Optimizing Ophthalmic Delivery of a Poorly Water Soluble Drug from an Aqueous

***In situ* gelling system**

F. G. Senjoti, P. Timmins, B. R Conway, and A. M. Smith[*]

Department of Pharmacy, School of Applied Sciences, University of Huddersfield, Queensgate,

Huddersfield, HD1 3DH, UK.

*Correspondence:

Prof. Alan M. Smith

Tel: +44-1484-472-305

Fax: +44-1484-472-305

a.m.smith@hud.ac.uk

Abstract

Poorly soluble drugs are often unsuitable to incorporate in ocular *in situ* gelling systems due to the aqueous based gelling formulations and low volumes administered. For such formulations to be successful, the administered drug must have sufficient solubility to diffuse from the formulation to the eye and should not affect the gelation of the *in situ* gelling material. Drug salt forms can improve the solubility of poorly soluble drugs, however, as *in situ* gel forming formulations are often designed to be crosslinked by salts (present the lacrimal fluid) it can make salt forms difficult to formulate. The aim of this study was to develop an *in situ* gel forming ophthalmic formulation of a poorly soluble drug flurbiprofen (FBP) through cyclodextrin complex formation and to analyse the impact on gelation, release and permeation through the cornea. Hydroxypropyl-beta-cyclodextrin (HβCD) was used as a complexing agent and low acyl gellan gum was added to the FBP- HβCD complex as a water soluble *in situ* gelling polymer. Measurements were performed using rheo-dissolution, which utilises a rheometer with a modified lower plate that has the unique ability to allow rheological measurement and analysis of drug release simultaneously. An *ex-vivo* permeation study was also performed using porcine cornea. Rheological measurements in terms of elastic (G′) and viscous (G″) modulus showed rapid gelation of the formulation upon contact with simulated lacrimal fluid (SLF). Approximately, 97% FBP was released when 10% HβCD was used and release was decreased to 79% when the amount of HβCD was increased to 20%. The percentage of drug permeation through the cornea was 55% in 300 min whereas the marketed non gelling eye drop formulation containing FBP sodium showed only 37% permeation. The data presented here, revealed that not only could a poorly soluble drug be complexed with cyclodextrin and loaded into an *in situ* gelling system without interfering with the gelation, but also permeability the of the drug improved.

1. Introduction

Improving the bioavailability of the therapeutics for ophthalmic delivery has been a major research focus over the past few decades. The distinctive anatomy and physiology of the eye make improving bioavailability of ophthalmic drugs one of the greatest challenges in drug delivery, whether that be delivering to the anterior or posterior regions of the eye. One of the best strategies to improve ocular bioavailability is to use *in situ* gel forming ophthalmic drug delivery systems. These dosage forms exhibit pseudoplastic flow behaviour to aid dispensing and then undergo an *in situ* sol-gel transition, which reduces clearance from blinking and nasolacrimal drainage, resulting in a prolonged retention time [1–3]. Poorly soluble drugs however, are generally unsuitable for incorporation in such delivery systems due to the aqueous based gelling formulations, making it difficult to dissolve the full dose in the low volumes administered in eye drop formulations. The polysaccharide gellan gum is one such *in situ* gel former that forms strong gels on exposure to monovalent and divalent cations (K^+, Na^+, Ca^{2+} and Mg^{2+}) that are found in sufficient concentrations in lacrimal fluid. It is currently used in the marketed *in situ* gelling formulation Timoptol® LA to increase retention time on the surface of the eye, improving the delivery of the water soluble drug timolol maleate for the treatment of glaucoma. For such formulations to be successful, the administered drug must have sufficient solubility to diffuse into the eye [1,4] (with the ideal drug having both lipophilic and hydrophilic properties) and should not interfere with the performance of the *in situ* gel former. Hence poorly soluble drugs are not usually used in such formulations, despite the potential useful therapeutic effect of the drug. In addition, drug salt forms which can improve the solubility of a poorly soluble drugs can interact with gellan gum increasing the viscosity or even inducing gelation before administration. Flurbiprofen (FBP) is an example of such a drug. It is an aryl propionic nonsteroidal anti-inflammatory drug (NSAID) having analgesic, anti-inflammatory and antipyretic properties. According to the Biopharmaceutical Classification

System (BCS), FBP is considered as a Class II drug exhibiting high permeability and low water solubility [5]. FBP is used as the sodium salt in anti-inflammatory eye drops (marketed as Ocufen®) to enhance the solubility. This salt form however is difficult to formulate as an *in situ* gel forming formulation using gellan gum because of the tendency of the polymer to crosslink with sodium ions.

A number of other strategies have been employed to increase the solubility of poorly soluble drugs, such as, use of solubilizing systems such as liposomes and microemulsions to increase the solubility in the aqueous tear fluid, formulating as nanogels to enhance the residence time on the surface of the eye [4] and use of drug-cyclodextrin inclusion complexation to enhance the aqueous solubility, stability and bioavailability of ocular drugs [6,7]. Cyclodextrin (CD) complexation in particular, has been successfully employed in recent years and not only have shown to improve solubility but also can increase the rate of dissolution [5]. Moreover, a recent study by Jansook et al (2019) demonstrated increased transcorneal permeation of the NSAID celecoxib using mucoadhesive polymers containing CD nanoaggregates [8]. CDs are cyclic oligosaccharides having hydrophobic/lipophilic internal cavity and hydrophilic outer surface. The hydrophobic cavity allows poorly soluble drug molecules to enter and formation of inclusion complex without forming any covalent bonds [5,9]. Amongst all cyclic oligosaccharides, HβCD is the most commonly applied CD in ophthalmic formulations. HβCD is well tolerated , even at concentration as high as 45% in humans and animals [7,10].

Here we have investigated flurbiprofen (FBP) which is a poorly soluble drug but shows increased solubility in buffered solution (pH 7.4) [11]. Formulating the *in situ* gelling formulation of FBP in a buffer solution, may however, cause the premature gelation of the gellan, due to its ability to form a solid gel when exposed to low concentrations of group 1 and group 2 metal ions. So a drug-cyclodextrin inclusion complex was created using 2-hydroxypropyl-β-cylodextrin (HβCD) that could be dissolved in gellan gum without addition

of salts to formulate an *in situ* gelling formulation. Simultaneous measurements of rheology and drug release were performed using a rheo-dissolution cell developed in our laboratories [12] and a drug permeation was measured through an *ex-vivo* corneal model.

2. Martials and Methods

2.1 Materials

FBP, HβCD, low acyl gellan gum (Gelrite®) were purchased from Sigma Aldrich, UK. Sodium bicarbonate was purchased from Fisher Scientific (UK). Calcium chloride dihydrate, sodium chloride, sulphuric acid, hydrochloric acid and phenol were purchased from Sigma-Aldrich (UK), All materials were used as received. Fresh porcine cornea was donated by a local abattoir.

