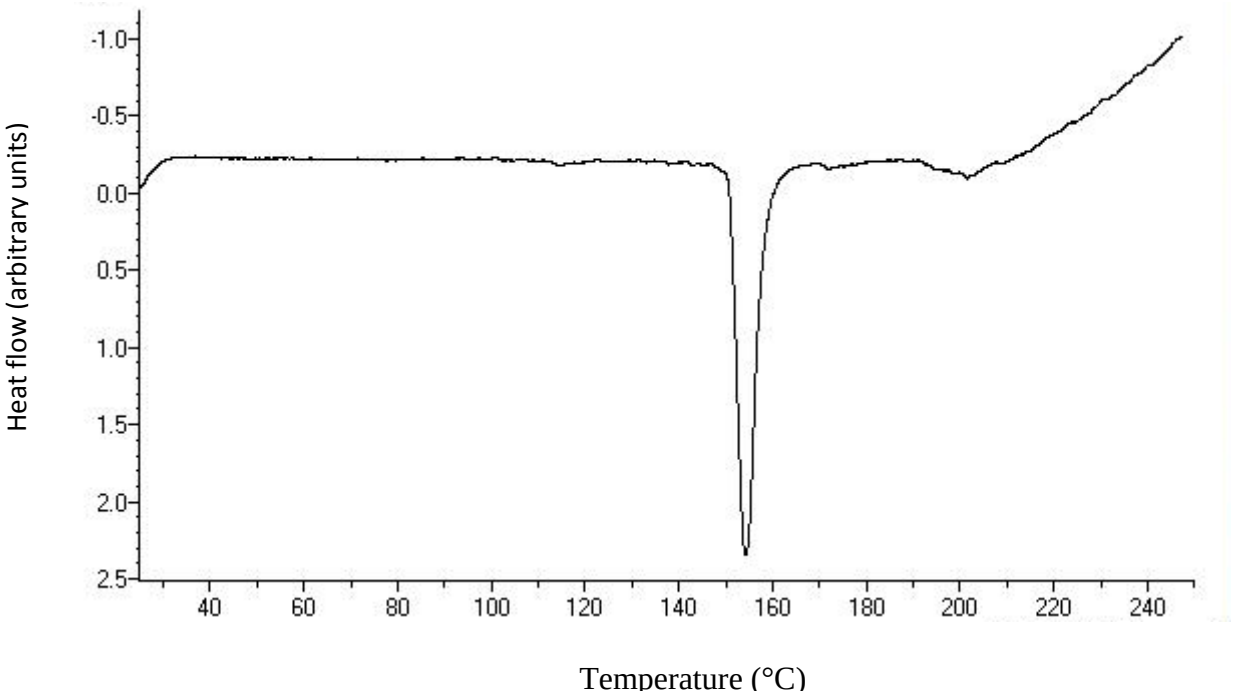

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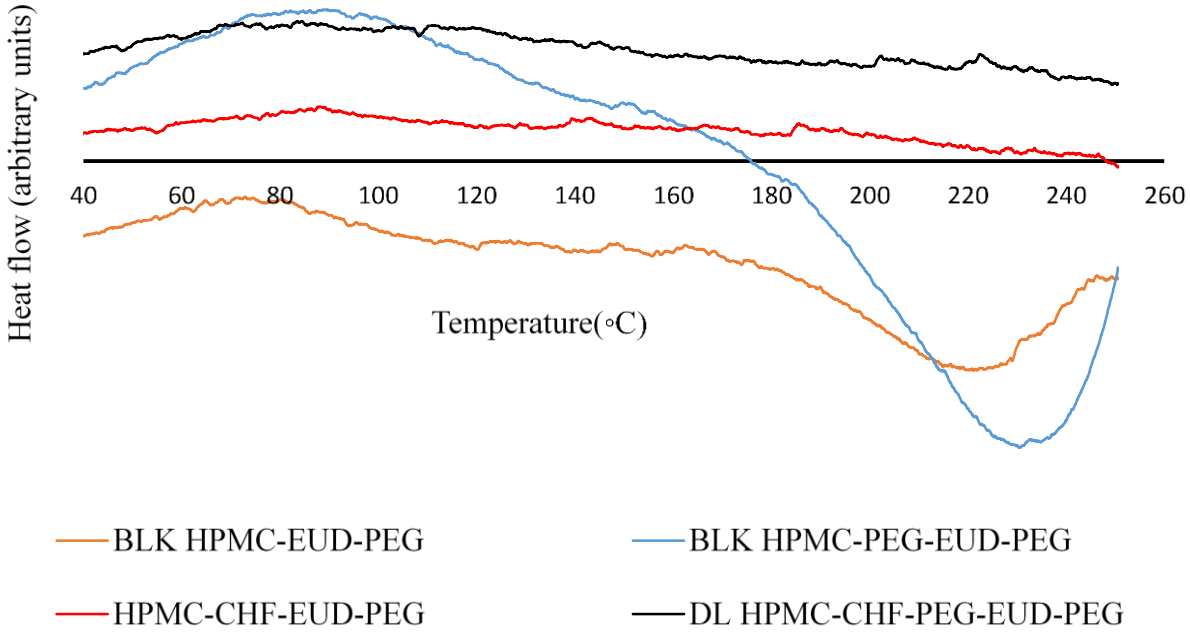

