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Original Article

PREPARATION AND CHARACTERIZATION OF ECONAZOLE NITRATE INCLUSION COMPLEX FOR OCULAR DELIVERY SYSTEM

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ABSTRACT

Objective: Econazole nitrate (ECZ) is one of the triazole antifungal drugs with poor aqueous solubility and dissolution rate; there is a need for enhancement of solubility. Therefore; inclusion complexation with β cyclodextrin (βCD) was performed.

Methods*:* In this study kneading method and co-evaporation method of preparation of inclusion complex between βCD and ECZ using two molar ratios of βCD. The solubility of these complexes in isotonic saline solution and distilled water was studied. Complexes prepared by kneading method were used for the preparation of different ophthalmic gel formulas using carbomer (CB) and sodium carboxymethylcellulose (sod CMC) as a gelling agent. The release profile and the rheological behaviour of the gel were studied.

Results: The solubility of ECZ was enhanced by complexation with β CD, and both complexation methods showed Ap type solubility curve, but the solubility of ECZ was significantly enhanced by complexation using kneading method over co-evaporation. EZC-βCD complex prepared by kneading method with 0.88 $*10^{-3}$ MβCD molar ratio and formulated in a gel using CB 0.75% w/w and sod CMC 0.25% w/w may be considered as a good candidate for ECZ ophthalmic gel dosage form, which showed Super case II transport release profile, and pseudo-plastic shear thinning behavior.

Conclusion: Kneading method was found to be the best method for inclusion of ECZ into βCD, which significantly enhanced ECZ solubility; enabling to be formulated into an ophthalmic gel using CB as a polymer, for further development.

Keywords: Econazole nitrate, β cyclodextrin, Kneading method, Ophthalmic gel

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INTRODUCTION

Ocular fungal infections, or ophthalmic mycoses, are being increasingly recognized as an important cause of morbidity and blindness; certain types of ophthalmic mycoses may even be life-threatening. Keratitis (corneal infection) is the most frequent presentation, but the orbit, lids, lacrimal apparatus, conjunctiva, sclera, and intraocular structures may also be involved Candida are one of the main causative agents of fungal keratitis, especially in diabetic and immunocompromised patients, or on long-term use of topical steroids [1]. Candida which acidifies the host environment, leading to the production of potent virulence factors, to enhance adherence to the host and compromising the host defence mechanism [2]. Econazole (ECZ), an imidazole with a similar molecular structure to miconazole is used primarily in the treatment of superficial mycoses, with some studies involving systemic use. It has been little studied in the treatment of eye infections, but there are some reports of topical administration to treat fungal keratitis [3]. Mahashabde *et al.* suggested the use of ECZ ointment as a prophylactic treatment after ocular trauma with the risk of fungal infection [4]. ECZ was formulated as ophthalmic ointments 1%w/w in an ointment base and found to be stable over 2 y in dark containers [5].

ECZ has low aqueous solubility because of its hydrophobic structure; this can have a negative impact on antifungal efficacy, pharmaco-kinetic variability (90% of topically applied cream remain on the skin surface) [6] and development of drug resistance [7]. The solubility of poorly water-soluble ECZ can be altered in many ways; such as the addition of co-solvents, [8] addition of surfactants, [9, 10] modification of drug crystal forms by solid dispersion and complexation with cyclodextrin, [11] or inclusion in a mesoporous silicate complex [12].

Among these possibilities, the cyclodextrin approach is of particular interest. Cyclodextrins are cyclic (α-1,4)-linked oligosaccharides of α-d-glucopyranose, (oligomer of glucose) containing a relatively hydrophobic central cavity and hydrophilic outer surface. Owing to the lack of free rotation about the bonds connecting the glucopyranose units, the cyclodextrin are not perfectly cylindrical molecules but toroidal or cone-shaped. Based on this architecture, the primary hydroxyl groups are located on the narrow side of the cone shape, while the secondary hydroxyl groups are located on the wider edge. The hydrophobic cavity of cyclodextrin is capable of trapping a variety of molecules within to produce inclusion complexes, β cyclodextrin β CD (seven α-d-glucopyranose oligomers) is the most widely used and available cyclodextrin, and its cavity size is suitable for wide range of guest molecules, although it has the least solubility; therefore many soluble derivatives of β cyclodextrin were produced [13, 14].