2.2 Preparation of Simulated Lacrimal Fluid

Simulated lacrimal fluid (SLF) was used in the evaluation of the formulation. Correct amount of $NaHCO_3$ (0.023 M), $CaCl_2 \cdot 2H_2O$ (0.001 M) and NaCl (0.114 M) were dissolved in in 500 ml deionized water with continuous stirring for 30 minutes [13]. The pH of the solution was 7.5.

2.3 Phase Solubility Studies

Conventional phase solubility studies were conducted according to Higuchi and Connors method [14] to show how CD influences the solubility of a drug [15]. For performing phase solubility studies, samples were prepared by adding excess amount FBP to 10 ml SLF (pH 7.5) in sealed glass containers containing 0,5, 1, 2, 5, and 10% (3.4, 6.8, 13.7, 34.2, 68.5 and 137 mM) of HβCD. The solutions were mixed at a constant temperature (25°C) for 24 hours using electromagnetic stirrer at 200 rpm. The solutions were then filtered (pore size 0.45 µm) and diluted accordingly. The solutions were analysed spectrophotometrically (λmax 247 nm) to

determine the drug concentration. A phase solubility diagram was created by plotting the concentration of total dissolved drug against the concentration of HβCD.

2.4 Experimental Setup for Rheological Measurements

All rheological measurements were performed using Kinexus Rheometer (Malvern Panalytical) fitted with a 40 mm serrated plate geometry. A circulatory system was used for the analysis which contained a modified rheo-dissolution cell attached to the lower plate of the geometry **(fig 1).**
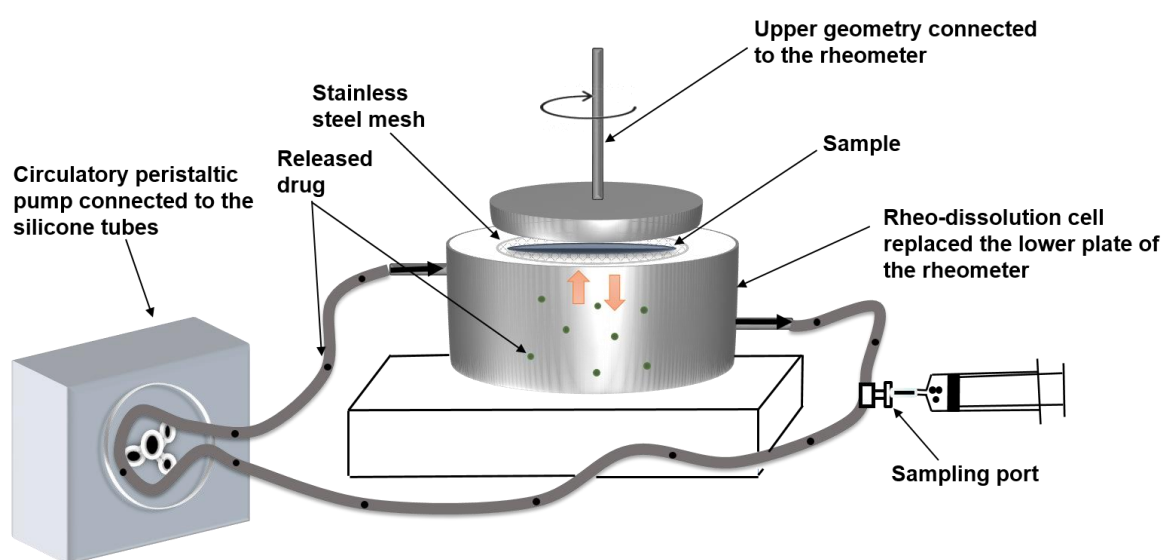
Fig. 1. Schematic diagram of the experimental set up of rheo-dissolution cell which replaced the lower plate of rheometer and connected to a peristaltic pump (adapted from [12])

The cell was constructed from acrylonitrile butadiene styrene using a Makerbot Replicator™ 2 3D printer (New York, USA). The circular cell was designed with an opening top covered with a stainless steel mesh where the sample was placed. The cell was connected to a circulating peristaltic pump (flow rate of 1 ml/min) to maintain the flow of SLF ensuring sink conditions. Gelation was induced by injecting the SLF into the flow through system, which allowed the ions of SLF to come into contact with the sample *in situ*. The rheological studies were evaluated

in terms of elastic modulus (G′) and viscous modulus (G″) at a frequency of 1 rads^{-1} and 0.5% strain which was within the linear viscoelastic region.

2.5 Interaction Studies between HβCD and Gellan gum

HβCD solutions were prepared by adding the appropriate amounts of HβCD in deionized water at room temperature to prepare 0.5%, 1%, 2%, 5% and 10% w/v final concentration. The HβCD solutions were then heated up to 85°C and gellan gum was added while stirring at 500 rpm to produce 0.4% w/v final gellan concentration. 0.4% concentration was selected according to the marketed Timoptol LA®. Once the gellan was dissolved, stirring was stopped and solutions were allowed to cool at room temperature. Oscillatory rheological analysis in terms of G′ and G″ (Pa) were carried out using the experimental set up described in 2.4 to examine the gelation behaviour of gellan gum in presence of different concentration of HβCD.

2.6 Preparation of *In Situ* Gel Forming Ophthalmic Formulation of FBP with HβCD and Gellan Gum

In the majority of CD based eye drop formulations investigated for clinical evaluation, 10% to 30% w/v of CD was used for solubilisation of the drugs [16–20]. However, to prepare the formulation, required quantities of HβCD was added to deionized water at room temperature. Precise amount of FBP (0.029%) was added to the solution based on the marketed product Ocufen® which contains FBP sodium 0.03% w/v. Once dissolved, the solution was heated to 85°C while stirring and required amount of gellan gum was added to prepare a 0.4% w/v solution. The stirring was stopped after complete dissolution of gellan gum and the formulation was cooled to room temperature. The pH of the prepared formulations was 4.3. It should be noted that the buffering action of lacrimal fluid *in vivo* depends on the chemical buffering capacity which is 0.434 mol/L/ΔpH. So it can be assumed that 25-30 µl formulation of pH 4.3 will not interfere with the physiological pH of 30 µl (maximum volume of lacrimal fluid in cul-de-sac) lacrimal fluid and therefore should not cause any irritation if applied to the eye surface

due to any pH disturbance. In these preliminary studies, the formulations were not sterilized and no antimicrobial preservatives were added.

