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Preparation and Characterization Studies of Dorzolamide Loaded Ophthalmic Implants for the Treatment of Glaucoma

Short title: Dorzolamide Loaded Ophthalmic Implants

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ABSTRACT
Objectives: This study aimed to construct dorzolamide loaded ophthalmic implants for extended drug delivery and increased drug retention.
Materials and Methods: Carboxymethyl cellulose and Chitosan were used to formulate the ophthalmic implants. The implants were prepared by solvent casting technique in existence of polyethylene glycol 6000 as plasticizer. Physiochemical characterization studies including mechanical characteristics (tensile strength, elongation at break, and Young's modulus), bioadhesion studies, *in vitro* and *ex vivo* drug release studies were conducted.
Results: Tensile strength of drug loaded ophthalmic implants were 10.70 and 11.68 MPa respectively. Elongation at break of Carboxymethyl cellulose and Chitosan implants were 62.00% and 59.05% respectively. The *in vitro* release profiles fit into the Higuchi type kinetic model. *Ex vivo* release study results for both implants were correlated with *in vitro* release investigations.
Conclusion: Carboxymethyl cellulose and Chitosan based implants provide extended drug delivery. Implants were prepared by using carboxymethyl cellulose provide significantly slower *in vitro* release rate, and the drug retention on ocular surfaces has been increased. Thus, it has been concluded that, dorzolamide loaded carboxymethyl cellulose implants could provide effective treatment for glaucoma.
Keywords: Dorzolamide; Carboxymethyl Cellulose; Chitosan; Ocular Implant

INTRODUCTION

Glaucoma is a progressive optic neuropathy with the result of high intra-ocular pressure (IOP). This condition is stated as the main reason of irreversible blindness after diabetic retinopathy.[1] Purpose of the therapy is prevention of the optic nerve damage. Glaucoma is treated basically with the use of drugs (pharmaceutical therapy). Pharmacologically, beta receptor antagonists, prostaglandin analogs, alpha-2 agonists and carbonic anhydrase inhibitors are chosen for treatment options.[2] Dorzolamide (DRZ) is one of the carbonic anhydrase inhibitors that decreases the secretion of aqueous humor, thus the IOP is lowered.[3] Dorzolamide eye drop is available in the market under trade name as Trusopt® (Merck, N.J., USA). The dosage form contains 2% of dorzolamide aqueous buffered solution at pH 5.6. Dorzolamide 2% eye drops have exhibited the highest and optimal therapeutic effect in clinical trials.[4]

Effective ocular delivery of drug substances is a challenging process. Topical ophthalmic solutions are mostly preferred preparations by clinicians due to its ease of application. However, topical conventional delivery systems remain insufficient due to multifactorial restrictions of the ocular anatomy. These can be mainly categorized as intraocular microenvironment, static, dynamic, and metabolic barriers.[5] Intraocular environment includes blood–

aqueous and blood–retina barrier. The static barriers include biological structures such as; corneal epithelium, sclera and conjunctiva. The metabolic barriers contain the metabolic enzymes. Dynamic barriers are listed as; blinking, tear turnover and nasolacrimal drainage which are remarkably decrease the drug bioavailability.[6]
Lots of sophisticated strategies have been introduced for bypassing the ophthalmic barriers such as nanoparticles for enhancement of corneal permeation[7-9], liposomal carriers for enhancement of ocular absorption and precorneal retention.[10,11] Micro or nano emulsions for increasing the precorneal residence time and providing sustained release.[12,13] Hydrogels and ocular inserts are used as either primary or secondary delivery systems. The incorporation of nano carriers to the hydrogels or ocular inserts makes them secondary delivery system. Hydrogels or ocular inserts can be used as primary drug delivery system for the treatment of ophthalmic problems.[14,15]
Chitosan (CHI) and carboxymethyl cellulose (CMC) are biodegradable and bio adhesive polymers that are used for the production of hydrogel or ocular implant or insert formulations.[16,17] These polymers are compatible materials with drug substances and biological surfaces. In this study, it was aimed to construct an ophthalmic implant for the extended delivery of DRZ to achieve efficient glaucoma treatment. For this purpose, polymeric ophthalmic implants were formulated and physiochemically characterized, then *in vitro* and *ex vivo* drug release profiles were investigated.

MATERIALS AND METHODS

Materials

CMC, and CHI were obtained from Sigma-Aldrich (Germany). Polyethylene glycol 6000 (PEG 6000), acetic acid, potassium dihydrogen phosphate (K_2PO_4) and methanol were obtained from Merck-Millipore (USA). DRZ was kindly donated from Deva Pharmaceuticals (Turkey). The other materials were of analytical quality.