Many advantages of drugs complex with cyclodextrin have been reported in the scientific literature, especially the more soluble derivatives, which includes increased solubility, [15, 16] enhanced bioavailability [17, 18], etc. the more soluble cyclodextrin derivatives were used in numerous topical ophthalmic solutions [19].

Ophthalmic solutions are inefficient due to low bioavailability of medicines, caused by rapid turnover of tears, and poor patient compliance due to repetitive applications. Viscous liquid and semisolid preparations; give alternative therapeutic options, by increasing the residence time of drugs in an ocular cul-de-sac and prolonging the drug to eye contact duration and consequently enhancing the bioavailability of ophthalmic medications and reducing the patient non-compliance problem. Gel delivery systems have several advantages such as the ease of administration, none greasy, acceptable consistency, patient compliance, the high residence time in eye and better drug release, using various polymers like sodium carboxymethyl-cellulose SCMC, hydroxypropylmethylcellulose HPMC etc [20-22].

The aim of this study was to investigate the use of a complexing agent β-cyclodextrin in two ratios and using two methods of preparation to enhance the solubility of ECZ Then these complexes were formulated as ophthalmic gels. The developed gels were evaluated for their *in vitro* release of ECZ and rheological behaviour.

MATERIALS AND METHODS

Materials

Econazole nitrate ECZ was supplied by Al-Safa factory (mol wt. 444.7), β-cyclodextrin (HiMedia lab. Pvt. Ltd, Mumbai, India) (Mol.

Wt. 1135), carbomer carbopol 940 (CB) (HiMediaLab. Pvt Ltd, Mumbai, India), sodium carboxymethylcellulose (Sod CMC) (BDH Chemicals, Ltd. Poole, England), boric acid (CARLO ERBA Reagents Strada Rivoltana Italy), EDTA (Schalau Chemie S. A. Spain), triethanolamine (TEA) (Hopkins and Williams Ltd. England).

Methods

Preparation of inclusion complexes

ECZ with increasing concentration of β CD were mixed together using two different methods of complexation.

A-kneading method

A physical mixture of ECZ and βCD was prepared by mixing ECZ with βCD and levigated in a mortar with a small volume of water-ethanol (50/50 v/v) to form a slurry, the sample was kept overnight in a desiccator to remove trace of solvent. [23].

B-Co-evaporation method

ECZ was dissolved in sufficient quantity of ethanol and while βCD was dissolved in sufficient quantity of distilled water at 25 °C; and after the powders were completely dissolved in each solvent; the two solutions were combined together. The solvents were then removed using a drying oven (Memmert, GmbH Germany) at 40 °C to get the dry powder complex [24].

Solubility and phase solubility diagram

The solubility of ECZ alone and ECZ-βCD complexes prepared by A and B methods were determined in both isotonic saline solution and in distilled water by adding an excess of powder into flasks containing the above media. The flasks were sealed and shaken in a water bath (Memmert, Germany) at 25 °C for 48 h [25]. Samples were withdrawn, filtered and analyzed using Biotech 9200 UV Visible Spectrophotometer for the amount of ECZ solubilized at its λmax 271 nm [26].

The apparent stability constant of the ECZ-βCD complexes; was calculated from the slope of the plot ECZ solubility vs. βCD concentration, usingEq.1 and Eq.2 from the drug solubility in absence of cyclodextrin, intrinsic solubility [S_0] (the intercept) and [St] and [Lt] which are the concentrations of ECZ and βCD in solution, respectively [27] and the complexation efficacy CE was calculated [28] as follows

K 1:1 $$K_{1:1} = \frac{slope}{S_0(1 - slope)} \quad (1)$$

K 1:2 $$\frac{[S_t] - [S_0]}{[L_t]} = K_{1:1}[S_0] + K_{1:2}[S_0][L_t] \quad (2)$$

Complexation efficiency $$CE = \frac{slope}{(1 - slope)} \quad (3)$$

Preparation of the ophthalmic gel

According to the results of the solubility study, complexes obtained by kneading method were used to prepare different ophthalmic gel formulas, as shown in table 1.