2.7 Confirmation of Complexation

Thermal analysis was performed using differential scanning calorimetry (DSC) to confirm the complexation between drug and HβCD (Mettler Toledo, DSC 1, STARe system). The formulations of FBP-HβCD and FBP-HβCD with gellan were prepared for freeze drying. The formulations were stored in a freezer at -20ºC for overnight. The frozen samples were then dried using a (Christ Alpha 2-4 L Dplus) freeze drier. The drying procedure was performed for 24 hours at -84.6ºC with the vacuum set at 0.001 mbar. DSC measurements were performed on all the samples (FBP, gellan, HβCD, physical mix and freeze dried samples) following the method described by Pralhad and Rajendrakumar (2004) [21]. Briefly, samples were accurately weighed (Mettler AT201) and heated in crimp sealed aluminium pans at 10°C per min between 50 to 150°C. The nitrogen gas flow was 200 ml/min. Thermograms of FBP, HβCD, gellan, physical mix were compared with the freeze dried samples.

2.8 Simultaneous Determination of Rheology and Dissolution of the Drug (Rheo-dissolution study)

Rheo-dissolution studies were performed using the experimental setup described in section 2.4. The samples were allowed to form gels *in situ* in presence of SLF and measurements of G′ and G″ were performed as an indication of gelation. To measure FBP release during the rheological analysis, aliquots of SLF were withdrawn at pre-determined time intervals (60, 120, 180, 240 and 300 min) and replaced with equal amount of fresh SLF. Collected samples were diluted and analysed using UV spectrophotometer (Agilent Cary 60 UV-Vis) at 247 nm. The percentage of FBP released from the *in situ* gel was determined from linear regression equation obtained from the UV standard calibration curve. All experiments were performed in triplicate.

2.9 HβCD Dissolution Studies

HβCD release was measured using phenol-sulphuric acid assay on the withdrawn samples as reported previously [22]. Briefly, 1 ml of 0.5% phenol solution was added to the filtered samples (1 ml) followed by adding 5 ml of concentrated H_2SO_4 and mixed vigorously for 10 min. The resultant solutions were placed in a water bath at 25-30°C for 20 min. The solutions were analysed at 490 using UV spectrophotometer [23]. The concentration of HβCD dissolved from the sample was determined from a standard calibration curve.

2.10 *Ex- vivo* Permeation Studies using Porcine Cornea

Permeation studies were conducted for the formulation containing 10% HβCD and marketed Ocufen® eye drop (pH 6.68) formulation. Corneas used in the permeation studies were obtained from fresh porcine eye balls. The fat and muscles around the eye balls were trimmed and an incision was made between optic nerve and cornea. The sclera was cut all the way around the cornea and the vitreous humor, lens and ciliary body were removed. Finally, the cornea was removed carefully to avoid any distortion (method adapted from Fatahalla et al., 2016) [24]. Permeation studies were performed using Franz diffusion cells containing a 4 ml fixed volume receiver compartment with a sampling port. The diffusion surface area of the cell was 1.76 cm², where the cornea was placed between the donor and receiver compartments. It was possible to place the cornea flat on the diffusion surface area. The endothelial side of the cornea faced the receiver compartment. The receiver compartments were filled with SLF (pH 7.5) and 35°C was maintained throughout the experiments with a magnetic stirrer bar used to ensure an isothermal temperature was maintained throughout the receiver fluid. Test samples, were placed directly on the cornea in the donor compartment and samples of the receiver fluid were withdrawn at different time intervals and were replaced with fresh medium. The amount of FBP permeated across the cornea was determined by the UV spectroscopy. Three corneas were used (n=3) for each of the formulations.

2.11 Statistical Analysis

The results were expressed as mean ± standard deviation (n=3), were statistically analysed using a Student's t-test for paired data. Statistical significance was accepted where $P < 0.05$.

3. Results and Discussion

3.1 Phase Solubility Studies

To initially determine how HβCD influenced the solubility of FBP, phase solubility studies were performed. These experiments revealed that the solubility of FBP increased linearly with increased amount of HβCD **(fig 2)**. The solubility of FBP was 48.53 mM at maximum concentration of HβCD (137 mM or 20%) which was in good agreement with the reported solubility of FBP in phosphate buffer system (pH 7.4) by Felton et al., (2014) [6]. According to Higuchi & Connors [14], the graph was type A which indicated the formation of a soluble complex. The linear portion of the graph (R^2=0.9934) indicated A_L type and allowed us to assume that 1:1 inclusion complex of FBP-HβCD was present in the media. 0.029 % (1.23 mM) was selected as a concentration of FBP for the final formulation based on Ocufen® eye drops. Although it was apparent that any concentration of HβCD ranging from 3.4 (0.5%) to 137 mM (20%) was able to solubilize 1.23 mM of FBP, the time taken for this to occur varied considerably. At concentrations above 34.2 mM (5%) HβCD solubilisation took approximately 30 minutes whereas at concentrations less than 34.2 mM required stirring overnight to fully solubilise the FBP. Therefore, further experiments were performed using 68.5 mM (10%) and 137 mM (20%) of HβCD.
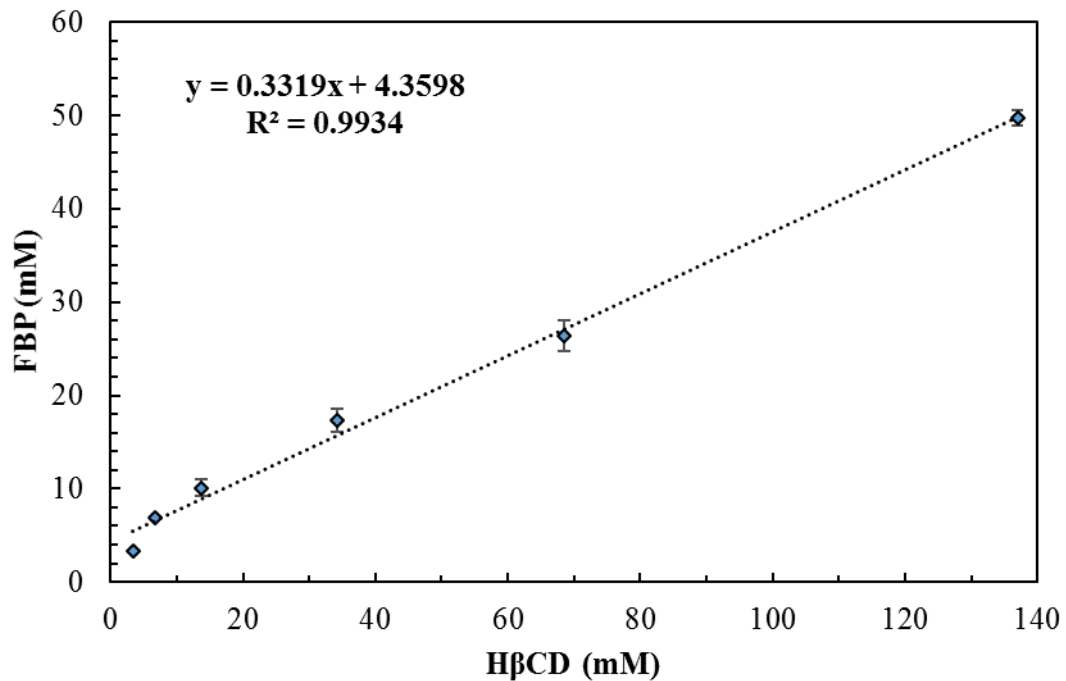
$$y = 0.3319x + 4.3598$$
$$R^2 = 0.9934$$