Methods

Preparation of ophthalmic implants

In this study, CMC and CHI were used as polymers, PEG 6000 was selected as plasticizer (to provide elasticity) and DRZ was used as active pharmaceutical ingredient which is dissolved in aqueous polymer-plasticizer dispersion. CHI based implants include: one-gram CHI was dispersed in 100 mL aqueous acetic acid solution (1%, w/w). CMC based implants include: one-gram CMC was dispersed in 100 mL water. Both of the implant formulations have 0.1 g PEG 6000 in 100 mL total dispersion volume in order to improve mechanic properties.
The dispersions were poured into the empty contact lens containers (1-Day Acuvue® Moist contact lens container). The lens containers have a 14.2 mm diameter and have a space to hold a gram of mass. Dispersion-loaded containers were left for drying under the fume hood for 24 hours at room temperature. According to the placebo weight of containers, the amount of DRZ was adjusted, then the strength was obtained as 2 mg DRZ/implant.

Analytical Quantification of DRZ

Quantification of DRZ was performed with using a high performance liquid chromatography (HPLC) (Agilent 1100 Series, Germany). The reported analytical method has been slightly changed.[18] In brief, the HPLC equipped with multiple wavelength ultraviolet/visible detectors. Separation was conducted by using a C-18 column (5 µm, 4.6 × 150 mm) (AgilentTech, Germany) at 25 ± 0.5 °C. A potassium dihydrogen phosphate (K_2PO_4) (pH:2.5): Methanol (MeOH) mixture (90:10, v/v) was used as the mobile phase. The quantification was achieved at 0.8 mL/min of flow rate. This analytical method was validated by using universal parameters.

Characterization Studies of Ocular Implants

Bioadhesion Studies

A previously stated technique was slightly adapted for the ocular tissues.[19] Bioadhesive characteristics of implants were detected by applying the texture analyzer (TA-XT Plus Texture Analyzer, Stable Micro System, UK). Swine eyes were obtained from the laboratory animals center (the ethical status has been presented in supplementary file) then, the cornea was isolated from the ocular tissues. The implant was stabilized on a probe of the instrument by using adhesive tape and the cornea was placed on the other probe of the instrument. DRZ loaded implant was contacted to the tissue. The time of contact, the rate and applied force was 60 s, 1 mm/s and 0.2 N, respectively. The work of bioadhesion has been calculated by using force-distance graph.

Mechanical Characteristics

Mechanical characteristics of ocular implants were investigated by using a texture analyzer (TA-XT Plus Texture Analyzer, Stable Micro Systems, UK). Calibration of force was achieved by using 2 kg of weight and calibration of height was achieved for Tensile Grips (Stable Micro Systems, UK). The samples were arranged by cutting 10mm x

10mm were stabilized in grip with primary distance of 50 mm and crosshead speed kept at 2 mm/sec tension mode. Tensile strength (TS) in MPa was measured by dividing the peak load improved during the analysis by the film's cross-sectional area. Tensile strength (TS, MPa), maximum elongation percentage at break (EAB, %), and Young's modulus (YM, MPa) were computed by using equations. 1, 2, and 3 respectively. Tests were achieved in triplicates and the outcomes were described as mean value (± SD).

$$\mathrm{TS} = \frac{Fmax}{A} \quad \text{(Eq.1)}$$

$$\mathrm{EAB} = \frac{Lmax}{L0}\ x\ 100 \quad \text{(Eq.2)}$$

$$\mathrm{YM} = S\,x\,\frac{L0}{A} \quad \text{(Eq.3)}$$

F_{max} indicates the maximum force, A is implant's cross sectional area, L_{max} and L0 are maximum deformation before rupture and primary length respectively, S is slope of force deformation.[20]

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The spectrums of ocular implants were obtained in range of 650 - 4000 cm^{-1} by using FTIR spectrometer (NICOLET iS50, Thermo Scientific, USA). Ocular implants were nicely divided and the specimens were directly applied over the crystal of the spectrometer. Scans were performed for each specimen and the force over the specimen was arranged to obtain satisfactory transmittance results.

Thermal Analysis

Previous method was applied to detect the thermal behavior of all materials.[21] Specimens were arranged as small parts and (approximately 5 mg) transferred in covered aluminum pans. The temperature was elevated up to 300 °C under a cover of nitrogen gas (50ml/s) with heating rate of 10 °C/min using Differential Scanning Calorimetry (DSC) instrument (Setaram, DSC131, France).