(b)

Figure 4

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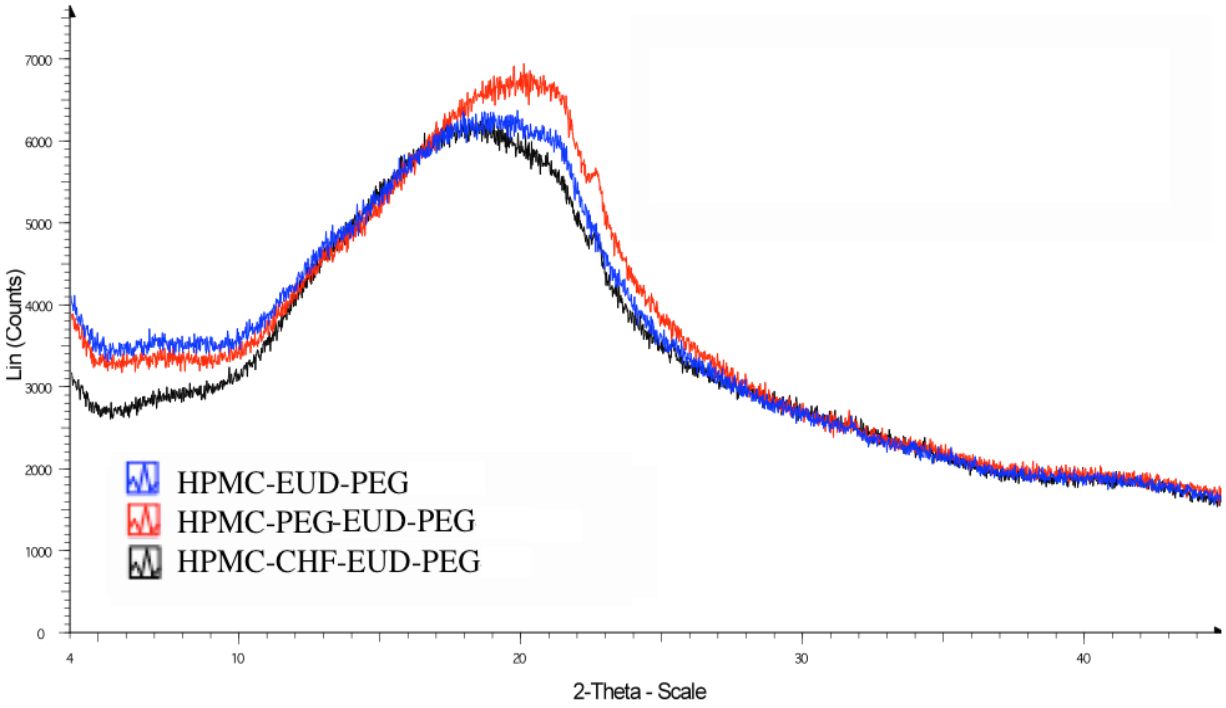

