Chitosan/poly(2-ethyl-2-oxazoline) films for ocular drug delivery: Formulation, miscibility, in vitro and in vivo studies

Article

Accepted Version

Chitosan/poly(2-ethyl-2-oxazoline) films for ocular drug delivery: formulation, miscibility, *in vitro* and *in vivo* studies

Guzel K. Abilova,[a] Daulet B. Kaldybekov,[a,b] Elvira K. Ozhmukhametova,[c] Aisulu Zh. Saimova,[c] Diara S. Kazybayeva,[a] Galiya S. Irmukhametova,[a] Vitaliy V. Khutoryanskiy[b,*]

[a] *Department of Chemistry and Chemical Technology, Al-Farabi Kazakh National University, 050040 Almaty, Kazakhstan*

[b] *School of Pharmacy, University of Reading, Whiteknights, RG6 6AD Reading, United Kingdom*

[c] *Semey State Medical University, 071400 Semey, Kazakhstan*

***Corresponding author:**

Postal address: School of Pharmacy, University of Reading, Whiteknights, PO Box 224, RG6 6AD Reading, United Kingdom

E-mail address: v.khutoryanskiy@reading.ac.uk

Telephone: +44(0) 118 378 6119

Fax: +44(0) 118 378 4703

ABSTRACT

Polymeric films were prepared based on chitosan and its blends with poly(2-ethyl-2-oxazoline) by casting from aqueous solutions. These materials were characterised using a number of physicochemical techniques, including Fourier-transform infrared spectroscopy, thermal gravimetric analysis, differential scanning calorimetry, wide angle x-ray diffraction, tensile testing and scanning electron microscopy. All these studies indicate that there is a weak intermacromolecular hydrogen bonding between these polymers, which facilitates their complete miscibility in solid state. These films were formulated with sodium fluorescein as a model drug and were evaluated for their potential application in ocular drug delivery both *in vitro* and *in vivo*. It was established that the films are biocompatible and mucoadhesive; they are capable of providing a sustained drug release when administered topically on the cornea.

Keywords: *chitosan, poly(2-oxazoline), films, miscibility, mucoadhesion, ocular drug delivery*

1. INTRODUCTION

Ability of hydrophilic polymers to stick to wet surfaces in the human body, defined as mucoadhesion, has been widely used for designing dosage forms for transmucosal administration. The current applications of mucoadhesive dosage forms include drug delivery to the eye, nose, oral cavity, gastrointestinal tract, vagina, rectum and urinary bladder [1–5]. These routes of drug administration offer a number of advantages and the use of mucoadhesive carriers facilitates better dosage form retention on mucosal surfaces resulting in improved drug bioavailability, possibility of targeting particular organs, ease of application and avoidance of painful injections.

Typically, all water-soluble polymers have some mucoadhesive properties; however, either positively or negatively charged polyelectrolytes exhibit better ability to stick to mucosal tissues compared to non-ionic macromolecules [2]. Chitosan as a polysaccharide of cationic nature is considered as one of the materials with excellent mucoadhesive properties and its numerous

applications in transmucosal drug delivery have been demonstrated [6–8]. Many attempts have been reported to modify mucoadhesive properties of chitosan through its chemical modification, as discussed in a recent review by Ways et al [9]. Additionally, properties of chitosan could also be altered by simple blending with other polymers. For example, Luo et al [10] demonstrated that chitosan forms miscible blends with hydroxyethylcellulose, which resulted in reduction of mucoadhesive properties of the buccal films based on the mixtures of these polymers. Freag et al [11] reported the development of mucoadhesive sponges based on blends of chitosan with hydroxypropylmethylcellulose and demonstrated that the materials prepared from 1:1 polymer mixture exhibited the best physicochemical characteristics suitable for buccal administration. Sizílio et al [12] fabricated mucoadhesive films by blending chitosan with poly(N-vinyl pyrrolidone) and evaluated their application for delivery of betamethasone-17-valerate used in the therapy of recurrent aphthous stomatitis.

Poly(2-oxazolines) is an emerging class of polymers highly promising for biomedical applications due to their non-toxicity, bio-inert nature and unique physicochemical properties [13–17]. Water-soluble representatives of this class such as poly(2-methyl-2-oxazoline), poly(2-ethyl-2-oxazoline), and poly(N-propyl-2-oxazoline) have received a lot of attention of researchers due to their unique physicochemical properties such as the ability to form hydrogen-bonded complexes with polycarboxylic acids and tannins [18–20] as well as their temperature-responsive properties [21]. Due to their bio-inert nature, low molecular weight poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) (5 kDa) were reported to facilitate mucus-penetration of silica nanoparticles through porcine stomach mucosa [22,23] and to reduce their mucoadhesion to rat intestinal tissues [24]. Poly(2-ethyl-2-oxazolines) with larger molecular weights (50, 200 and 500 kDa) exhibited weak mucoadhesive properties and their simple blends and complexes with Carbopols 971 and 974 also resulted in reduction of dosage forms mucoadhesiveness compared to pure Carbopols® [25].

Polymer blending and miscibility of poly(2-oxazolines) with other polymers is studied insufficiently. Earlier publications reported the miscibility studies of poly(2-oxazolines) with

hydroxyl-containing polymers [26–28] and some conventional plastic materials such as poly(vinyl chloride), polystyrene, polypropylene and poly(vinylidene fluoride) [29]. Despite the growing biomedical importance of both chitosan and poly(2-oxazolines) the studies of miscibility in their blends are limited only to a very few publications [30].

In the present work we have prepared chitosan/poly(2-ethyl-2-oxazoline) films by casting from aqueous mixtures of these polymers; studied the physicochemical properties of these blends using Fourier transform infrared spectroscopy, thermal analysis and X-ray diffraction methods, tensile properties, and scanning electron microscopy; and evaluated the mucoadhesive potential and retention of these films on freshly excised bovine cornea *ex vivo* and on rabbit ocular cornea *in vivo*.

2. EXPERIMENTAL SECTION

2.1. Materials

A high molecular weight chitosan (CHI, M_W ~ 310 – 375 kDa, degree of deacetylation: 75 – 85%), poly(2-ethyl-2-oxazoline) (POZ, M_W ~ 50 kDa and PDI 3 – 4), hydrochloric acid solution (HCl, 1 M), fluorescein sodium salt (NaFl) and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich (Gillingham, UK). All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of films

Chitosan solution was prepared by first dissolving 3.75 g of chitosan in 25 mL of 1 M HCl, then the total volume was made up to 500 mL with deionised water (CHI 0.75% w/v, pH ~ 4.0) and stirred magnetically overnight at room temperature. Before casting CHI solution was sonicated in a sonication bath (FS200b, Decon Laboratories Ltd., UK) for 30 min. Poly(2-ethyl-2-oxazoline) solutions (0.75% w/v) were prepared by dissolving 3.75 g polymer powder in 500 mL of deionised water (pH ~ 6.8) for 1 h under continuous stirring. The film-forming solutions (FFS) with and without NaFl (0.1 mg/mL) were obtained by mixing CHI and POZ aqueous solutions at different volume ratios, where part of CHI was gradually replaced with POZ, up to 60%. Formulations were named

indicating the CHI/POZ volume ratio as CHI (100), (80:20), (60:40), (40:60) and POZ (100), respectively. The pH values of combined solutions were in the range of 4.0 – 4.2. FFS were magnetically stirred for 3 h until fully homogeneous mixture formed, after that, 45 mL of each solution was poured into 90 mm diameter Petri dishes and dried at room temperature for several days.

2.3. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were recorded on Nicolet iS5 FTIR spectrometer (Thermo Scientific, UK) using an attenuated total reflectance (ATR) accessory equipped with a diamond crystal. The transmission mode was used and the resolution was 1 cm^{-1}.

2.4. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of CHI, POZ and CHI/POZ blend film samples was conducted using Q50 TGA analyser (TA Instruments, UK) in the range between 20 and 600 °C at a heating rate of 10 °C/min under nitrogen atmosphere. Moisture content in each film was determined from the weight loss corresponding to the first step weight loss in their TGA curves (up to about 150 °C).

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were performed on TA-Q2000 DSC instrument (TA Instruments, UK). DSC thermograms of each film were recorded from the second heating run at 20 °C/min, after the first run of heating up to 80 °C and cooling down to 25 °C at 10 °C/min, under nitrogen atmosphere, in order to estimate the glass transition temperatures (Tg).

2.6. X-Ray diffraction (XRD)

X-Ray diffraction patterns of the polymers and their blends were evaluated using Oxford Diffraction Gemini Ultra diffractometer fitted with CuKα radiation and Saturn detector (Oxford Diffraction Ltd., UK). Film samples were cut in 1×1 cm, loaded, and scanned at diffraction ranges from 6 to 120° with a scan step of 0.01°, generating characteristic diffractograms at the rate of 2.5 scans min^{-1}.

2.7. Scanning electron microscope (SEM)

SEM experiments used a FEI Quanta 600 FEG Environmental Scanning Electron Microscope instrument (FEI UK Ltd., UK) with an acceleration voltage of 20 kV. The images were taken from the fracture surface of the materials, which were preliminary frozen in liquid nitrogen and coated with gold sputter to facilitate high resolution imaging.