Fig. 2. FBP solubility as a function of HβCD

3.2 Interaction Studies between HβCD and Gellan Gum

Oscillatory rheological analysis was performed to examine the *in situ* gelling behaviour of the polymer upon contact with SLF and to determine if the presence of HβCD caused any changes in gelation behaviour **(fig 3)**. Figure **3A** shows the viscoelasticity of 0.4% gellan on exposure to SLF where the onset of gelation was fast (within 2 min) and G′ was significantly dominant over G″ with a terminal value of ~320 Pa. Addition of increasing concentration of HβCD (0.5, 1, 2, 5 and 10%) to the gellan solution did not alter the rheological characteristics of gellan in terms of onset of gelation and average final gel strength of ~310 Pa indicated an absence of physical interactions between gellan and HβCD.
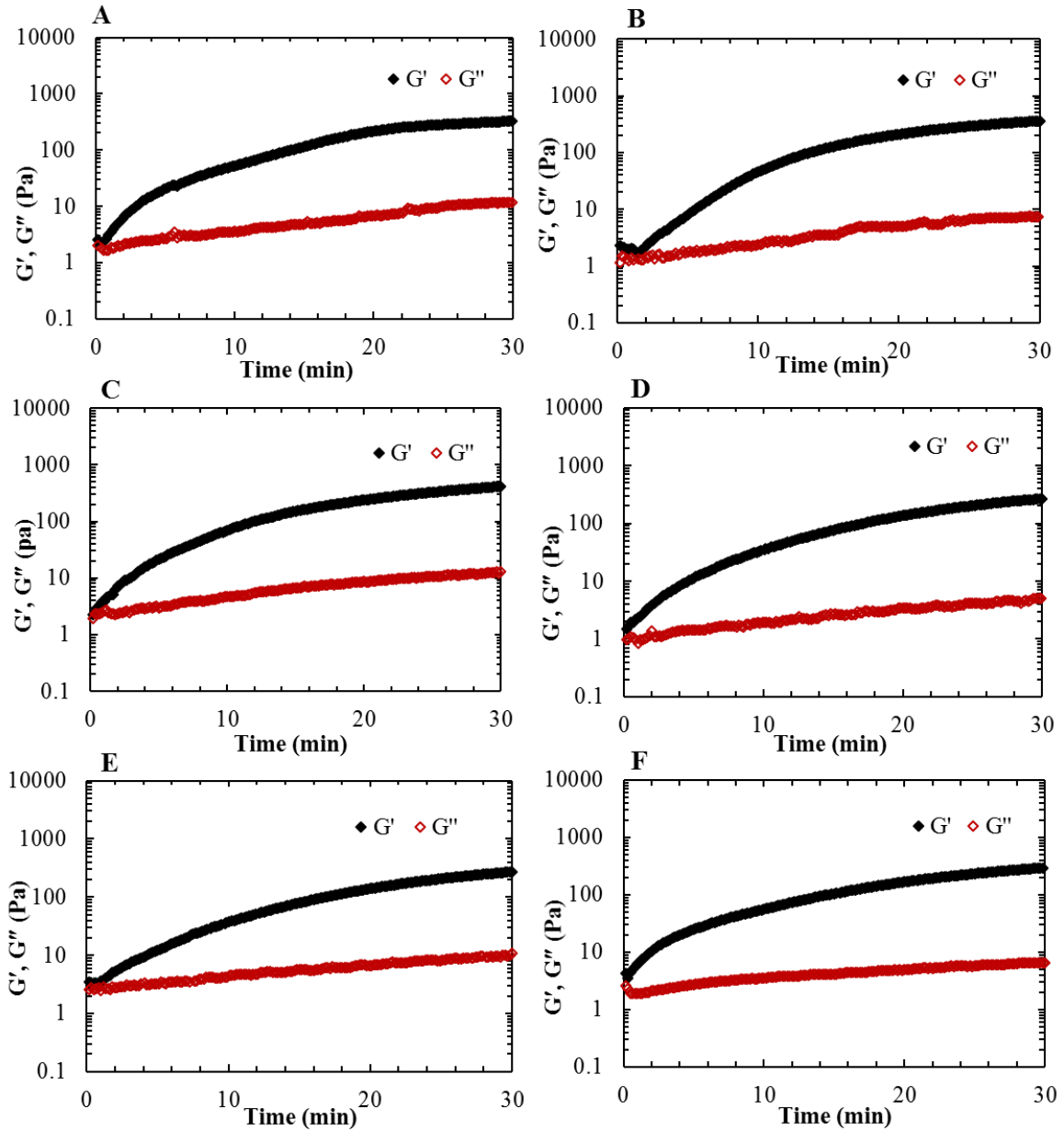
Fig. 3. *In situ* gelation of 0.4% gellan showing G′ and G″ on exposure to SLF (A) 0.4% gellan only and in the presence of (B) 0.5% HβCD (C) 1% HβCD (D) 2% HβCD (E) 5% HβCD and (F) 10% HβCD

3.3 Confirmation of Complexation by DSC

Thermal analysis was conducted to confirm the complexation of FBP- HβCD with gellan. Thermograms of FBP, gellan and HβCD alone, physical blends and the freeze dried samples revealed a readily apparent melting endotherm for FBP alone and there were no melting transition for gellan and HβCD as expected **(fig 4)**. The well-defined melt transition of FBP