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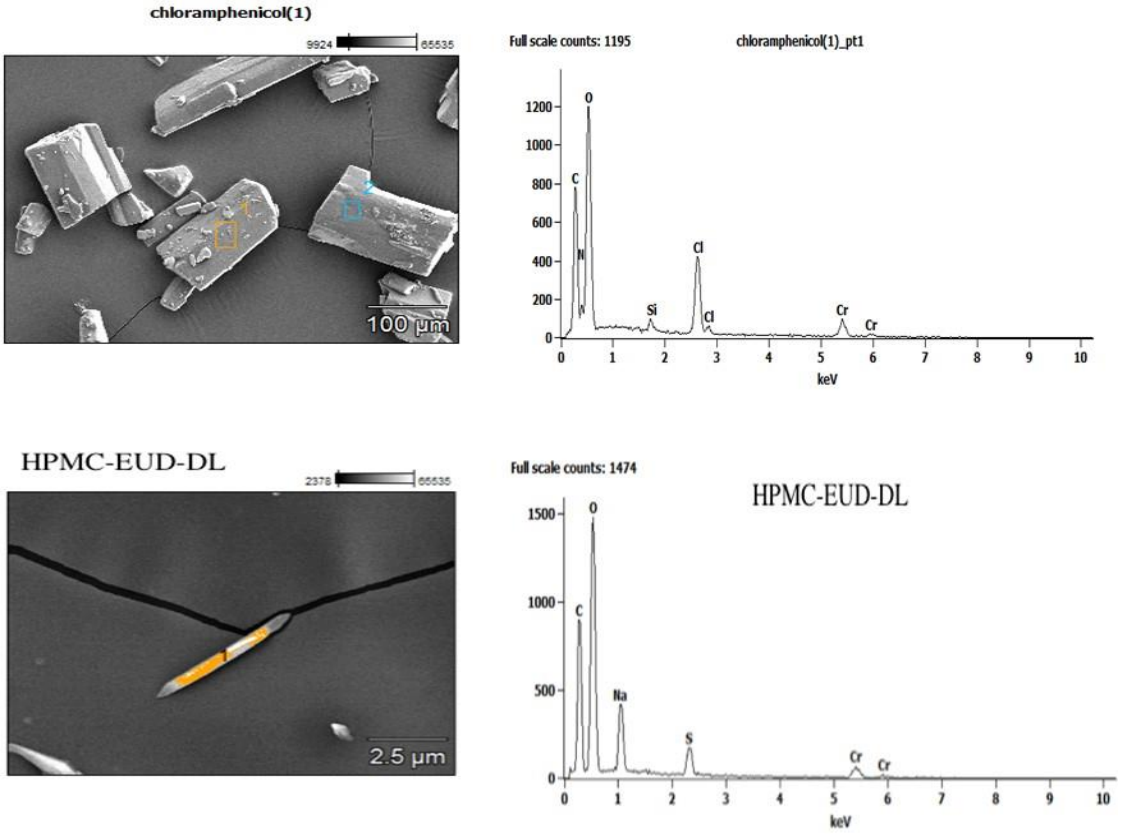

(b)

Figure 5

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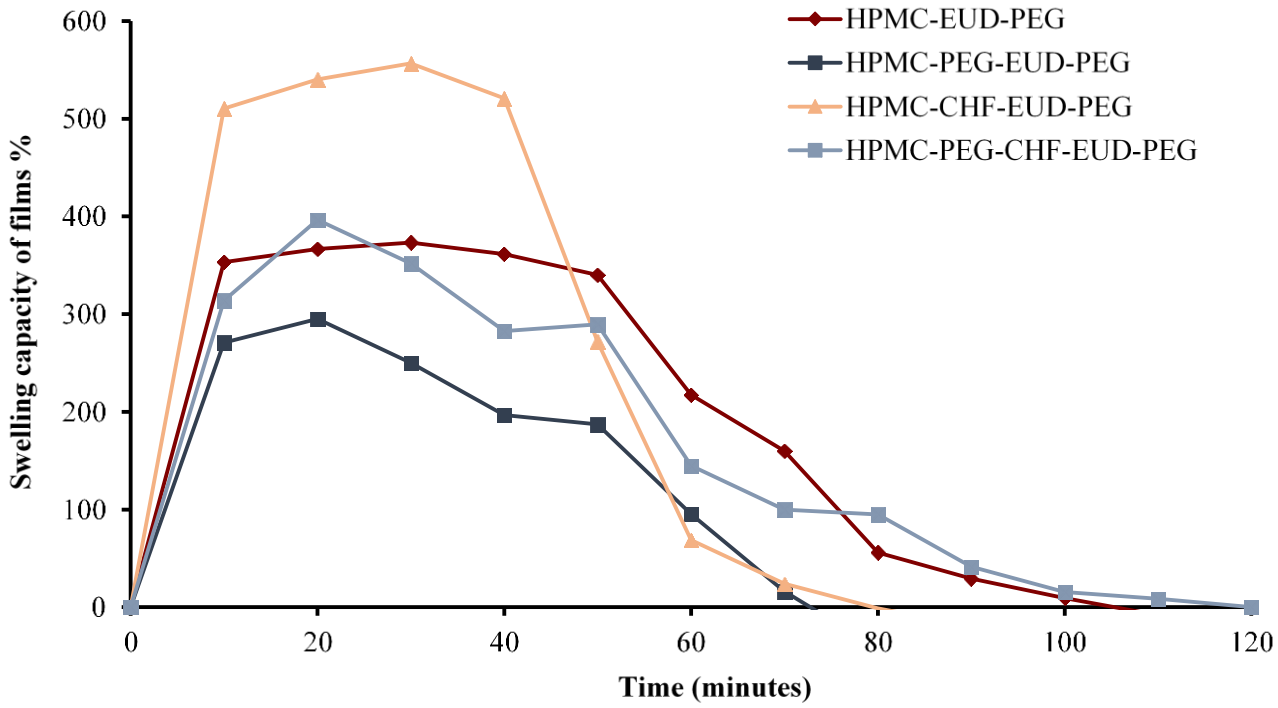