2.8. Mechanical analysis

Puncture strength (PS) and elongation at break (EB) of the films were measured using a TA.XT Plus Texture Analyser (Stable Micro Systems Ltd., UK) in compression mode at room temperature [31,32]. Film thickness was measured with a hand-held micrometer; six replicates were taken for each sample in different places and the mean values were calculated accordingly. The thickness of the films was about 0.065 ± 0.002 mm. The square shaped film samples (30 x 30 mm) fixed by screws between two plates with a cylindrical hole of 10 mm diameter (area of the sample holder hole: Ar_s = 78.54 mm^2) and compressed by the upper load 5 mm stainless steel spherical ball probe (P/5S) at a test speed 1.0 mm/sec. The plate was stabilised to avoid movements using two pins. The measurements started after the probe was in contact with the sample surface. The probe moved at a constant speed until each film sample was broken [33]. These tests were performed with the following settings: pre-speed test 2.0 mm/sec; test-speed 1.0 mm/sec; post-test speed 10.0 mm/sec; target mode – distance; distance 5 mm; trigger type auto; trigger force 0.049 N. The film samples were punctured and the force required in Newtons was recorded and puncture strength was calculated using the following equation [33,34]:

$$\mathrm{PS} = \frac{\mathrm{Force}}{Ar_s} \quad (1)$$

where Force is the maximum applied force recorded during strain.

Elongation at break (EB) is the ratio between the extension of the film at the point of rupture and the initial length of the sample and is expressed in percentage:

$$EB = \left(\frac{\sqrt{a'^2 + b^2} + r}{a} - 1\right) \times 100\% \quad (2)$$

where a' – the initial length of the film sample that is not punctured by the probe; b – the penetration depth/vertical displacement by the probe; r – the radius of the probe; and a – radius of the film in the sample holder opening.

All experiments were conducted 5 times and the mean values ± standard deviations were calculated and evaluated statistically.

2.9. *Ex vivo* mucoadhesion studies on bovine cornea

The retention of polymeric films on bovine eyes was studied using the protocols previously reported by our group with some modifications [35,36]. The bovine eyes are commonly used in ocular drug delivery and irritation testing because of their availability, suitable dimensions and structural similarity to human eyes [37]. These were acquired from P.C. Turner Abattoirs (Farnborough, UK) immediately after animal slaughter and were transported to the laboratory in a cold polystyrene container. Bovine corneas were dissected within 4 h of eyes delivery, where the whole cornea with 2-3 mm of sclera was carefully excised using a sharp blade. Each cornea was rinsed with PBS solution, placed in Petri dishes, wrapped with cling film to reduce dehydration and stored at 4 °C to be used on the following day. Each experiment was performed in triplicate using different corneas.

Experiments were carried out with a cornea mounted on a glass slide placed on half cut falcon tubes already mounted at an angle of 45° and maintained at 37 °C in an incubator. Prior to each experiment, spherically shaped polymeric discs (4 mm in diameter) containing NaFl were quickly soaked in 0.9% NaCl saline solution (1 sec) and then placed on cornea previously rinsed with 1 mL of simulated tear fluid (STF: 3.35 g NaCl; 1 g $NaHCO_3$; and 0.0305 g $CaCl_2$ made up to 500 mL with deionised water). The background microscopy images were recorded for each cornea prior to administration of a fluorescent film. Then 12 mL of STF solution was dripped for 1 h on a corneal surface at a flow rate of 200 µL/min using a syringe pump. Fluorescence microscopy images of whole tissue were recorded after each wash every 5 min using a Leica MZ10F stereo-microscope (Leica

Microsystems, UK) with Leica DFC3000G digital camera at 0.8× magnification and 20 ms exposure time (gain 3.0×), fitted with a GFP filter (blue, $\lambda_{emission}$ = 512 nm). The microscopy images were then analysed with ImageJ software by measuring the fluorescence pixel intensity after each washing cycle. The pixel intensity of the blank samples (corneal mucosa without a fluorescent film) was deducted from each measurement and data were normalised and converted into fluorescent intensity values using the following equation:

$$Fluorescence\ intensity = \frac{I - I_b}{I_0 - I_b} \times 100\% \quad (4)$$

where I is the fluorescence intensity of a given tissue sample with a mucoadhesive film after each washing; I_b is the background fluorescence intensity of that tissue sample (a blank sample); and I_0 is the initial fluorescence intensity of that sample (a tissue sample with mucoadhesive film on it prior to the start of first washing).

In parallel, STF solution flowing through the corneal epithelium was collected at pre-determined time points and used for determination of the percentage of NaFl washed off the corneal surface. All the collected samples were diluted with STF, making up the total volume to 30 mL. The amount of NaFl in each sample was then quantified using a FP-6200 spectrofluorometer (Jasco, UK) at $\lambda_{excitation}$ and $\lambda_{emission}$ wavelengths of 460 and 512 nm, respectively. A standard curve used to determine the amount of NaFl released from the films can be found in Supplementary information (Figure S1).

All measurements were conducted in triplicate and the mean values ± standard deviations were calculated and evaluated statistically.

2.10. *In vivo* experiments

In vivo experiments on ocular administration of fluorescent films (CHI and CHI/POZ) were conducted using chinchilla rabbits (2.5 – 4.0 kg) according to a previously described protocol [38]. These experiments were approved by Semey State Medical University (Kazakhstan) ethics committee

and were conducted following the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Before the start of experiments, rabbits were housed in standard cages and allowed free access to food and water. In the beginning of each experiment rabbits were sedated with 0.2 mL (100 mg/mL) sodium thiopental (Arterium Corporation, Ukraine), previously dissolved in 10 mL of 0.9% NaCl saline solution, administered through the lateral auricular vein. Approximately 5 min after administration of sodium thiopental, polymeric discs containing NaFl (10 mm in diameter) were quickly soaked (1 sec) in a saline solution (0.9% NaCl) and carefully placed on rabbit left eye's cornea; their right eye always served as a control. The behaviour of each polymeric disc on the eye was controlled visually and images were taken at different time intervals with a high resolution digital camera. In addition, a weak UV light from an UVGL-25 Compact UV handheld lamp (Ultra-Violet Products, UK) was shone into the eye to facilitate the detection of fluorescence.

Each type of polymeric film was tested in 3 rabbits and each experiment was conducted until a film was detached or dislodged. The mean residence time values ± standard deviations were calculated and assessed for statistical differences.

2.11. Statistical analysis

Data acquired during these experiments, i.e. mean values ± standard deviations were calculated and assessed for significance using two-tailed Student's *t*-test and a one-way analysis of variance (ANOVA) followed by Bonferoni *post hoc* test using GraphPad Prism statistical analysis software (version 7.0; GraphPad Software Inc.), where $p < 0.05$ was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Preparation and characterisation of films

CHI and CHI/POZ blend films were successfully developed and tested. These two polymers are well characterised for their safety and biocompatibility and known to be stable under normal processing and storage conditions. POZ solutions (0.75% w/v) used to prepare films were easy to handle and no heating was required during their dissolution in deionised water. Chitosan solution can

be obtained only *via* solvent casting from acidified water. All films were prepared without any plasticisers and were homogenous and transparent.

The presence of amide carbonyl groups in poly(2-ethyl-2-oxazoline) suggests that this tertiary polyamide has potential for forming miscible blends with a variety of polymers containing complementary functional groups such as carboxylic, phenolic or alcoholic hydroxyl groups [39–41]. Previous studies demonstrated that carbonyl groups of POZ exhibit proton-accepting properties and participate in hydrogen bonding with proton-donating groups of other functional polymers [40,42]. Chitosan, in contrast to poly(2-ethyl-2-oxazoline), has in its structure numerous hydroxyl groups that can act as proton-donors with respect to the proton-accepting groups of POZ and form intermolecular hydrogen bonds. In order to establish the possibility of hydrogen bonding in chitosan/POZ blends all films were studied using FTIR spectroscopy (Figure 1). The FTIR spectrum of pure CHI film shows the presence of the broad peak appeared above 3000 cm^{-1} that is due to OH- stretching, which overlaps with NH-stretching in the same region. The peaks at 2923 cm^{-1} and 2889 cm^{-1} correspond to CH_2- and CH- stretching vibrations. The absorption bands at 1627 cm^{-1} and 1520 cm^{-1} are C=O stretching (amide I) and NH-bending (amide II), respectively. The absorption band at 1416 cm^{-1} is attributed to CH- and OH- vibrations [10]. The band that appeared at 1377 cm^{-1} is assigned to the acetamide groups, which demonstrate that chitosan is not totally deacetylated and the peak at 1316 cm^{-1} can be due to C–N stretching (amide III) [43]. According to Bonilla et al [44] the peak at 1250 cm^{-1} corresponds to amino groups. The peak occurred at 1151 cm^{-1} is the anti-symmetric stretching of the C–O–C bridge, 1062 cm^{-1} and 1024 cm^{-1} are the skeletal vibrations involving the C–O stretching, which are characteristics of chitosan polysaccharide structure [45].

The FTIR spectrum of POZ shows the presence of the broad absorption peak at 3488 cm^{-1}, which is an indication of the presence of bound water that was not eliminated from the film completely. The absorption bands at 2977 cm^{-1} and 2939 cm^{-1} correspond to CH_2-stretching vibrations. The characteristic bands at 1624 cm^{-1} and 1419 cm^{-1} are assigned to C=O stretching (amide I) and CH_3 bending, respectively [22]. The absorption bands at 1470, 1374 and 1322 cm^{-1} (CH

bending) as well as 1237, 1194 and 1061 cm^{-1} (C-C stretching) are in good agreement with FTIR data on POZ reported in the literature [46].

All the characteristic bands of the component polymers are present in the spectra of their blends and the intensities of the bands and the shape of the peaks depend on the polymers ratio in the blend. The spectra of the miscible CHI/POZ blends show significant changes in hydroxyl stretching region, suggesting a redistribution in the arrangement of the hydroxyl group associations. When comparing the spectra corresponding to the same system as a function of composition, a shift of this band toward higher wavenumbers is observed for increasing content of POZ. This behaviour suggests that a significant part of the hydroxyl groups involved in CHI are hydrogen-bonded to amide carbonyl groups in POZ. This is in good agreement with the data reported by Fang et al [30].