appeared in the physical blends indicating incomplete complexation by HβCD. However, in the freeze dried samples melting endotherms were absent. The absence of this peak can be is an indication that complete inclusion complexation of FBP-HβCD was achieved [6]. Furthermore, the thermogram of the complex with gellan indicated that gellan did not interfere with the complex formation.
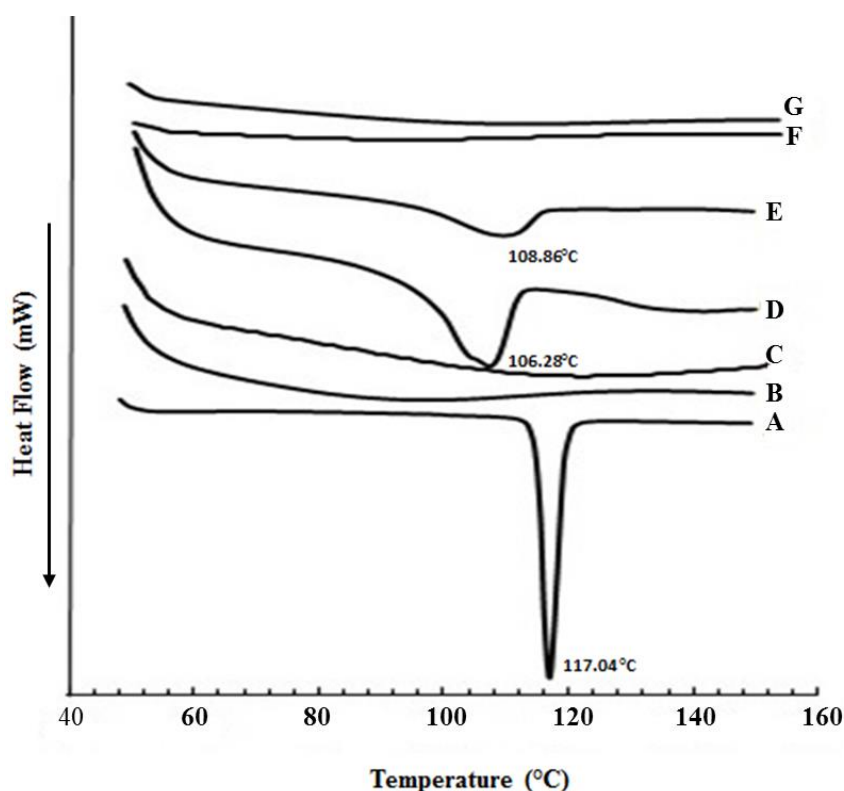

Fig. 4. DSC thermogram of (A) FBP (B) HβCD (C) gellan (D) physical mix of FBP and HßCD (E) physical mix of FBP, gellan and HßCD (F) freeze dried formulation of FBP and HßCD (G) freeze fried formulation of flurbiprofen, gellan and HPßCD

3.4 Simultaneous Determination of Rheology and Dissolution of the Drug (Rheo-Dissolution Study)

Following the exposure of FBP-HβCD complex and gellan to the SLF, gelation was initiated, as indicated by an increase of G' and G″ over first 10 min of exposure **(fig 5A and B).** The modulus reached a steady state after complete gelation with $G' > G''$ indicating the formation of strong gel [25]. The FBP release curve reveals an initial burst release of FBP (~25% at 60

min) while the gel was forming and the release followed a similar trend even after complete gelation. Approximately 97 % of FBP was released in 300 min **(fig 5A)**. The release curve of FBP when 20% HβCD was used showed comparatively lower release at 300 min (~ 80%) although it was not significantly different (p > 0.05) **(fig 5A)**.
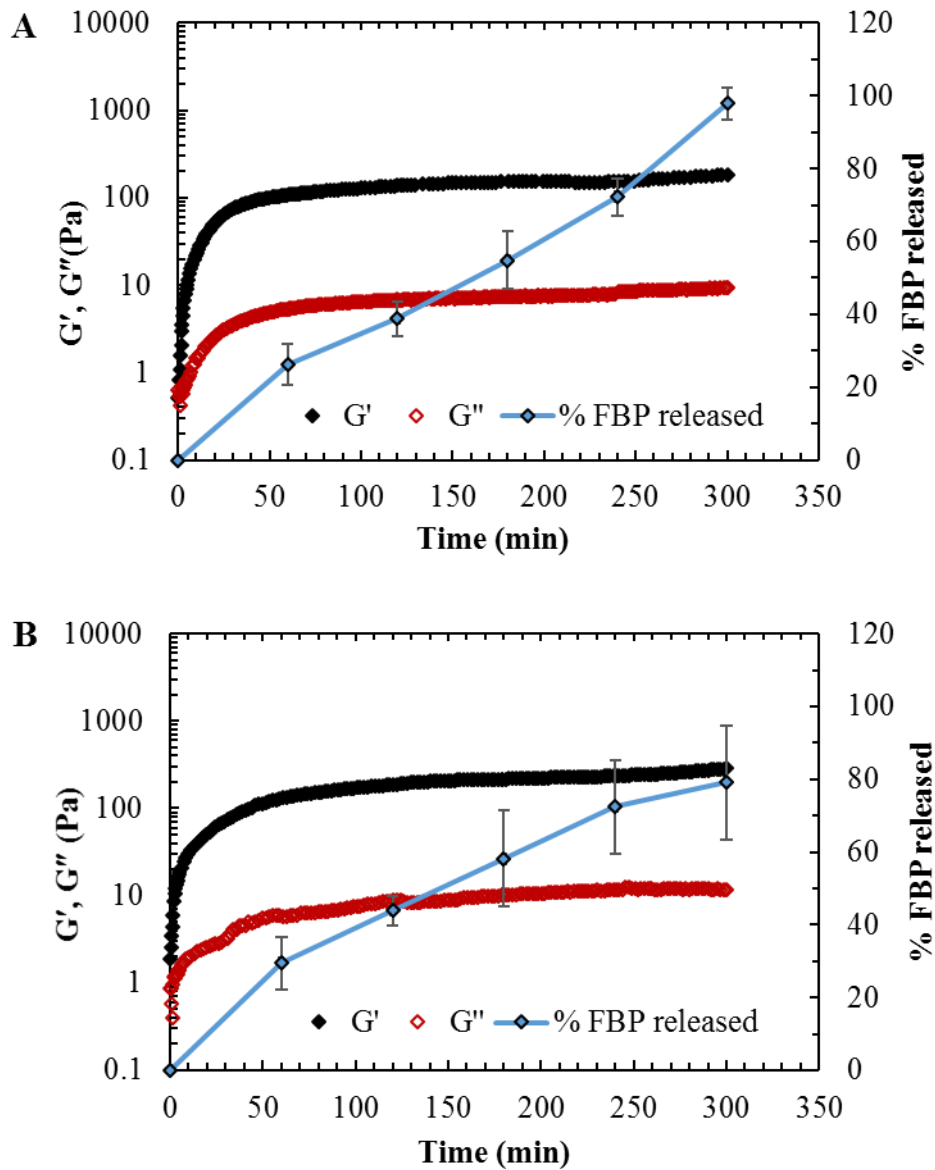

Fig. 5. Simultaneous determination of rheological changes (G′ and G″) and drug release study of the formulation containing (A) 10% HβCD (B) 20% HβCD