Morphological Analysis

Morphological analysis of ocular implants was achieved by using a scanning electron microscope (SEM) (Quattro S, Thermo Scientific, USA). SEM used with an accelerating voltage of 15.00 kV and surfaces of the isolated specimens were covered with gold and palladium by using sputter (LEICA EM ACE200, Leica Microsystems, Germany) at 3 kV for 60 s. SEM micrographs were captured by applying a high vacuum.

Solubility studies

Prior to the *in vitro* and *ex vivo* studies, solubility of DRZ was investigated by using previously described method.[19] Briefly, the excessive amount of DRZ was added into flasks (10ml volume of each) that contains distilled water (pH: 7), phosphate buffer (pH: 7.4), normal saline solution (0.9% of NaCl, pH:5.5). The flasks were shaken for 48 h and saturated solutions were filtered through 0.22 µm filters and quantified by using HPLC.

In vitro drug release

In vitro DRZ release profile was investigated by using previously reported paddle over disk method.[21] The specimens were placed in the vessels that contain 250 ml phosphate buffer solution at pH 7.4. Temperature was kept constant at 37.5 °C. At predetermined time intervals 2 ml of samples were withdrawn from the release medium and completed with same volume of fresh buffer solution. The samples were analyzed by using the validated HPLC method. Release kinetics were also assessed by using Zero-order, First-order, Hixson Crowell, Higuchi, and Korsmeyer-Peppas models.

Ex-vivo studies

Ex vivo studies were conducted by using removed swine eyes. The eyes were placed into a small beaker (25 ml volume) and 20 ml buffer solution (pH 7.4) was added up to covering the surface of the eye. Then, the ophthalmic implant was placed onto the eye. At predetermined time intervals 1 ml of sample was withdrawn and replenished by using fresh buffer.

An eye drop of DRZ was prepared by using normal saline solution (NS-DRZ) at 2% (w/v) of concentration. Similarly, the same *ex vivo* protocol was applied to the eye drops. Briefly, 2 drops (2mg of DRZ) of preparation were applied to the swine eyes (placed in 20 ml buffer solution at pH 7.4). At predetermined time intervals, 1 ml of sample was withdrawn and replenished by using a fresh buffer.

The samples were analyzed by using the HPLC method. Then, the amount of drug penetrated was calculated retrospectively.

RESULTS AND DISCUSSION

Preparation of Ophthalmic Implants

Ophthalmic implants in shape of convex ocular hemispheres were prepared via using previously generated solvent casting method.[21] This technique involves preparing a polymer dispersion which is poured onto a concave mold (empty contact lens containers). Then, the solvent was removed by evaporation which causes the reorganization of polymer molecules and engagement with each other. Finally, formation of films was achieved by this phenomenon. After solvent casting, implants had similar surface with the mold shape. They were dry, elastic, and transparent films. Elastic films were easily taken from the contact lens containers (molds), probably the presence of plasticizer.

Mechanical Characteristics

Mechanical characteristics of the specimens, including TS, EAB, and YM were shown in Table 1. EAB is defined as the ability of film to extend before it breaks. For that reason, if the EAB is high, the structure of implant might be thought as flexible and soft.[22] TS is described as the maximum load power used to break the film. Rigid and fragile materials exhibit high resistance.[23] EAB and TS of unloaded CMC films were 71.68% and 7.81 MPa respectively, and the values of loaded CMC films were 62.00% and 10.70 MPa respectively. The similar values are also available for CHI films, unloaded CHI films were 69.78% and 8.21 MPa respectively, and the values of loaded CHI films were 59.05% and 11.68 MPa respectively. The decline of the EAB and increment of the TS could be explained as the drug molecules were interposed the linkages of the polymers.

Similar formulations based on CMC were investigated, it was observed that mechanical properties changed according to the concentration of active substance. In a study, 1 % (w/w) of CMC film was formulated with different concentrations of active substance and it was found that addition of the active substance increases the TS of lean film from 17.75 MPa to 58.85 MPa. Also, the amount of casting mass and thickness of the final formulation has a direct effect on the mechanical properties. The increment of mechanical strength is compatible with literature data.[24]

Table 1: Mechanical Characteristics of Inserts

Sample	Work of Bioadhesion (mJ/cm^2)	TS (MPa)	YM (MPa)	EAB (%)
CMC 1% Unloaded	0.143 ± 0.046	7.81 ± 0.017	10.77 ± 0.317	71.68 ± 0.049
CMC 1% Loaded	0.427 ± 0.163	10.70 ± 0.031	13.80 ± 0.226	62.00 ± 0.03
CHI 1% Unloaded	0.255 ± 0.032	8.21 ± 0.02	11.32 ± 0.165	69.78 ± 0.211
CHI 1% Loaded	0.434 ± 0.072	11.68 ± 0.012	14.39 ± 0.244	59.05 ± 0.101