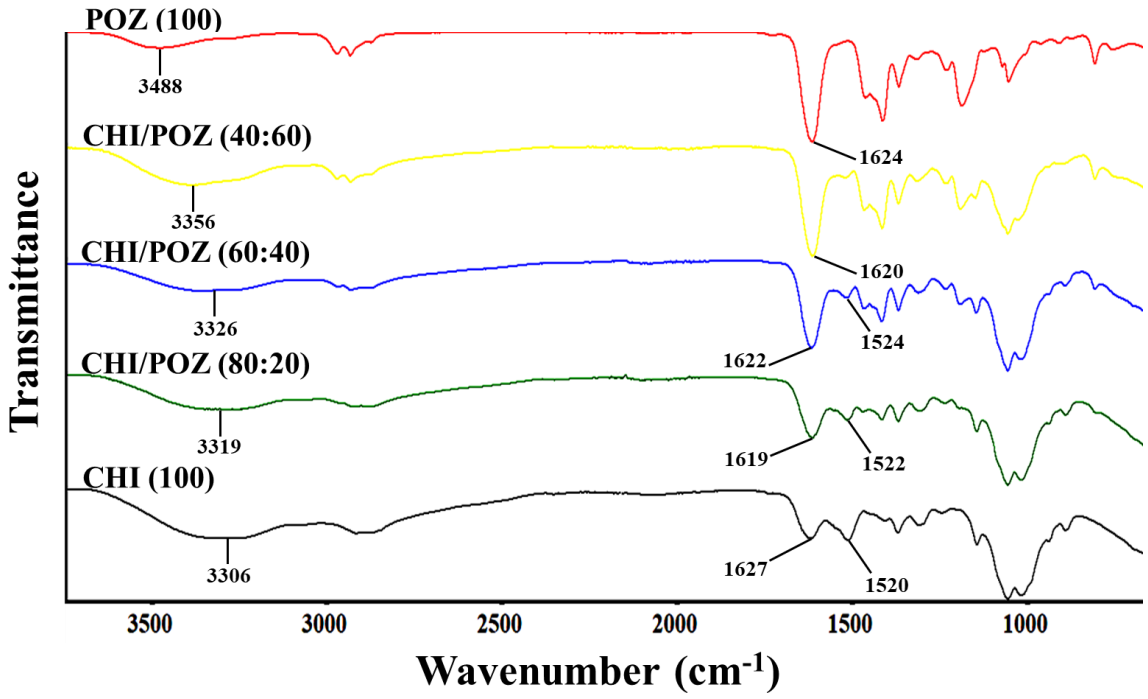

Figure 1. FTIR spectra of chitosan, POZ and CHI/POZ blends.

Thermogravimetric analysis indicates that degradation of pure chitosan film proceeds via two main stages (Figure 2): first, it starts to loose physically-bound water at >30 °C and this process finishes at 135 °C. The amount of this physically bound water in pure CHI film is about 13 %. The

next thermal decomposition stage appears at 178 – 300 °C (with a maximum degradation rate observed at 188 °C); this degradation results in 52% loss of chitosan weight. This is caused by depolymerisation of chitosan chains and pyranose rings through dehydration and deamination and finally ring-opening reaction [47,48]. The pure POZ film shows greater thermal stability compared to 100 % CHI: the first thermal event begins above 30 °C, which is related to the evaporation of physically-bound water (approximately 8%). The second stage of thermal decomposition of POZ starts above 315 °C (with the maximal degradation rate observed at 395 °C) reaching 92% of the total weight loss at 430 °C. A single decomposition stage of dry POZ at 400 °C was previously reported by Beruhil Adatoz et al [49], which broadly agrees with our data. The decomposition profiles of CHI/POZ blends are characterised by three stages: (1) 25 – 150 °C, corresponding to the loss of physically bound water, (2) 175 – 275 °C, corresponding to the degradation of CHI, and (3) 275 – 425 °C, corresponding to the degradation of POZ. It should be noted that the temperatures, at which the degradation rates of stages (2) and (3) were maximal, showed a good correlation with the composition of the blends (Figure S2, Supplementary information). This indicates that the presence of more thermally stable POZ in the blend improves the thermal stability of less stable chitosan, which may be due to the presence of weak hydrogen bonding between these polymers.

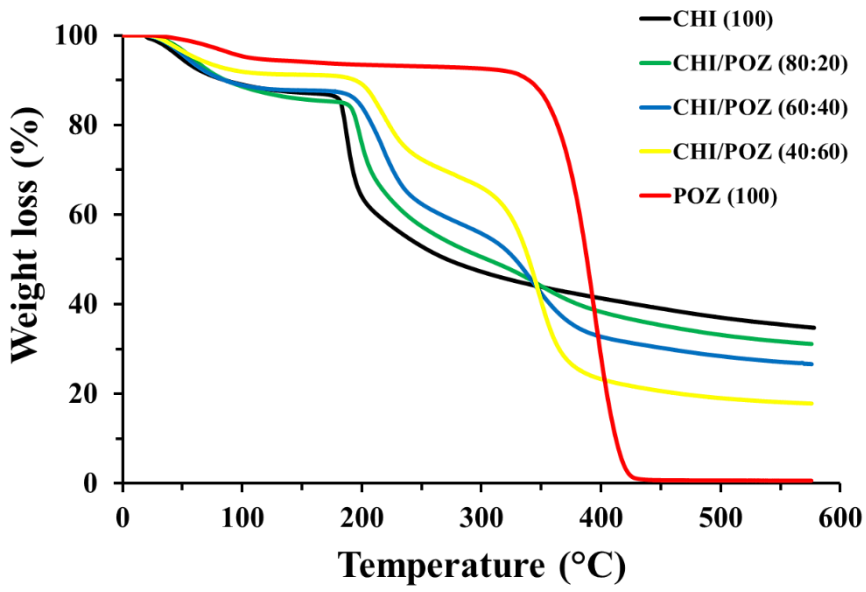

Figure 2. TGA curves of pure CHI, pure POZ and CHI/POZ blend films.

Differential scanning calorimetry (DSC) was used to characterize the miscibility between the polymers. Usually the presence of a single glass transition temperature, situated between Tg values of individual polymer components indicates a complete miscibility. Figure 3 shows that the presence of a single glass transition in the blends, which depends on the composition of the polymer mixture. All glass transition temperatures of the blends are between the Tg values of individual POZ (56 °C) and chitosan (131 °C), which is in good agreement with the data reported by Fang et al [30].

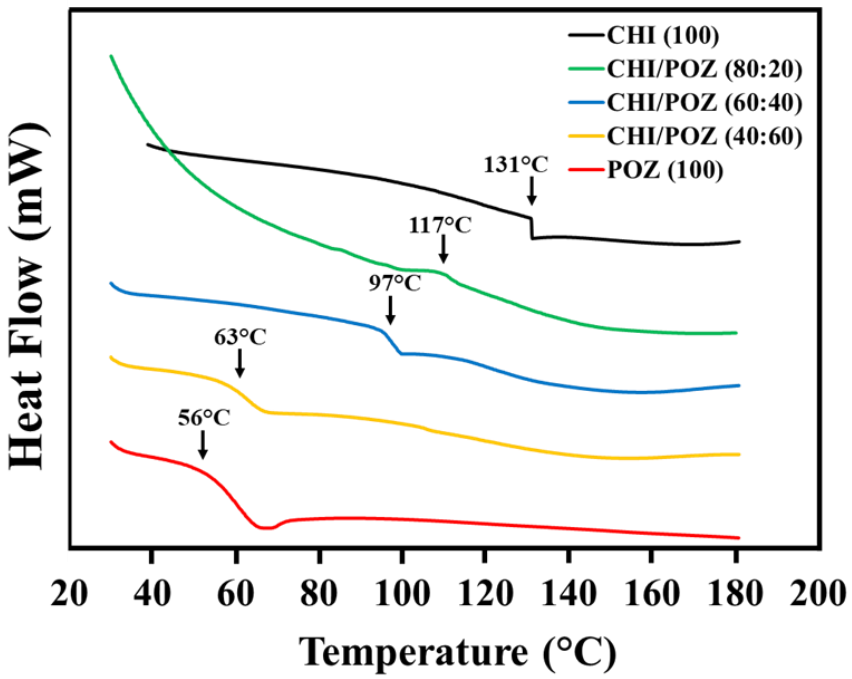

Figure 3. DSC thermograms of pure CHI, pure POZ and CHI/POZ blend films.

X-ray diffraction (WAXD) method was used to probe the effect of blending on crystallinity of polymers (Figure 4). WAXD diffractograms of pure CHI and POZ films show a broad halo typical for predominantly amorphous polymers. Pure CHI still shows the presence of four diffraction peaks at 2θ = 8.5°; 11.8°; 18.1° and 23.8°, which is typical for crystalline domains in this polysaccharide and is consistent with our previous report [50]. The diffractogram of pure POZ film shows the presence of two broad amorphous humps centred at 2θ = 10.4° and 18.8°, indicating non-crystalline nature of this polymer, which is also consistent with the literature data [51,52].

Diffraction peaks characteristic of chitosan are also present in the blend film, however a shift of a broad diffraction peak from 23.3° to 19.2° is observed. This may indicate that chitosan is involved in some weak interaction with POZ that affects the formation and structure of its crystalline domains.