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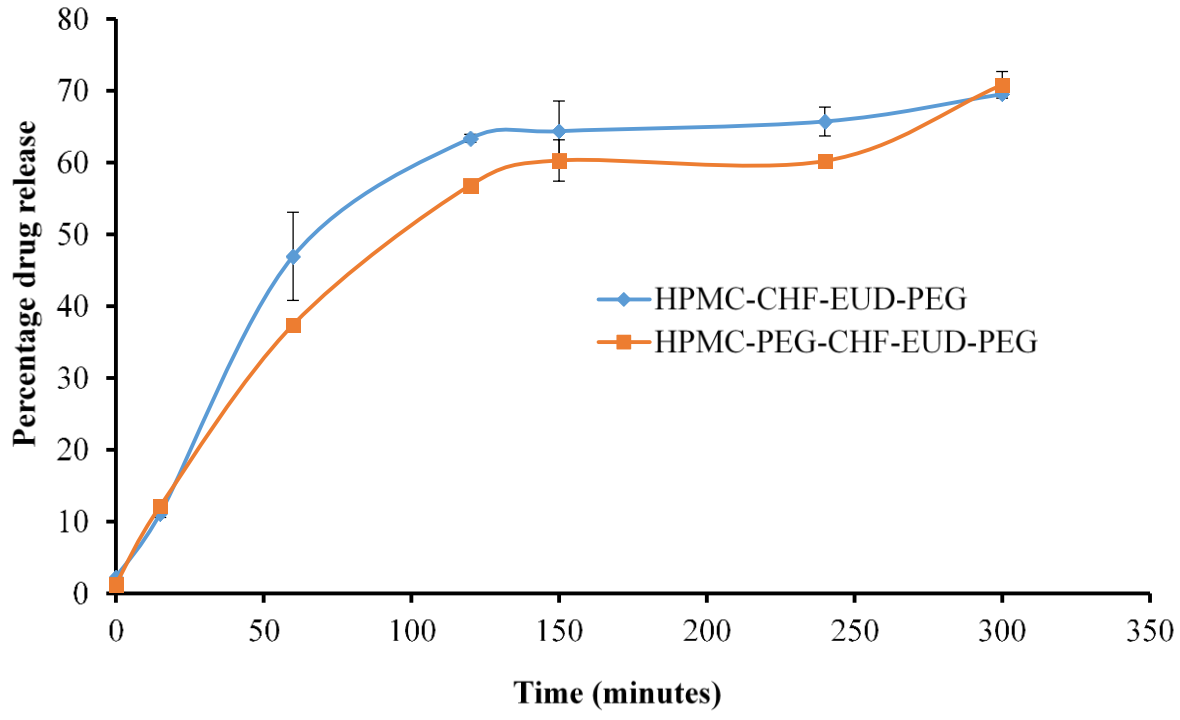

(b)

Figure 6

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