For the mechanical assessment of CHI-based formulations, it was observed that polymer concentration and active substance amount have direct impacts on the mechanical properties. In a research study, thin-film formulations of CHI have been prepared. Then, it was found that mechanical strength has been elevated (7.1 N, 21.6 N, 36.5 N) by increased polymer concentrations (1%, 1.5%, 2%), and elastic properties were found to be declined. In the same report, it was observed that incorporation of the active substance exhibited similar mechanical behavior.[25]

In the pre-formulation part, it is not possible to remove the lean implants (contains only polymers) from the molds because of their fragileness. Thus, a plasticizer (PEG 6000) was added onto the lean CMC and CHI dispersions to augment mechanical characteristics. YM is related with film rigidity and ability to undergo elastic deformation under applied stress.[23] The addition of DRZ has been increased the YM. The data gathered from the mechanical experiments (EAB, TS and YM) has been found to be correlated with each other.

Bioadhesive assessment of the implant formulations are demonstrated in Table 1. Unloaded implants exhibited lesser work of bioadhesion than loaded formulations. Thus, drug loaded formulations could be promising delivery systems for the eye. The chemical structure of the drug substance in salt form, thus may affect the bioadhesive properties of implants. The existence of salts has been reported as one of the factors that affect the bioadhesive properties of polymeric drug delivery systems for topical or mucosal administration.[26]

The *in vivo* bioadhesion mechanism of the polymeric films can be explained by the interaction with tear fluid or meibum which is secreted from holocrine meibomian glands.[27] The early stages of mucosal adhesion include the hydration of the polymer via normal physiological conditions of the eye surface. The hydrogen bonding capacity of polymers (CMC and CHI) have also contributed to mucosal adhesion due to the presence of hydroxyl groups. This

functional group has also contributed to the wettability and hydration. Physiologically, the contents of the meibum (ester content) could have great potential to increase the adhesion properties as *in vivo*.

FTIR analysis

FTIR reflects the interaction between the contents of ocular implants and the DRZ. These possible interactions will directly affect the characteristics of the ocular implant.[3] FTIR spectra of unloaded CMC and CHI implants treated with DRZ are shown in Figure 1. The 4 inserts exhibited similar main peaks, but the amplitude varied dramatically, with some of them moving. The Figure 1 depicts the FTIR spectra of the inserts. In the DRZ spectrum, the characteristic SO_2 bonds of sulphonamide, shifted from 1342 cm^{-1} to 1782 cm^{-1} and 1766 cm^{-1} for %1 CMC-DRZ and %1 CHI respectively. Other characteristic bands of -NH2+ stretching at 1281 cm^{-1}, shifted to 1396 cm^{-1} for %1CHI-DRZ and become widespread for %1 CMC-DRZ.[28,29] %1 CMC-DRZ and %1 CHI-DRZ spectrums show that the peak intensities of the CMC were better than the CHI formulations. In the %CHI spectrum, the characteristic bands of -CH_2 stretching at 1083 cm^{-1}, become widespread for %1 CHI-DRZ and %CHI spectrum, the characteristic bands of -C=O stretching at 1603 cm^{-1}, intensity decreased with the addition of DRZ for %CHIDRZ.[30,31] The peak intensity of the films with DRZ was better than that of the films without DRZ. In addition, the double spectra at 2900-2800 cm^{-1} observed in both CMC and CHI formulations are due to the vibrations of the -COO group in CMC and CHI.[32] Therefore, we confirmed that the addition of DRZ to and use of CMC film can facilitate uniform mixing in the film.