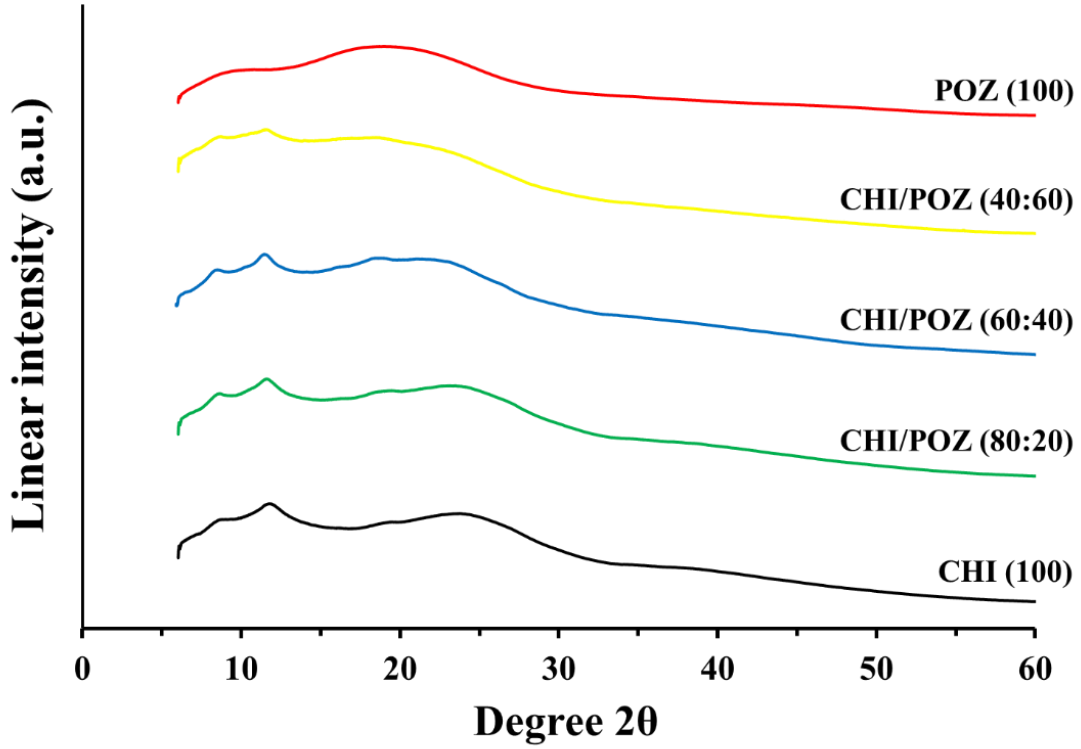

Figure. 4. WAXD diffractograms of CHI and CHI/POZ blend films.

The morphology of the polymer film cross-sections and surface were studied by scanning electron microscope (SEM). The investigation of the sample cross-sections at high magnification (2000×) reveals that the films have fully homogeneous structure with no signs of phase separation and interface boundaries (Figure 5). Thus, the SEM data provides another evidence for miscibility between CHI and POZ in the solid state at different ratios.

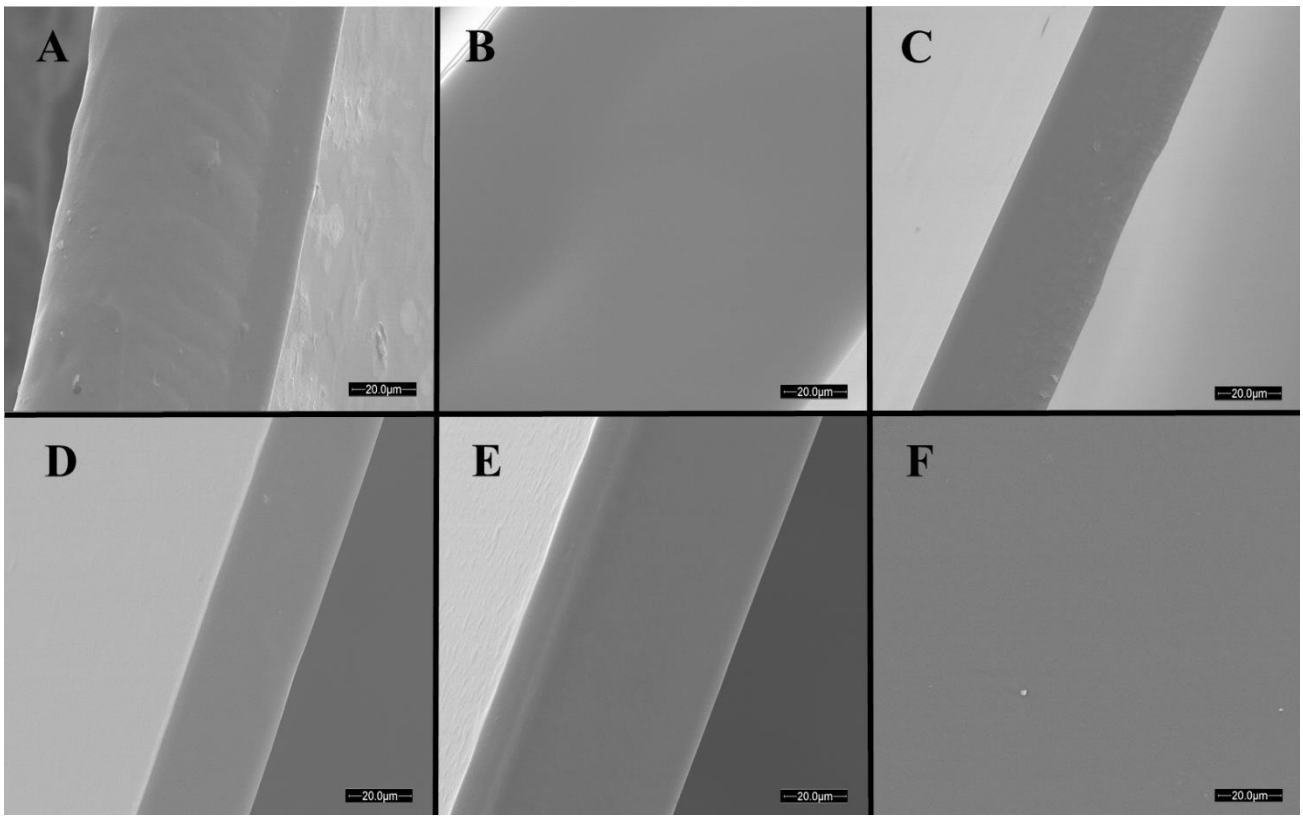

Figure 5. SEM images of (A) CHI, (B) pure POZ, and their blend (C, D, E) cross- sections and (F) surface. Content of POZ in the blends: 20 (C), 40 (D) and 60 % (E, F). Scale bars are 20 µm.

The films composed of the blends with different CHI and POZ ratios were examined by elongation and puncture strength analysis and the results are shown in Figure 6. A film composed of pure POZ was not suitable for this type of analysis because of its extreme brittleness. Comparing the mechanical properties of pure CHI films with the polymer blends indicates that an increase in POZ content in the sample results in gradual reduction in the elongation at break and puncture strength values.

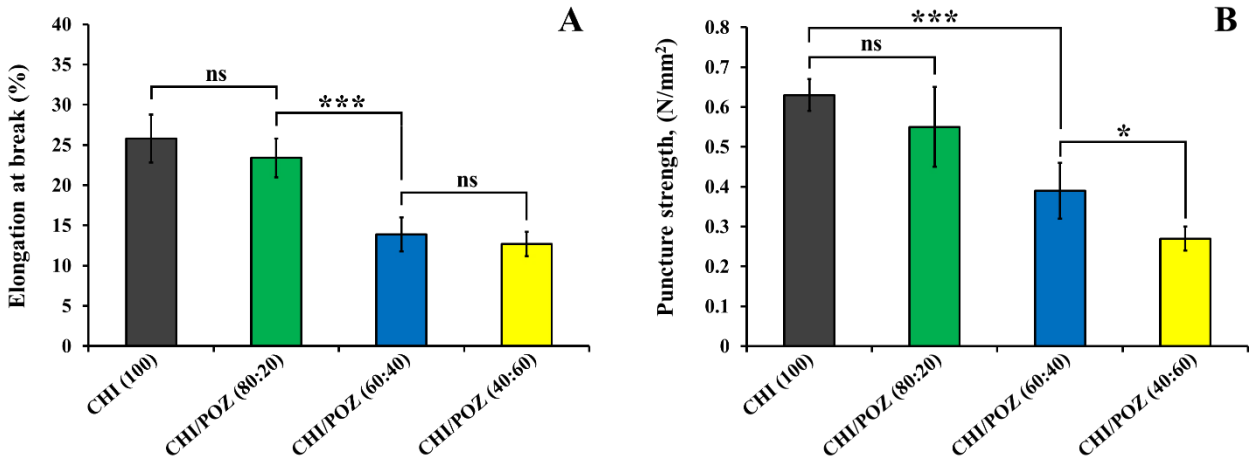

Figure 6. Mechanical analysis of CHI and CHI/POZ blend films: elongation at break (A) and puncture strength (B). Data are expressed as mean ± standard deviation (n = 5). Statistically significant differences are given as: *** – $p < 0.001$; * – $p < 0.05$; ns – no significance.

3.2. Mucosal retention of blend films on *ex vivo* corneal tissue

Soluble or swellable polymeric films have been used in ocular therapeutics either to improve the efficiency of drug delivery (compared to conventional eye drops), or to protect the injured cornea [53–55]. These films should exhibit some mucoadhesive properties to have a prolonged residence on the mucosa; however, eventually these materials are supposed to dissolve in the tear fluid completely or should allow their non-traumatising detachment from the ocular surface. These dosage forms should have excellent mechanical properties and a balanced mucoadhesiveness. Materials with insufficiently mucoadhesive properties will not be suitable because of the difficulties in their attachment to the ocular surface and, on the contrary, excessively films will cause discomfort because they may interfere with blinking [56]. Chitosan has previously been reported as a potential material for preparation of mucoadhesive ocular films [57–60]. It exhibits superior mucoadhesive properties compared to many other pharmaceutical polymers, which often needs to be moderated to make it suitable for a particular application. In the present work we have evaluated the suitability of CHI/POZ blends as materials for application as mucoadhesive ocular films.