CD complexation is a dynamic process with drug molecules in a dynamic equilibrium with the CD, constantly associating and disassociating from the complex, therefore there is always

presence of free drug and CD complexed drug. Release of FBP from the *in situ* gel is thought to occur by parallel diffusion of FBP-HβCD complexes and free drug. Once the complexes are released from the gel the FBP dissociates from the complex before dissolving into the simulated lacrimal fluid release medium, due to its higher pH (pH 7.5) facilitating dissolution of FBP **(fig 6).** The amount of drug detected in the release media was dependent on the concentration of HβCD. The release of FBP was greater when 10% HβCD was used with approximately 97% released in 300 min **(fig 5A)**.
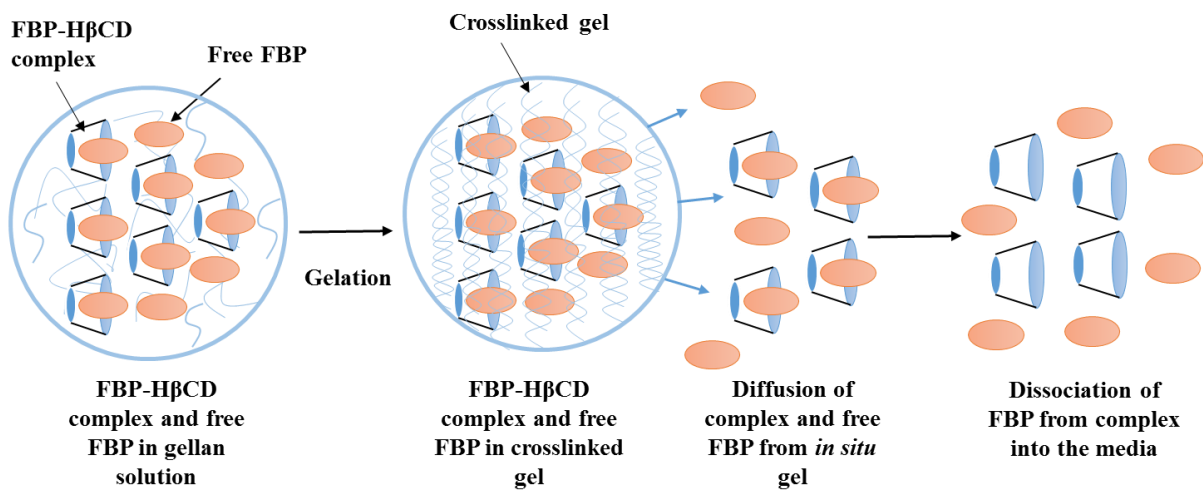

Fig. 6. Schematic representation of diffusion of FBP- HβCD complex through the gel and dissociation of FBP from the complex

The comparatively lower release of FBP from the formulation containing 20% HβCD **(fig 5B)** was likely due to the tendency of CD derivatives (like HβCD) and their complexes to self-associate and form aggregates in aqueous solutions [26]. To investigate this, release of HβCD from the *in situ* gel forming formulation containing varying concentration of HβCD (10% and 20%) was quantified using the phenol sulphuric acid assay **(fig 7).**
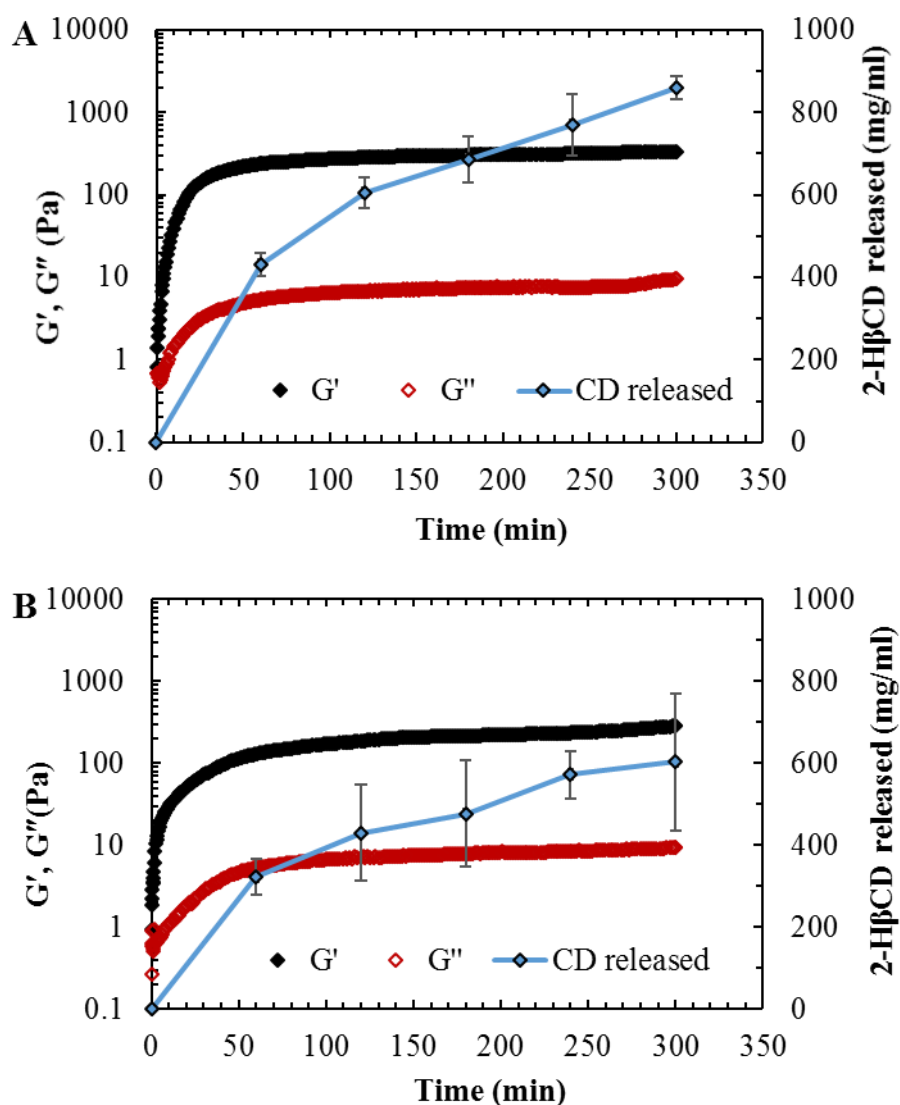
Fig. 7. Simultaneous determination of rheological changes (G′ and G″) and HβCD release study using phenol sulphuric acid assay of the formulations containing (A) 10% HβCD (B) 20% HβCD

The release of HβCD from the gel containing 20% HβCD **(fig 7B)** was significantly lower (P < 0.05) compared with the formulation containing 10% HβCD **(fig 7A)** supporting the explanation postulated for reduced FBP release when 20% HβCD was used. CDs self-assemble and form small clusters that can then aggregate further and form larger clusters **(fig 8)**. The aggregates usually have a diameter between 20 to 200 nm and the size of the aggregates increase with increasing the concentrations of CDs [27]. Intermolecular hydrogen bonding

between the OH groups of CDs and surrounding water molecules is the driving force for assembly and further aggregation of the molecules.
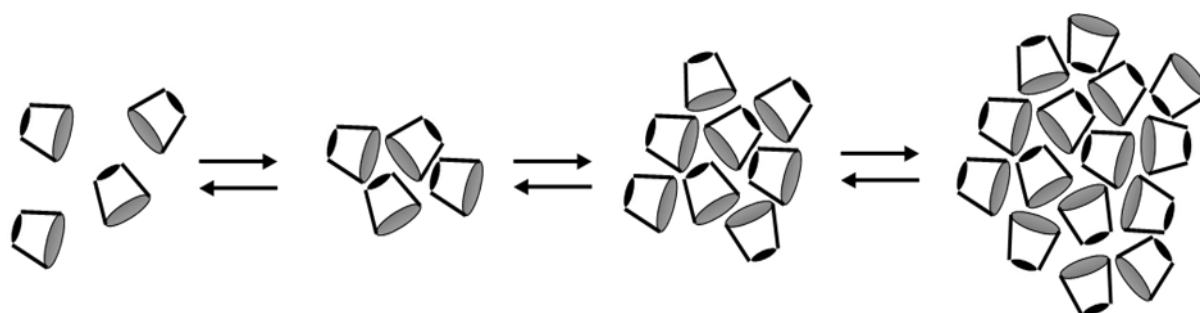