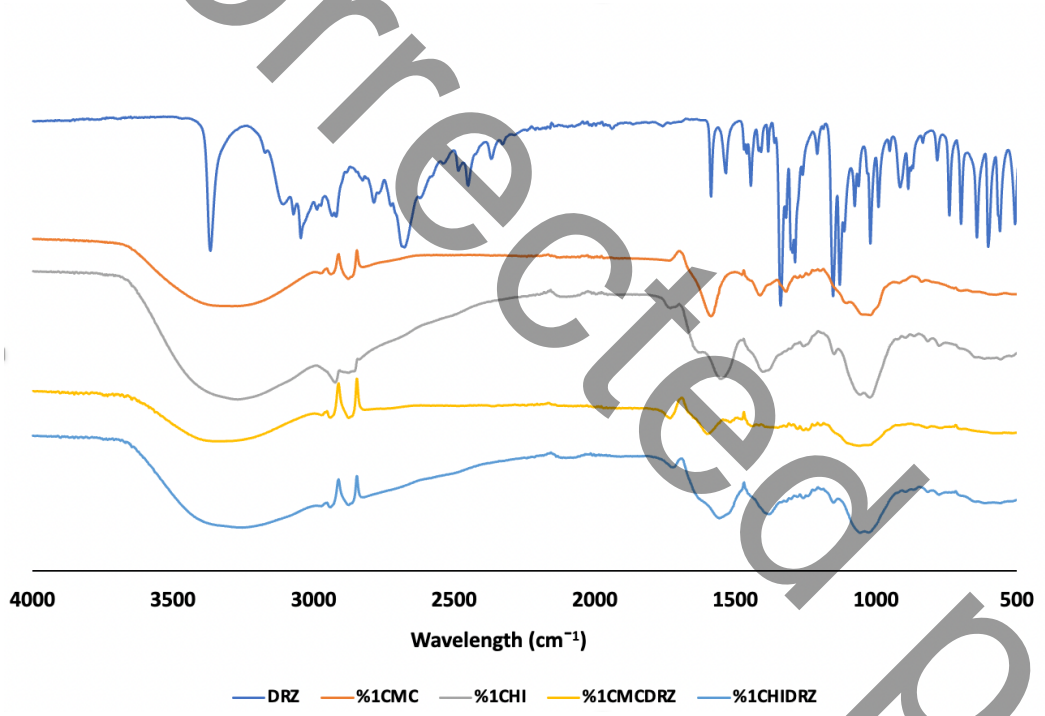

Figure 1. FT-IR spectra of active substance and formulations

DSC Analysis

Determination of solid-state interactions has been performed by using DSC. The thermograms are shown in Figure 2 and melting points, enthalpies and crystallinity indices are presented in Table 2. The enthalpy values of DRZ, CMC %1, CMC-DRZ %1, CHI %1 and CHI-DRZ %1 was 9.455, 29.016, 28.702, 23.892 and 21.233 J/g, and the melting temperatures of the DRZ, CMC %1, CMC-DRZ%1, CHI%1 and CHI-DRZ%1 were 260.41, 219.82, 206.56, 249.14 and 239.53 °C respectively.