Fluorescein sodium (NaFl) was used as a model drug to demonstrate the potential use of

CHI/POZ films for the application in ocular drug delivery. The retention of CHI and CHI/POZ films containing 0.1% NaFl on freshly isolated bovine cornea was assessed using a flow-through *in vitro* technique with fluorescent detection. This methodology has been previously used to study the retention of various materials on different mucosal surfaces, including ocular tissues, and it was validated against the other techniques established in assessment of mucoadhesive properties [35,36,38,61,62]. Figure 7 illustrates exemplary fluorescent images of the retention of polymeric films on bovine corneal mucosa irrigated with STF (200 µL/min). The fluorescence intensity on the mucosa was monitored following each washing cycle over 60 min. After analysing the fluorescent images using ImageJ software, it can be seen that CHI and CHI/POZ films showed an initial increase in fluorescence intensity after the first wash (Figure 8). This can potentially be related to the moisture effect on fluorescent films, causing an increased brightness that leads to >100% fluorescence intensity values. Despite of this initial increase in the fluorescence intensity, the subsequent washes resulted in the reduction of fluorescence intensity due to the dissolution of the films and subsequent wash out of NaFl. It was established that 100% CHI and CHI/POZ (80:20 and 60:40) exhibited significantly greater retention ($p < 0.01$) in the initial washing cycles (except for CHI after 60 min washing, 12 mL STF, $p < 0.05$) compared to CHI/POZ (40:60). It could be concluded that introduction of POZ made the films less retentive. This observation is consistent with our previous studies reporting that decorating nanoparticles with POZ makes them less mucoadhesive [22–24]. Moreover, the retention of CHI/POZ films (80:20 and 60:40) on ocular mucosa was found not to be significantly different from CHI ($p > 0.05$) demonstrating a similar retention profile until the end of washing cycles (Figure 8).

In parallel, STF solution flowing down the corneal surface during each washing cycle was collected at pre-determined time intervals and analysed using fluorescent spectrometry. An insert in Figure 8 depicts the cumulative percentage release of fluorescein sodium from CHI and CHI/POZ films. It was revealed that CHI/POZ (40:60) films released the highest amount of NaFl after total washing cycle over 60 min compared to 100% CHI and CHI/POZ (80:20 and 60:40). In the course

of work, the use of NaFl with the concentration higher than 0.1 mg/mL led to the formation of strong complexes with chitosan and precipitation of complexes were observed. Fluorescein sodium is a negatively charged compound and therefore may form complexes with the positively charged backbones of chitosan due to the electrostatic attraction forces. Hence, NaFl with lower concentration was used to prepare fluorescent films based on different ratios of CHI and POZ. Considering the composition of CHI/POZ fluorescent films, therefore, the lower content of chitosan in the blend film, the more NaFl is released. The fact that 100% CHI released less NaFl could be explained by partial entrapment of fluorescein sodium within the corneal epithelium, or/and by mucoadhesive effects of chitosan, i.e. specific interactions of chitosan macromolecules with the ocular surface.

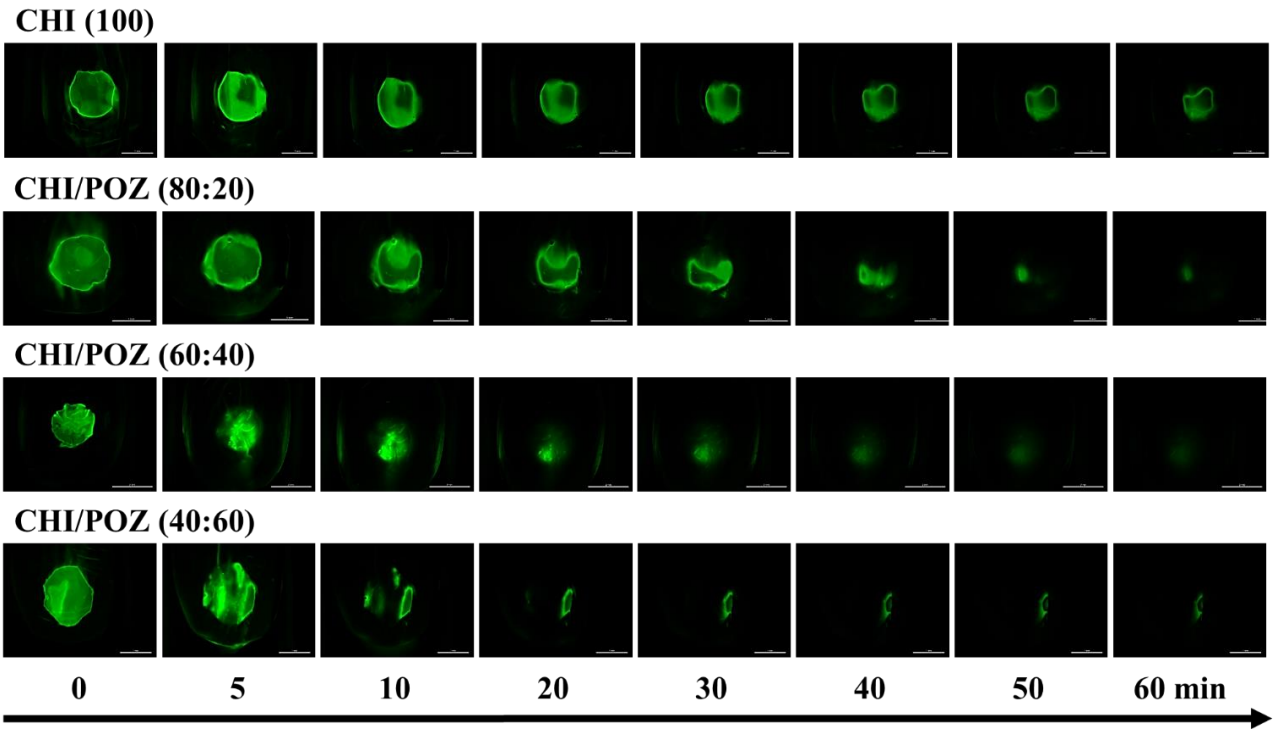

Figure 7. Exemplary fluorescent microphotographs showing mucosal retention of CHI and CHI/POZ films on freshly excised bovine cornea washed with different volumes of STF (200 µL/min) over 60 min. Scale bars are 1 µm.

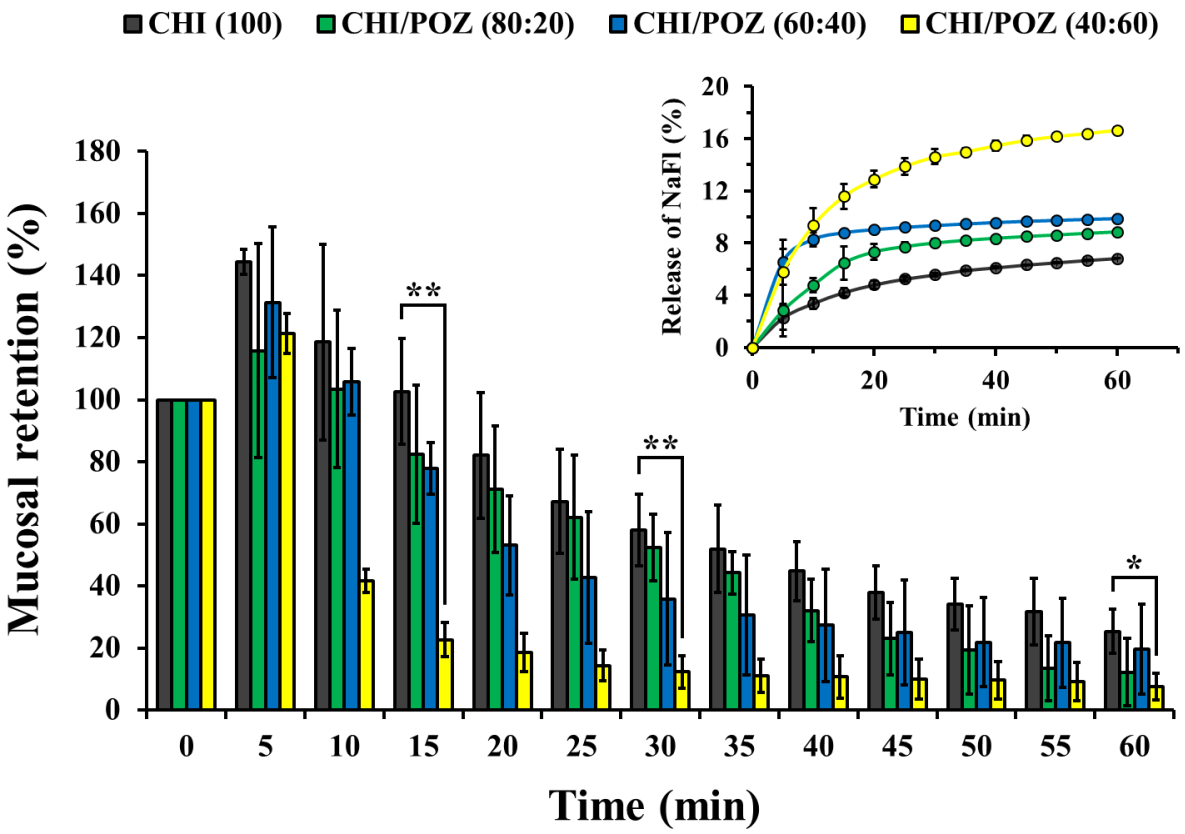

Figure 8. Mucosal retention of CHI and CHI/POZ films on fresh bovine cornea after irrigating with different volumes of STF (200 µL/min) over 60 min. All values are the means ± standard deviations of triplicate experiments. Statistically significant differences are given as: ** – $p < 0.01$; * – $p < 0.05$.