Fig 8: Schematic presentation of self-assembling of CD molecules to form small clusters that associate to create larger aggregates

There is therefore, a possibility that larger CD aggregates formed at high concentration of HβCD (20%) obstructed diffusion of the free drug and smaller complexes through the gellan which resulted in reduced drug release into the medium. To demonstrate that the gellan did not dissolve into the release media and interfere measurements of HβCD release, a blank rheo-dissolution analysis was also performed using 0.4% gellan alone. Only a negligible amount of gellan (<4%) was detected in the release medium (data not shown) indicating that gellan did not interfere with measurements of HβCD release.

3.5 *Ex-vivo* Permeation Study

The permeation study highlighted that the percentage of FBP permeated through the porcine cornea was higher in case of the formulation containing HβCD and gellan compared with that of FBP sodium (Ocufen®) **(fig 9)**. This can be explained by the ability of CDs' to act as carrier and deliver the unionised drug to the surface of a biological membrane [28].
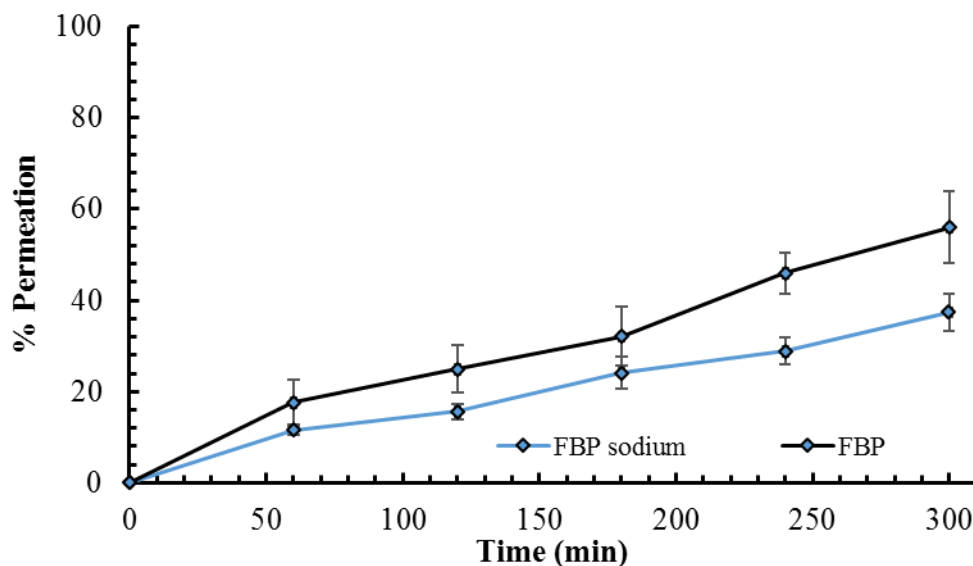
Fig. 9. Amount of FBP permeated from the formulation containing FBP with 10% HβCD and gellan compared with the commercial product Ocufen® containing flurbiprofen sodium (n=3)

The FBP-HβCD inclusion complex diffused through the *in situ* gel to the surface of the cornea and FBP then dissociated from the complex to permeate through the cornea. It is well known that free acid drugs can permeate biological membranes faster than the equivalent salt form [29]. The pH of the Ocufen® (pH 6.7) eye drop formulation was much greater than the pK_a of FBP (pK_a 4.2) causing more than 99% FBP to be ionized. Whereas the in the FBP-HβCD formulation the pH is 4.3, which is almost equivalent to the pKa of the FBP (pK_a 4.2) delivering ~50% of the drug in the unionised form, and therefore, enhancing permeability when compared with the ionised FBP in the Ocufen®. This formulation approach to improving permeability of poorly soluble drugs could potentially be utilised with other water based ionotropic *in situ* gelling biopolymers such as sodium alginate, carrageenan or pectin.

3. Conclusion

This study has demonstrated a method of developing an *in situ* gel forming ophthalmic formulation of the poorly soluble drug FBP by forming inclusion complex with HβCD. Formation of an inclusion complex enhanced the solubility of FBP and allowed addition of

gellan gum in order to form a gel *in situ*. This study also utilized a system to monitor real time gelation and dissolution of FBP simultaneously on exposure to the crosslinking solution. Data obtained from the rheo-dissolution study for the formulation containing 10% HβCD showed 97% FBP release in 5 hours. Comparative permeation studies indicated improved permeation of FBP as a complex compared to the sodium salt of FBP. All the data obtained indicated that gellan gum did not interfere the complex formation and the complex diffused successfully through the *in situ* gel. Thus, this particular poorly soluble drug can be included as the free acid in the *in situ* gelling system by forming an inclusion complex with HβCD. Moreover, the ability to monitor real time gelation and drug release simultaneously, can be a useful tool when designing *in situ* gelling drug delivery systems.

References

[1]   D. Achouri, K. Alhanout, P. Piccerelle, V. Andrieu, Recent advances in ocular drug delivery, Drug Dev. Ind. Pharm. 39 (2013) 1599–1617. https://doi.org/10.3109/03639045.2012.736515.

[2]   A.H. El-Kamel, In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate, Int. J. Pharm. 241 (2002) 47–55. https://doi.org/10.1016/S0378-5173(02)00234-X.

[3]   J.C. Lang, Ocular drug delivery conventional ocular formulations, Adv. Drug Deliv. Rev. 16 (1995) 39–43. https://doi.org/10.1016/0169-409X(95)00012-V.

[4]   T. Loftsson, E. Stefánsson, Effect of cyclodextrins on topical drug delivery to the eye, Drug Dev. Ind. Pharm. 23 (1997) 473–481. https://doi.org/10.3109/03639049709148496.

[5]   M. Cirri, C. Rangoni, F. Maestrelli, G. Corti, P. Mura, Development of fast-dissolving tablets of flurbiprofen-cyclodextrin complexes, Drug Dev. Ind. Pharm. 31 (2005) 697–707. https://doi.org/10.1080/03639040500253694.