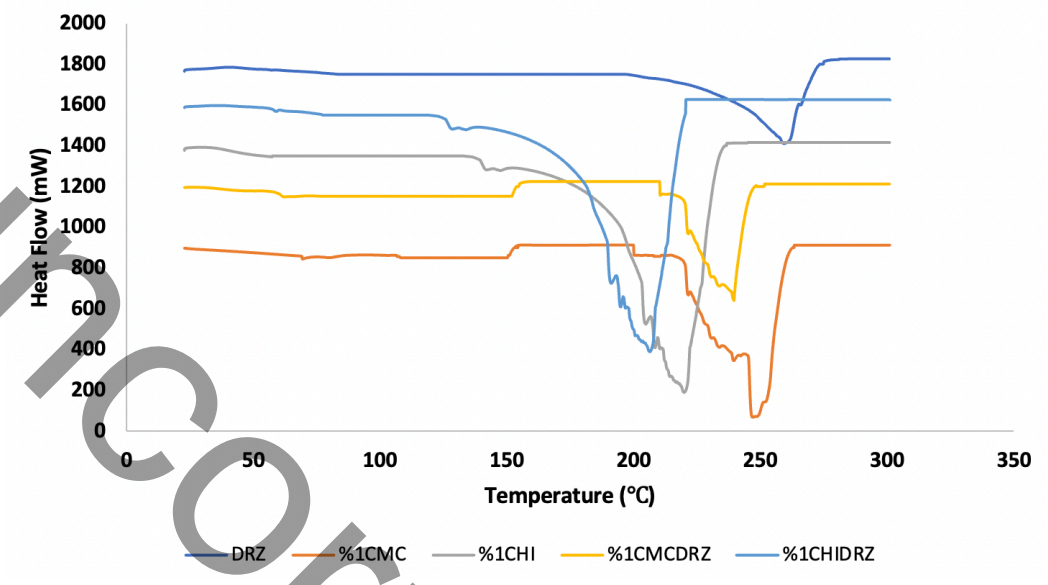

Figure 2. DSC curves of DRZ, CMC %1, CMC-DRZ%1, CHI%1 and CHI-DRZ%1

Table 2 shows that results proved the CMC of the amorphous structure of polymers and obstructed crystallization.[33,34] Melting points of ocular films have exposed a decline to lower temperatures with larger peaks compared to the bulk polymer by giving variable enthalpy values indicating several thermal transitions as well.[35] Decline in melting points of CHI%1 and CHI-DRZ%1 formulation was detected as 10 °C when added the carboxymethyl cellulose instead of CHI caused more decrease in case of CMC%1 and CMC-DRZ1%. The reduction in CI of CMC%1 and CMC-DRZ%1 compared to CHI%1 and CHI-DRZ%1 could be attributed to the crystal order in CMC%1 and CMC-DRZ%1 greatly disturbed due to CMC. In a study, salicylic acid was loaded into the CHI-based films and reported that the crystallization index of the salicylic acid loaded formulations increased by about 10% compared to the unloaded films.[36]

Table 2: Thermal parameters of active substance and films

Formulation	Melting point (°C)	Enthalpy ΔH (J/g)	Crystallinity indice (CI) (%)
DRZ	260.41	9.455	100
CMC%1,	219.82	29.016	32.57
CMC-DRZ%1	206.56	28.702	32.94
CHI%1	249.14	23.892	39.58
CHI-DRZ%1	239.53	21.233	44.529

SEM Analysis of ocular implants

Images of ocular inserts are presented in Figure 3. Obtained data verify that the surface of the designed inserts is smooth and plain. Therefore, it could be considered that the implants would not block the vision.

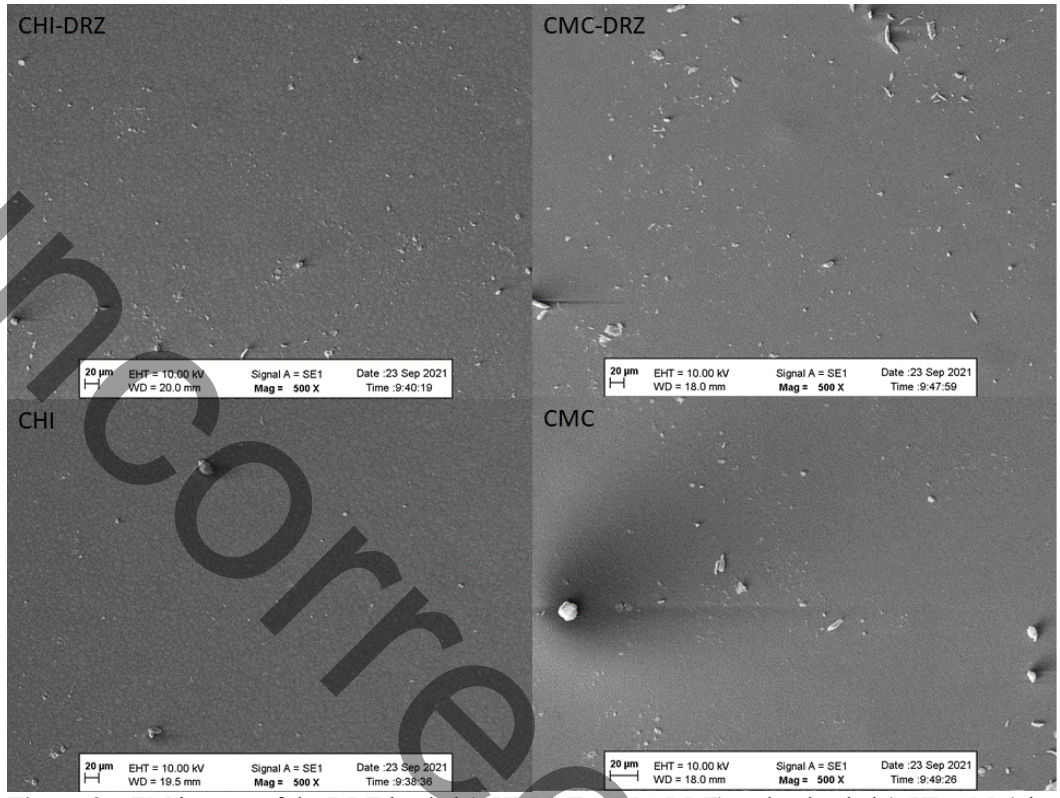

Figure 3. SEM images of the DRZ loaded (CHI-DRZ; CMC-DRZ) and unloaded (CHI; CMC) implants.