3.3. *In vivo* retention studies

Taking into consideration the known non-toxic and non-irritant nature of both chitosan and POZ [14,63] the mucoadhesive films were tested directly in vivo without prior evaluation of their biocompatibility in cell culture or other in vitro irritation models.

In vivo experiments were carried out in rabbits (n = 3) with round-shaped 10 mm CHI and CHI/POZ films containing 0.1 mg/mL NaFl. Each formulation was placed on corneal mucosa of a chinchilla rabbit's left eye and their retention was monitored visually by taking photographs at regular time points until the films detached. A UV lamp was used to enhance the detection of fluorescence.

Figure 9 shows exemplary images of rabbit eyes with fluorescent films administered *in vivo* providing even coverage of the cornea and subsequent changes were observed, i.e. time of

detachment, physical changes such as films swelling and wrinkling. The results for all other formulations are presented in Figures S3-S6 (Supplementary information). Films made of 100% POZ were not suitable for *in vivo* experiments due to their extreme brittleness. The *in vivo* results indicate that pure CHI and CHI/POZ films could achieve from at least 10 min to up to 50 min residence on the ocular surface, which is consistent with *in vitro* data. Generally, all the films exhibited excellent adhesion to the cornea. However, the presence of a nictitating membrane on chinchilla rabbit cornea often led to the dislodging or a complete removal of the films from the corneal surface into the lower fornix of conjunctiva. This greatly affected *in vivo* data reproducibility. No significant differences ($p > 0.05$) were observed between all film formulations in terms of their retention on the rabbit's cornea (Figure 10). It is expected that these films will exhibit much better retention in human tests because these dosage forms will not be voluntarily dislodged or removed. It should also be noted that the films did not cause any observable discomfort, irritation, inflammatory reactions or excessive tear production in rabbit eyes, which indicate that these materials are biocompatible and potentially suitable for ocular administration.

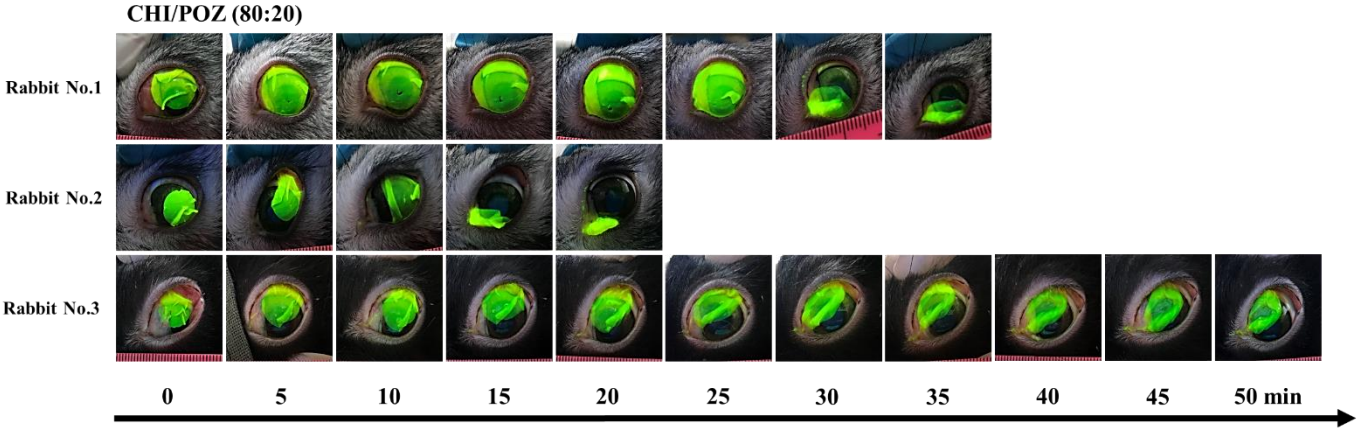

Figure 9. Exemplary photographs of *in vivo* mucosal retention of CHI/POZ (80:20) films on rabbit eyes taken at different time intervals.

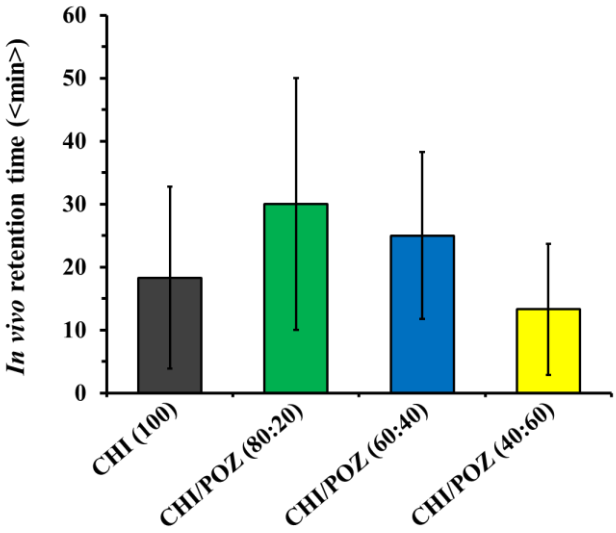

Figure 10. *In vivo* mucosal retention of CHI and CHI/POZ blend films on rabbit eye. Data are expressed as mean ± standard deviation (n = 3).

4. CONCLUSIONS

Polymeric blends of chitosan and poly(2-ethyl-2-oxazoline) were prepared in the form of flexible and transparent films using casting from aqueous solutions with subsequent solvent evaporation. The structure and physicochemical properties of these films were evaluated using Fourier-transformed infrared spectroscopy, thermal gravimetric analysis, differential scanning calorimetry, wide angle x-ray diffraction, tensile testing and scanning electron microscopy. These studies indicated a complete miscibility between the polymers in the blends. Blending of chitosan and poly(2-ethyl-2-oxazoline) leads to a significant reduction of the films mechanical properties (elongation at break and puncture strength). The films based on pure chitosan and blends with poly(2-ethyl-2-oxazoline) were formulated with sodium fluorescein and evaluated as potential dosage forms for ocular drug delivery both *in vitro* and *in vivo*. The results indicate that these films are biocompatible and do not cause any irritation to the eye. They also exhibit ability to adhere to the cornea and to retain for up to 50 min, providing a sustained drug release.

ACKNOWLEDGEMENTS

Chemical Analysis Facility (University of Reading) is thanked for providing access to DSC, SEM, TGA and PXRD instruments. The authors are grateful to Amanpreet Kaur and Nick Spencer (University of Reading) for their help with scanning electron microscopy and X-ray diffraction experiments, respectively. G.K.A. acknowledges Ministry of Education and Science of the Republic of Kazakhstan for the research grant (No. AP05133221). D.B.K. gratefully acknowledges the British Council Newton−Al-Farabi Partnership Programme, the Researcher Links Post-Doctoral Mobility Grant (No. 216046068) for financial support of his 2-years of postdoctoral fellowship at the University of Reading. P.C. Turner Abattoirs (Farnborough, United Kingdom) is also thanked for providing bovine eye tissues for experiments. The authors are grateful to Shariat Bolatova (Semey State Medical University, Kazakhstan) for her technical assistance with *in vivo* experiments.

DATA AVAILABILITY AND SUPPLEMENTARY INFORMATION

The Supplementary Information is available free of charge in PDF format. It contains a standard curve used to determine the amount of freed fluorescein sodium from CHI and CHI/POZ films (Figure S1); correlation between the maximal decomposition rate temperature and composition of polymer blends (Figure S2); and exemplary photographs of *in vivo* mucosal retention of CHI and CHI/POZ films on rabbit eyes taken at different time points (Figure S3-S6).

ABBREVIATIONS

CHI, chitosan; DSC, differential scanning calorimetry; EB, elongation at break; PBS, phosphate buffered saline; POZ, poly(2-ethyl-2-oxazoline); PS, puncture strength; SEM, scanning electron microscope; STF, simulated tear fluid; NaFl, fluorescein sodium salt; TGA, thermogravimetric analysis; WAXD, wide-angle X-Ray diffraction.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ORCID ID of authors

Daulet B. Kaldybekov: https://orcid.org/0000-0002-7191-5465

Aisulu Zh. Saimova: https://orcid.org/0000-0002-9564-732X

Galiya S. Irmukhametova: https://orcid.org/0000-0002-1264-7974

Vitaliy V. Khutoryanskiy: https://orcid.org/0000-0002-7221-2630

REFERENCES

[1] G.P. Andrews, T.P. Laverty, D.S. Jones, Mucoadhesive polymeric platforms for controlled drug delivery, Eur. J. Pharm. Biopharm. 71 (2009) 505–518. doi:https://doi.org/10.1016/j.ejpb.2008.09.028.

[2] V. V. Khutoryanskiy, Advances in mucoadhesion and mucoadhesive polymers, Macromol. Biosci. 11 (2011) 748–764. doi:10.1002/mabi.201000388.

[3] V. V. Khutoryanskiy, Mucoadhesive Materials and Drug Delivery Systems, John Wiley & Sons, Ltd, Chichester, UK, 2014. doi:10.1002/9781118794203.

[4] S. Mansuri, P. Kesharwani, K. Jain, R.K. Tekade, N.K. Jain, Mucoadhesion: A promising approach in drug delivery system, React. Funct. Polym. 100 (2016) 151–172. doi:https://doi.org/10.1016/j.reactfunctpolym.2016.01.011.

[5] A.R. Mackie, F.M. Goycoolea, B. Menchicchi, C.M. Caramella, F. Saporito, S. Lee, K. Stephansen, I.S. Chronakis, M. Hiorth, M. Adamczak, M. Waldner, H. Mørck Nielsen, L.