Table 1: The composition of various econazole nitrate ophthalmic gels (%w/w)

ECZ: βCD	SCMC	CB	EDTA	Boric acid	Water q. s.
1	0.25	0.5	0.1	1.5	100
1	0.25	0.75	0.1	1.5	100
1	0.25	1	0.1	1.5	100

ECZ: βCD is the econazole: cyclodextrin complex, SCMC is sodium carboxymethylcellulose, CB is carbomer 940

CB, SCMC and a weighed amount of complex equivalent to 1% of the drug with βCD molar ratios of either (0.88×10^{-3} M)or (1.76×10^{-3} M) were dispersed in distilled water with continuous mixing using mortar and pestle until a homogenous mixture is obtained. Aqueous solution of EDTA and boric acid was added to the previous mixture with continuous mixing and the final weight of the gel was adjusted to 100 grams using distilled water. The final pH of the prepared gels was brought to eye pH by using TEA. [29] The prepared ECZ gels were inspected visually for their color. The pH of the gel was measured using pH meter. (Hanna instruments pH 211 Microprocessor, Italy).

Drug content

The drug content was determined by diluting 1 g of the formulation to 100 ml with an isotonic saline solution [30]. Aliquot of 1 ml was withdrawn and further diluted to 10 ml with the same solution ECZ concentration was then determined spectrophotometrically [31]

***In vitro* release study of ECZ ophthalmic gels**

The *in vitro* release study of ECZ from the prepared formulation was studied by using a modified USP dissolution apparatus. (Copley Scientific TDL England).

1 gram of ECZ ophthalmic gel was placed in folded filter paper inside the dissolution basket. The basket was attached to the metallic drive shaft immersed in the dissolution media containing 250 ml of isotonic saline solution at 37 °C±1so that the filter paper is slightly suspended in dissolution media. The shaft was rotated at 50 rpm Aliquot each of 5 ml volume was withdrawn at regular time intervals and replaced by an equal volume of dissolution media [32]. The absorbance of each sample was measured at 271 nm and converted to concentration using calibration curve of ECZ in isotonic saline solution. Each test was done in triplicates.

Kinetics of drug release

Drug release data were fitted on zero (Eq. 4), Korsmeyer Peppas (Eq.5) and to Peppas Sahlin equation (Eq.5) to describe drug release from the polymeric system using a Microsoft Excel plug-in program DDSolver [33].

Zero-order $$F = K_0 t \quad (4)$$

Korsmeyer Peppas $$F = K_{KP} t^n \quad (5)$$

Peppas Sahlin $$F = K_1 t^m + K_2 t^{2m} \quad (6)$$

Where F denotes the fraction of drug release at time t, K denotes the proportionality constant of release accordingly and (n) and (m) are the release index. When n or m = 0.5 the drug release from a thin film polymer will correspond to Fickian release, and it may be less for cylinders (0.45) and spheres (0.43). If n or m =1 this indicate zero-order kinetics if 0.5<n or m<1 it indicates anomalous release kinetics. Lastly when n or m>1 Super case II transport is apparent for thin films, (>0.89) for cylinders, and (>0.85) for spheres [34]. While Peppas Sahlin describes two steps of release, the initial release from fixed shape platform, then a secondary release, this equation is similar to Korsmeyer Peppas, with two steps of release. The most appropriate equation with the goodness of fit R^2 will be selected as the release model [33].

Rheology studies

The viscosity of the prepared gels was measured at room temperature using (Myr VR 3000, Spain) cup and bob rotational viscosity (a viscometer comparable to a Brookfield viscometer). The samples were sheared with spindle R7 rotated at speeds starting from 20 to 200 rpm in ascending order; each sample was rotated for

2 min. before each reading, [35] then the sample was allowed to rotate in descending order [36, 37].

Statistical analysis

Solubility and release data were subjected to one–way (t-test) using Microsoft Excel 2010. The data were considered significant at (p<0.05).

RESULTS AND DISCUSSION

Solubility study and phase solubility diagram

The solubility of ECZ alone and ECZ-β CD complexes using two methods (kneading and co-evaporation) in both isotonic saline solution and distilled water were studied as seen in fig. 1.