In vitro drug release

The *in vitro* drug release profile of a drug delivery system is an important parameter for noticing the *in vivo* action of a drug substance. Generally, the release experiments are accomplished under sink conditions. European pharmacopoeia (EP) describes the sink conditions as a volume of release medium that is at least three to ten times of the active ingredient's saturation volume.[37] Solubility of DRZ was found as 6.65 mg/ml, 6.72 mg/ml, and 38.76 mg/ml in distilled water (pH:7), and phosphate buffer (pH:7.4) and normal saline solution (pH:5.5) respectively. The obtained solubility data are in agreement with the literature.[38,39] After that, the volume of release medium and content was determined, and other parameters were selected by considering the normal physiological conditions. According to the mathematical analysis of the *in vitro* release studies (Table 3, Figure 4), the profiles fitted to Higuchi type kinetic model. As indicated in the literature Higuchi type release kinetics could express the drug release from the polymeric matrices.[40] Moreover, there were some assumptions reported for the Higuchi type kinetics, in this case the probable assumptions could be: (i) drug diffusion is one-dimensional, making effects of margins are negligible, (ii) the diffusivity of the drug is fixed, (iii) the perfect sink conditions are reached.[40,41] The polymer type and its molecular weight directly change the release profile of formulations. There was a similar study that investigates the release patterns of sodium alginate, hydroxypropyl methylcellulose (HPMC), and CHI-based ocular inserts loaded with brimonidine.[42] The CHI and HPMC-based inserts exhibited more than 80% of drug release *in vitro* in the first 30 mins. The sodium alginate-based formulations exhibited approximately 80% of drug release *in vitro* in the first 120 mins. The goal of the present study was to observe the prolongation of drug contact with the ocular tissue via using biodegradable polymeric systems for daily application. Thus, the amount of DRZ was calculated (2mg DRZ/implant) according to the dose of the market product's daily application.

Table 3. The kinetic models of formulations

	Zero Order (R^2)	**First Order** (R^2)	**Higuchi** (R^2)	**Korsmeyer-Peppas** (R^2)	(n)	**Hixson-Crowell** (R^2)
1.0 % CMC-DRZ	0.8156	0.7312	0.9733	0.9313	0.301	0.9461
1.0 % CHI-DRZ	0.9352	0.6447	0.9918	0.7818	0.243	0.9172