460 Marcelloni, Innovative methods and applications in mucoadhesion research, Macromol.
461 Biosci. 17 (2017) 1600534. doi:10.1002/mabi.201600534.
462 [6] M. Amidi, E. Mastrobattista, W. Jiskoot, W.E. Hennink, Chitosan-based delivery systems for
463 protein therapeutics and antigens, Adv. Drug Deliv. Rev. 62 (2010) 59–82.
464 doi:https://doi.org/10.1016/j.addr.2009.11.009.
465 [7] A. Bernkop-Schnürch, S. Dünnhaupt, Chitosan-based drug delivery systems, Eur. J. Pharm.
466 Biopharm. 81 (2012) 463–469. doi:https://doi.org/10.1016/j.ejpb.2012.04.007.
467 [8] L. Casettari, L. Illum, Chitosan in nasal delivery systems for therapeutic drugs, J. Control.
468 Release. 190 (2014) 189–200. doi:https://doi.org/10.1016/j.jconrel.2014.05.003.
469 [9] T.M.M. Ways, W.M. Lau, V. V Khutoryanskiy, Chitosan and its derivatives for application
470 in mucoadhesive drug delivery systems, Polymers (Basel). 10 (2018) 267–304.
471 doi:10.3390/polym10030267.
472 [10] K. Luo, J. Yin, O. V Khutoryanskaya, V. V Khutoryanskiy, Mucoadhesive and elastic films
473 based on blends of chitosan and hydroxyethylcellulose, Macromol. Biosci. 8 (2008) 184–
474 192. doi:10.1002/mabi.200700185.
475 [11] M.S. Freag, W.M. Saleh, O.Y. Abdallah, Exploiting polymer blending approach for
476 fabrication of buccal chitosan-based composite sponges with augmented mucoadhesive
477 characteristics, Eur. J. Pharm. Sci. 120 (2018) 10–19.
478 doi:https://doi.org/10.1016/j.ejps.2018.04.041.
479 [12] R.H. Sizílio, J.G. Galvão, G.G.G. Trindade, L.T.S. Pina, L.N. Andrade, J.K.M.C. Gonsalves,
480 A.A.M. Lira, M. V Chaud, T.F.R. Alves, M.L.P.M. Arguelho, R.S. Nunes, Chitosan/PVP-
481 based mucoadhesive membranes as a promising delivery system of betamethasone-17-
482 valerate for aphthous stomatitis, Carbohydr. Polym. 190 (2018) 339–345.
483 doi:https://doi.org/10.1016/j.carbpol.2018.02.079.
484 [13] R. Hoogenboom, Poly(2-oxazoline)s: A polymer class with numerous potential applications,
485 Angew. Chemie Int. Ed. 48 (2009) 7978–7994. doi:10.1002/anie.200901607.

[14] R. Luxenhofer, Y. Han, A. Schulz, J. Tong, Z. He, A. V Kabanov, R. Jordan, Poly(2-oxazoline)s as polymer therapeutics, Macromol. Rapid Commun. 33 (2012) 1613–1631. doi:10.1002/marc.201200354.

[15] O. Sedlacek, B.D. Monnery, S.K. Filippov, R. Hoogenboom, M. Hruby, Poly(2-Oxazoline)s – Are they more advantageous for biomedical applications than other polymers?, Macromol. Rapid Commun. 33 (2012) 1648–1662. doi:10.1002/marc.201200453.

[16] H. Bludau, A.E. Czapar, A.S. Pitek, S. Shukla, R. Jordan, N.F. Steinmetz, POxylation as an alternative stealth coating for biomedical applications, Eur. Polym. J. 88 (2017) 679–688. doi:https://doi.org/10.1016/j.eurpolymj.2016.10.041.

[17] T. Lorson, M.M. Lübtow, E. Wegener, M.S. Haider, S. Borova, D. Nahm, R. Jordan, M. Sokolski-Papkov, A. V Kabanov, R. Luxenhofer, Poly(2-oxazoline)s based biomaterials: A comprehensive and critical update, Biomaterials. 178 (2018) 204–280. doi:https://doi.org/10.1016/j.biomaterials.2018.05.022.

[18] A. Sundaramurthy, M. Vergaelen, S. Maji, R. Auzély-Velty, Z. Zhang, B.G. De Geest, R. Hoogenboom, Hydrogen bonded multilayer films based on poly(2-oxazoline)s and tannic acid, Adv. Healthc. Mater. 3 (2014) 2040–2047. doi:10.1002/adhm.201400377.

[19] S. Hendessi, P.T. Güner, A. Miko, A.L. Demirel, Hydrogen bonded multilayers of poly(2-ethyl-2-oxazoline) stabilized silver nanoparticles and tannic acid, Eur. Polym. J. 88 (2017) 666–678. doi:https://doi.org/10.1016/j.eurpolymj.2016.10.039.

[20] C. Su, J. Sun, X. Zhang, D. Shen, S. Yang, Hydrogen-bonded polymer complex thin film of poly(2-oxazoline) and poly(acrylic acid), Polymers (Basel). 9 (2017) 363. doi:10.3390/polym9080363.

[21] R. Hoogenboom, H. Schlaad, Thermoresponsive poly(2-oxazoline)s, polypeptoids, and polypeptides, Polym. Chem. 8 (2017) 24–40. doi:10.1039/C6PY01320A.

[22] E.D.H. Mansfield, K. Sillence, P. Hole, A.C. Williams, V. V Khutoryanskiy, POZylation: a new approach to enhance nanoparticle diffusion through mucosal barriers, Nanoscale. 7

(2015) 13671–13679. doi:10.1039/C5NR03178H.

[23] E.D.H. Mansfield, V.R. de la Rosa, R.M. Kowalczyk, I. Grillo, R. Hoogenboom, K. Sillence, P. Hole, A.C. Williams, V. V Khutoryanskiy, Side chain variations radically alter the diffusion of poly(2-alkyl-2-oxazoline) functionalised nanoparticles through a mucosal barrier, Biomater. Sci. 4 (2016) 1318–1327. doi:10.1039/C6BM00375C.

[24] T.M.M. Ways, W.M. Lau, K.W. Ng, V. V Khutoryanskiy, Synthesis of thiolated, PEGylated and POZylated silica nanoparticles and evaluation of their retention on rat intestinal mucosa in vitro, Eur. J. Pharm. Sci. 122 (2018) 230–238. doi:https://doi.org/10.1016/j.ejps.2018.06.032.

[25] L. Ruiz-Rubio, M.L. Alonso, L. Pérez-Álvarez, R.M. Alonso, J.L. Vilas, V. V Khutoryanskiy, Formulation of Carbopol®/Poly(2-ethyl-2-oxazoline)s mucoadhesive tablets for buccal delivery of hydrocortisone, Polymers (Basel). 10 (2018) 175. doi:10.3390/polym10020175.

[26] J. Dai, S.H. Goh, S.Y. Lee, K.S. Slow, Complexation between poly(2-hydroxypropyl methacrylate) and three tertiary amide polymers, J. Appl. Polym. Sci. 53 (1994) 837–845. doi:10.1002/app.1994.070530701.

[27] J. Dai, S.H. Goh, S.Y. Lee, K.S. Siow, Miscibility and interpolymer complexation of poly(2-methyl-2-oxazoline) with hydroxyl-containing polymers, J. Polym. Res. 2 (1995) 209–215. doi:10.1007/BF01492772.

[28] J.R. Isasi, E. Meaurio, C. Cesteros, I. Katime, Miscibility and specific interactions in blends of poly(2-ethyl-2-oxazoline) with hydroxylated polymethacrylates, Macromol. Chem. Phys. 197 (1996) 641–649. doi:10.1002/macp.1996.021970219.

[29] S. Kobayashi, M. Kaku, T. Saegusa, Miscibility of poly(2-oxazolines) with commodity polymers, Macromolecules. 21 (1988) 334–338. doi:10.1021/ma00180a009.

[30] L. Fang, S.H. Goh, Miscible chitosan/tertiary amide polymer blends, J. Appl. Polym. Sci. 76 (2000) 1785–1790. doi:10.1002/(SICI)1097-4628(20000620)76:12<1785::AID-

APP8>3.0.CO;2-B.

[31] P.J.A. Sobral, F.C. Menegalli, M.D. Hubinger, M.A. Roques, Mechanical, water vapor barrier and thermal properties of gelatin based edible films, Food Hydrocoll. 15 (2001) 423–432. doi:10.1016/S0268-005X(01)00061-3.

[32] S. Mali, M.V.E. Grossmann, M.A. García, M.N. Martino, N.E. Zaritzky, Barrier, mechanical and optical properties of plasticized yam starch films, Carbohydr. Polym. 56 (2004) 129–135. doi:10.1016/j.carbpol.2004.01.004.

[33] M. Preis, K. Knop, J. Breitkreutz, Mechanical strength test for orodispersible and buccal films, Int. J. Pharm. 461 (2014) 22–29. doi:10.1016/J.IJPHARM.2013.11.033.

[34] R. Gnanasambandam, N.S. Hettiarachchy, M. Coleman, Mechanical and barrier properties of rice bran films, J. Food Sci. 62 (1997) 395–398. doi:10.1111/j.1365-2621.1997.tb04009.x.

[35] G.S. Irmukhametova, G.A. Mun, V. V Khutoryanskiy, Thiolated mucoadhesive and PEGylated nonmucoadhesive organosilica nanoparticles from 3-mercaptopropyltrimethoxysilane, Langmuir. 27 (2011) 9551–9556. doi:10.1021/la201385h.

[36] E.A. Mun, P.W.J. Morrison, A.C. Williams, V. V Khutoryanskiy, On the barrier properties of the cornea: A microscopy study of the penetration of fluorescently labeled nanoparticles, polymers, and sodium fluorescein, Mol. Pharm. 11 (2014) 3556–3564. doi:10.1021/mp500332m.