[6]   L.A. Felton, C. Popescu, C. Wiley, E.X. Esposito, P. Lefevre, A.J. Hopfinger, Experimental and Computational Studies of Physicochemical Properties Influence NSAID-Cyclodextrin Complexation, AAPS PharmSciTech. 15 (2014) 872–881. https://doi.org/10.1208/s12249-014-0110-2.

[7]   T. Loftssona, T. Järvinen, Cyclodextrins in ophthalmic drug delivery, Adv. Drug Deliv. Rev. 36 (1999) 59–79. https://doi.org/10.1016/S0169-409X(98)00055-6.

[8]   P. Jansook, P. Kulsirachote, R. Asasutjarit, T. Loftsson, Development of celecoxib eye drop solution and microsuspension: A comparative investigation of binary and ternary cyclodextrin complexes, Carbohydr. Polym. 225 (2019) 115209.

https://doi.org/10.1016/J.CARBPOL.2019.115209.

[9]   V.J. Stella, V.M. Rao, E.A. Zannou, V. Zia, Mechanisms of drug release from cyclodextrin complexes, Adv. Drug Deliv. Rev. 36 (1999) 3–16. https://doi.org/10.1016/S0169-409X(98)00052-0.

[10]   T. Loftsson, E. Stefánsson, Cyclodextrins and topical drug delivery to the anterior and posterior segments of the eye, Int. J. Pharm. 531 (2017) 413–423. https://doi.org/10.1016/j.ijpharm.2017.04.010.

[11]   S. Chandran, A. Roy, R.N. Saha, Effect of pH and Formulation Variables on In Vitro Transcorneal Permeability of Flurbiprofen: A Technical Note, AAPS PharmSciTech. 9 (2008) 1031–1037. https://doi.org/10.1208/s12249-008-9139-4.

[12]   F.G. Senjoti, M.U. Ghori, R. Diryak, B.. Conway, G.A. Morris, A.M. Smith, Rheo-dissolution: A new platform for the simultaneous measurement of rheology and drug release, Carbohydr. Polym. 229 (2019) 115541. https://doi.org/10.1016/J.CARBPOL.2019.115541.

[13]   M.R.C. Marques, R. Loebenberg, M. Almukainzi, Simulated biological fluids with possible application in dissolution testing, Dissolution Technol. 18 (2011) 15–28. https://doi.org/10.14227/DT180311P15.

[14]   T. Higuchi, K. Connors, Phase-solubility techniques, in: C. Reilley (Ed.), Adv. Anal. Chem. Instrum., John Wiley & Sons, Inc, New York, 1965: pp. 117–212.

[15]   T. Loftsson, M.E. Brewster, Pharmaceutical applications of cyclodextrins: Basic science and product development, J. Pharm. Pharmacol. 62 (2010) 1607–1621. https://doi.org/10.1111/j.2042-7158.2010.01030.x.

[16]   E.C. Kearse, O.L. Mcintyre, M.H. Johnson, C.I. Phillips, R. Lathe, W. Adams, K. Green, Influence of dehydroepiandrosterone on rabbit intraocular pressure, Ophthalmic Res. 33 (2001) 42–47. https://doi.org/10.1159/000055640.

[17]   T. Loftsson, P. Jansook, E. Stefánsson, Topical drug delivery to the eye: Dorzolamide, Acta Ophthalmol. 90 (2012) 603–608. https://doi.org/10.1111/j.1755-3768.2011.02299.x.

[18]   A.H.A. Mohamed-Ahmed, A. Lockwood, H. Li, M. Bailly, P.T. Khaw, S. Brocchini, An ilomastat-CD eye drop formulation to treat ocular scarring, Investig. Ophthalmol. Vis. Sci. 58 (2017) 3425–3431. https://doi.org/10.1167/iovs.16-21377.

[19]   N. Okamoto, Y. Ito, N. Nagai, T. Murao, Y. Takiguchi, T. Kurimoto, O. Mimura, Preparation of Ophthalmic Formulations Containing Cilostazol as an Anti-glaucoma Agent and Improvement in Its Permeability through the Rabbit Cornea, J. Oleo Sci. 59 (2010) 423–430. https://doi.org/JST.JSTAGE/jos/59.423 [pii].

[20]   M. Tanito, K. Hara, Y. Takai, Y. Matsuoka, N. Nishimura, P. Jansook, T. Loftsson, E. Stefánsson, A. Ohira, Topical dexamethasone-cyclodextrin microparticle eye drops for diabetic macular edema, Investig. Ophthalmol. Vis. Sci. 52 (2011) 7944–7948. https://doi.org/10.1167/iovs.11-8178.

[21]   T. Pralhad, K. Rajendrakumar, Study of freeze-dried quercetin-cyclodextrin binary systems by DSC, FT-IR, X-ray diffraction and SEM analysis, J. Pharm. Biomed. Anal. 34 (2004) 333–339. https://doi.org/10.1016/S0731-7085(03)00529-6.

[22] M. Dubois, K. Gilles, J. Hamilton, P. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356. https://doi.org/10.1021/ac60111a017.

[23] S.S. Nielsen, Phenol-Sulfuric Acid Method for Total Carbohydrates, in: Food Anal. Lab. Man., 2nd ed., Springer, New York, 2010: pp. 47–54. https://doi.org/10.1007/978-1-4419-1463-7.

[24] Z.M.A. Fathalla, K.A. Khaled, A.K. Hussein, R.G. Alany, A. Vangala, Formulation and corneal permeation of ketorolac tromethamine-loaded chitosan nanoparticles, Drug Dev. Ind. Pharm. 42 (2016) 514–524. https://doi.org/10.3109/03639045.2015.1081236.

[25] E.R. Morris, K. Nishinari, M. Rinaudo, Gelation of gellan - A review, Food Hydrocoll. 28 (2012) 373–411. https://doi.org/10.1016/j.foodhyd.2012.01.004.

[26] P. Saokham, C. Muankaew, P. Jansook, T. Loftsson, Solubility of cyclodextrins and drug/cyclodextrin complexes, Molecules. 23 (2018) 1–15. https://doi.org/10.3390/molecules23051161.

[27] T. Loftsson, M. Másson, M.E. Brewster, Self-Association of Cyclodextrins and Cyclodextrin Complexes, J. Pharm. Sci. 93 (2004) 1091–1099. https://doi.org/10.1002/jps.20047.

[28] T. Loftsson, Increasing the cyclodextrin complexation of drugs and drug biovailability through addition of water-soluble polymers, Pharmazie. 53 (1998) 733–740.

[29] P. Minghetti, F. Cilurzo, A. Casiraghi, L. Montanari, A. Fini, Ex vivo study of transdermal permeation of four diclofenac salts from different vehicles, J. Pharm. Sci. 96 (2007) 814–823. https://doi.org/10.1002/jps.20770.