R^2: correlation coefficient n: release exponent

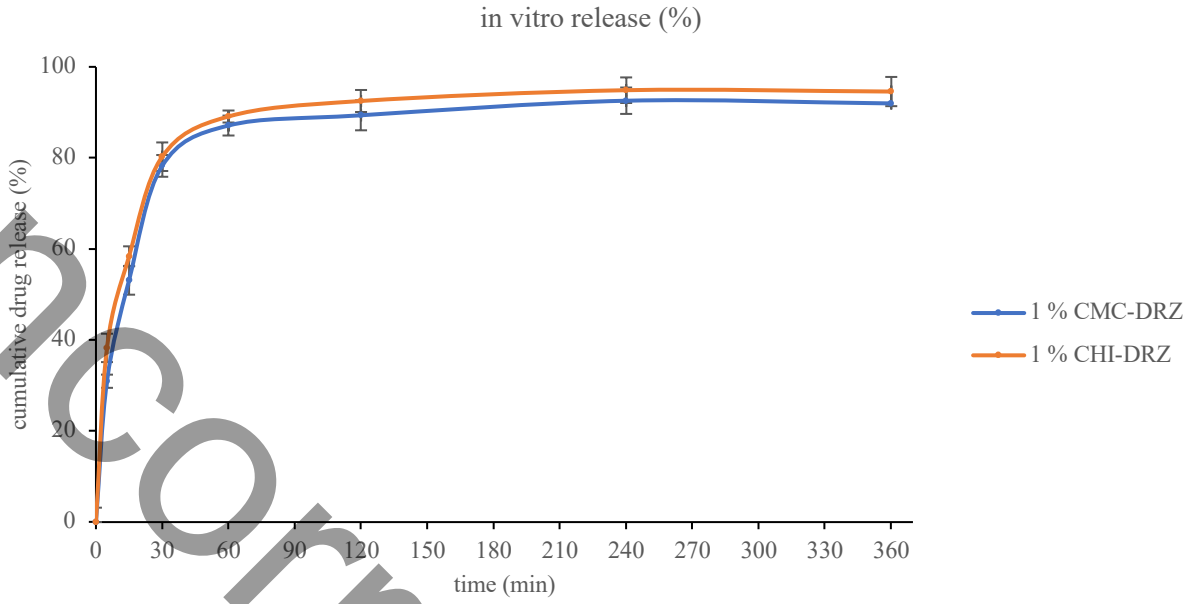

Figure 4. *in vitro* release profile of ocular implants

Ex vivo release study

The *ex vivo* drug release profile of a dosage form reflects the passage or retention of the drug substances throughout the tissue. The applied method detects the residual drug amount on the release medium. Thus, the plateau levels at the 3rd and 6th hours indicates the drug saturation levels (Figure 5). The eye drop (NS-DRZ, 2% DRZ) exhibited instant drug payload in the first 30 min (95.4% ± 1.3), then release percentages of NS-DRZ were found as: 93.04% and 92.66% at the 3rd and 6th hours, respectively. It was considered that, 7.34% of the drug estimated as retained or passed throughout the *ex vivo* tissue. The release percentages of CMC-DRZ were 81.5% and 80.9% at the 3rd and 6th hours, respectively. The release percentages of CHI-DRZ were 78.1% and 78.7% at the 3rd and 6th hours, respectively. Thus, the *ex vivo* release outcomes are correlated with the *in vitro* release studies. The implants designed with CHI has shown slightly faster *in vitro* release rate, so it could cause faster retention onside the *ex vivo* tissues. Between 21.3% and 18.5% of the drug estimated as retained or passed throughout the *ex vivo* tissue.

As previously stated, physiological factors hinder ocular drug absorption and bioavailability.[5,6] It was reported that less than 5% of an applied dose is absorbed into ocular tissues.[39] Therefore, nanoparticulate systems, *in situ* gelling systems, and biodegradable polymeric systems were developed to increase drug permeation, extend the presence of drug substances onto ocular tissues and prolong the drug release. The NS-DRZ (represents the traditional application) implant exhibits 2.5- and 3.0-fold lower *ex vivo* drug absorption than the CMC-DRZ and CHI-DRZ implants.

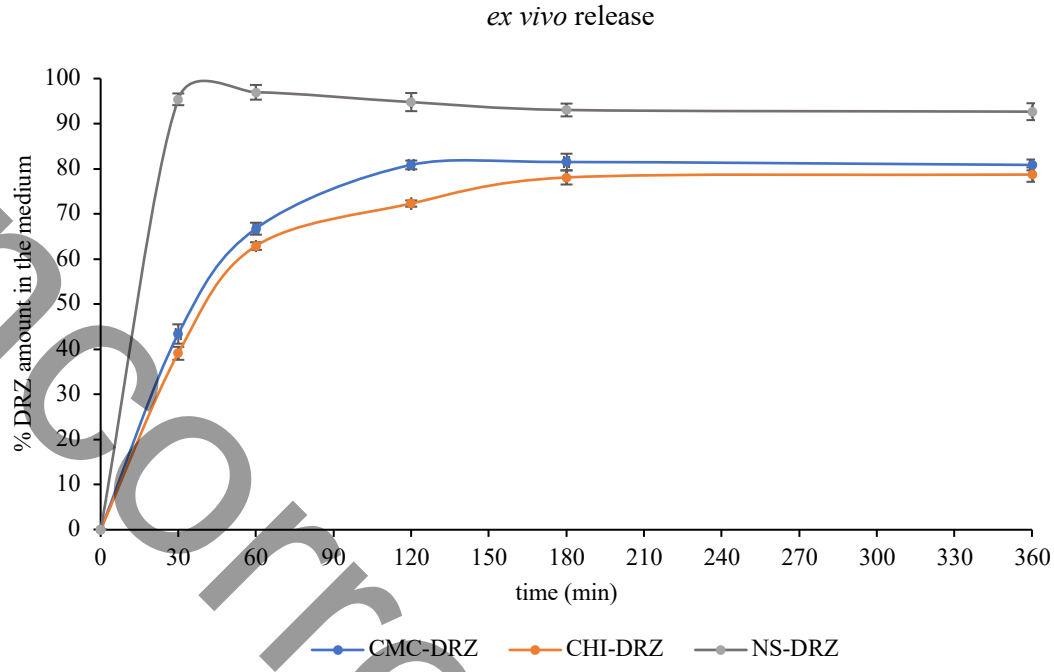

Figure 5. *ex vivo* release profile of ocular implants

CONCLUSION

The ophthalmic implants showed remarkable results in both *in vitro* and *ex vivo* investigations for better ophthalmic drug delivery in this study. The DRZ loaded CMC and CHI ocular implants were prepared in shape of transparent hemispheres not to interfere the vison. The *ex vivo* release data are correlated with the *in vitro* release outcomes. The implant designed CHI has exhibited slightly faster *in vitro* release rate, so it could cause faster retention onside the ocular tissues. The DRZ release from implants was biphasic, with an initial release lasting about 2 hours and then a continuous release lasting up to 6 hours. It can be inferred that DRZ-loaded ocular implants can be an effective ocular delivery strategy.

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