[37] P.W.J. Morrison, N.N. Porfiryeva, S. Chahal, I.A. Salakhov, C. Lacourt, I.I. Semina, R.I. Moustafine, V. V Khutoryanskiy, Crown ethers: Novel permeability enhancers for ocular drug delivery?, Mol. Pharm. 14 (2017) 3528–3538. doi:10.1021/acs.molpharmaceut.7b00556.

[38] K. Al Khateb, E.K. Ozhmukhametova, M.N. Mussin, S.K. Seilkhanov, T.K. Rakhypbekov, W.M. Lau, V. V Khutoryanskiy, In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery, Int. J. Pharm. 502 (2016) 70–79. doi:10.1016/j.ijpharm.2016.02.027.

564 [39] E. Meaurio, L. Cesteros, L.G. Parada, I. Katime, Blends of poly(mono N-alkyl itaconates)
565 with tertiary polyamides: Specific interactions and thermal degradation, Polym. J. 36 (2004)
566 84–90. doi:10.1295/polymj.36.84.
567 [40] L.G. Parada, E. Meaurio, L.C. Cesteros, I. Katime, Miscibility behaviour in blends of
568 copolymers of vinyl alcohol with poly(ethyloxazoline), Macromol. Chem. Phys. 199 (1998)
569 1597–1602. doi:10.1002/(SICI)1521-3935(19980801)199:8<1597::AID-
570 MACP1597>3.3.CO;2-W.
571 [41] S.H. Goh, S.Y. Lee, X. Zhou, K.L. Tan, Miscibility and specific interactions in two
572 polyelectrolyte/poly(2- ethyl-2-oxazoline) blend systems, Polymer (Guildf). 40 (1999) 2667–
573 2673. doi:10.1016/S0032-3861(98)00501-1.
574 [42] M. Maldonado-Santoyo, C. Ortíz-Estrada, G. Luna-Bárcenas, I.C. Sanchez, L.C. Cesteros, I.
575 Katime, S.M. Nuño-Donlucas, Miscibility behavior and hydrogen bonding in blends of
576 poly(vinyl phenyl ketone hydrogenated) and poly(2-ethyl-2-oxazoline), J. Polym. Sci. Part B
577 Polym. Phys. 42 (2004) 636–645. doi:10.1002/polb.10758.
578 [43] I. Leceta, P. Guerrero, I. Ibarburu, M.T. Dueñas, K. de la Caba, Characterization and
579 antimicrobial analysis of chitosan-based films, J. Food Eng. 116 (2013) 889–899.
580 doi:https://doi.org/10.1016/j.jfoodeng.2013.01.022.
581 [44] J. Bonilla, E. Fortunati, L. Atarés, A. Chiralt, J.M. Kenny, Physical, structural and
582 antimicrobial properties of poly vinyl alcohol–chitosan biodegradable films, Food Hydrocoll.
583 35 (2014) 463–470. doi:https://doi.org/10.1016/j.foodhyd.2013.07.002.
584 [45] C.L. Silva, J.C. Pereira, A. Ramalho, A.A.C.C. Pais, J.J.S. Sousa, Films based on chitosan
585 polyelectrolyte complexes for skin drug delivery: Development and characterization, J.
586 Memb. Sci. 320 (2008) 268–279. doi:https://doi.org/10.1016/j.memsci.2008.04.011.
587 [46] A. Colombo, F. Gherardi, S. Goidanich, J.K. Delaney, E.R. de la Rie, M.C. Ubaldi, L.
588 Toniolo, R. Simonutti, Highly transparent poly(2-ethyl-2-oxazoline)-TiO2 nanocomposite
589 coatings for the conservation of matte painted artworks, RSC Adv. 5 (2015) 84879–84888.

590 doi:10.1039/C5RA10895K.
591 [47] A. Pawlak, M. Mucha, Thermogravimetric and FTIR studies of chitosan blends,
592 Thermochim. Acta. 396 (2003) 153–166. doi:https://doi.org/10.1016/S0040-6031(02)00523-
593 3.
594 [48] T. Wanjun, W. Cunxin, C. Donghua, Kinetic studies on the pyrolysis of chitin and chitosan,
595 Polym. Degrad. Stab. 87 (2005) 389–394.
596 doi:https://doi.org/10.1016/j.polymdegradstab.2004.08.006.
597 [49] E. Beruhil Adatoz, S. Hendessi, C.W. Ow-Yang, A.L. Demirel, Restructuring of poly(2-
598 ethyl-2-oxazoline)/tannic acid multilayers into fibers, Soft Matter. 14 (2018) 3849–3857.
599 doi:10.1039/C8SM00381E.
600 [50] J. Yin, K. Luo, X. Chen, V. V Khutoryanskiy, Miscibility studies of the blends of chitosan
601 with some cellulose ethers, Carbohydr. Polym. 63 (2006) 238–244.
602 doi:10.1016/J.CARBPOL.2005.08.041.
603 [51] A. Shubha, S.R. Manohara, L. Gerward, Influence of polyvinylpyrrolidone on optical,
604 electrical, and dielectric properties of poly(2-ethyl-2-oxazoline)-polyvinylpyrrolidone blends,
605 J. Mol. Liq. 247 (2017) 328–336. doi:https://doi.org/10.1016/j.molliq.2017.09.086.
606 [52] H. Fael, C. Ràfols, A.L. Demirel, Poly(2-ethyl-2-oxazoline) as an alternative to
607 poly(vinylpyrrolidone) in solid dispersions for solubility and dissolution rate enhancement of
608 drugs, J. Pharm. Sci. 107 (2018) 2428–2438. doi:https://doi.org/10.1016/j.xphs.2018.05.015.
609 [53] H. Abdelkader, B. Pierscionek, R.G. Alany, Novel in situ gelling ocular films for the opioid
610 growth factor-receptor antagonist-naltrexone hydrochloride: Fabrication, mechanical
611 properties, mucoadhesion, tolerability and stability studies, Int. J. Pharm. 477 (2014) 631–
612 642. doi:https://doi.org/10.1016/j.ijpharm.2014.10.069.
613 [54] H.S. Mahajan, S.R. Deshmukh, Development and evaluation of gel-forming ocular films
614 based on xyloglucan, Carbohydr. Polym. 122 (2015) 243–247.
615 doi:https://doi.org/10.1016/j.carbpol.2015.01.018.

[55] S. Karki, H. Kim, S.-J. Na, D. Shin, K. Jo, J. Lee, Thin films as an emerging platform for drug delivery, Asian J. Pharm. Sci. 11 (2016) 559–574. doi:https://doi.org/10.1016/j.ajps.2016.05.004.

[56] O. V Khutoryanskaya, P.W.J. Morrison, S.K. Seilkhanov, M.N. Mussin, E.K. Ozhmukhametova, T.K. Rakhypbekov, V. V Khutoryanskiy, Hydrogen-bonded complexes and blends of poly(acrylic acid) and methylcellulose: Nanoparticles and mucoadhesive films for ocular delivery of riboflavin, Macromol. Biosci. 14 (2014) 225–234. doi:10.1002/mabi.201300313.

[57] G. de O. Fulgêncio, F.A.B. Viana, R.R. Ribeiro, M.I. Yoshida, A.G. Faraco, A. da S. Cunha-Júnior, New mucoadhesive chitosan film for ophthalmic drug delivery of timolol maleate: In vivo evaluation, J. Ocul. Pharmacol. Ther. 28 (2012) 350–358. doi:10.1089/jop.2011.0174.

[58] R.M.D. Byrro, G. de Oliveira Fulgêncio, A. da Silva Cunha, I.C. César, P.R. Chellini, G.A. Pianetti, Determination of ofloxacin in tear by HPLC–ESI-MS/MS method: Comparison of ophthalmic drug release between a new mucoadhesive chitosan films and a conventional eye drop formulation in rabbit model, J. Pharm. Biomed. Anal. 70 (2012) 544–548. doi:https://doi.org/10.1016/j.jpba.2012.05.003.

[59] G. de Oliveira Fulgêncio, F.A.B. Viana, R.O.S. Silva, F.C.F. Lobato, R.R. Ribeiro, J.R. Fanca, R.M.D. Byrro, A.A.G. Faraco, A. da Silva Cunha-Júnior, Mucoadhesive chitosan films as a potential ocular delivery system for ofloxacin: preliminary in vitro studies, Vet. Ophthalmol. 17 (2014) 150–155. doi:10.1111/vop.12140.

[60] K. Hermans, D. Van den Plas, S. Kerimova, R. Carleer, P. Adriaensens, W. Weyenberg, A. Ludwig, Development and characterization of mucoadhesive chitosan films for ophthalmic delivery of cyclosporine A, Int. J. Pharm. 472 (2014) 10–19. doi:https://doi.org/10.1016/j.ijpharm.2014.06.017.

[61] P. Tonglairoum, R.P. Brannigan, P. Opanasopit, V. V. Khutoryanskiy, Maleimide-bearing nanogels as novel mucoadhesive materials for drug delivery, J. Mater. Chem. B. 4 (2016)

642 6581–6587. doi:10.1039/C6TB02124G.
643 [62] O.M. Kolawole, W.M. Lau, V. V Khutoryanskiy, Chitosan/β-glycerophosphate in situ
644 gelling mucoadhesive systems for intravesical delivery of mitomycin-C, Int. J. Pharm. X. 1
645 (2019) 100007. doi:https://doi.org/10.1016/j.ijpx.2019.100007.
646 [63] S. Rodrigues, M. Dionísio, C.R. López, A. Grenha, Biocompatibility of chitosan carriers with
647 application in drug delivery, J. Funct. Biomater. 3 (2012). doi:10.3390/jfb3030615.
648