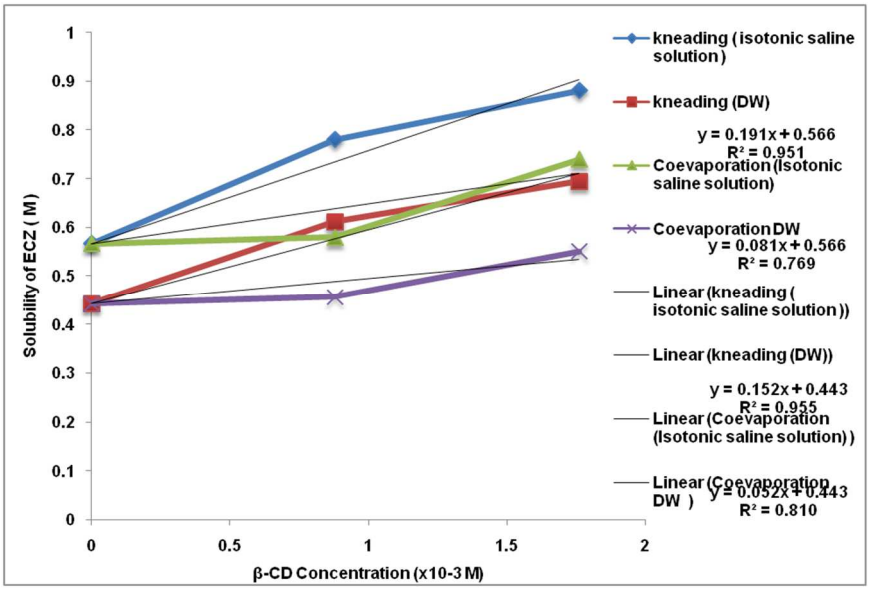

Fig. 1: The solubility of ECZ and ECZ-βCD complexes in isotonic saline solution and DW (mean±SD, n=3)

The solubility of ECZ was 0.252 mg/ml and 0.197 mg/ml in isotonic saline solution and distilled water respectively. ECZ exhibited about 1.3 times solubility in isotonic saline solution more than water, which may be attributed to the acidic nature of isotonic saline solution (pH 5.5) [38]. ECZ nitrate is very basic with a pKa of 6.67, therefore, it is more soluble in the more acidic solution. However, it may affect the complexation of the ionized ECZ with the neutral CD [39] and decrease the size of the stability constant [40].

Complexation by kneading method significantly enhanced the solubility of ECZ in both media (p<0.05) comparing with the solubility of ECZ alone. The driving force for complexation is the release of the enthalpy rich water molecules from the inside of the cone, and formation of non-polar bonds between the ECZ and the βCD, decreasing the ring strain of the cyclodextrin, leading to the formation of lower energy state, more stable, inclusion complex resulting in improvement of solubility [41, 42].

There was a significant difference (p<0.05) between the solubility of ECZ complexes prepared by kneading method compared with co-evaporation method. This result is similar to the results obtained by Al-Marzouqiet. *et al.* in which ECZ: βCD obtained by kneading method, showed drug peak disappearance in differential scanning colorimeter and X-ray diffraction, suggesting complex formation and/or sample amorphization while the product obtained by co-evaporation method showed a small reduction in intensity of the drug peak, suggesting small degree of drug-cyclodextrin interaction[43]. Complexation by kneading seems to more suitable for poorly-soluble guests in contrast to co-evaporation which is more suitable to non-water-soluble guests [44].

Solubility enhancement of ECZ using kneading method was significantly affected by the ECZ: βCD ratio (p<0.05), increasing β-CD ratio to ECZ, increased the solubility, [45] as seen in fig. 1.

The solubility diagram was both of AL type according to Higuchi and Connors classification, showing a linear increase of drug solubility with an increase in the concentration of βCD [46] both showed a type Ap curve which means CD is more effective at higher concentration.

The slopes of the lines were less than one referring to formation of (1:1) stoichiometry, but does not exclude formation of higher ratios [45 ,47]Pedersen *et al.* showed that ECZ was included in βCD at a supersaturated ratio of 2:3 ratio, and excellent antimycotic activity [48].

The stability constant K_S (K 1:1) of ECZ: βCD complex prepared by kneading method was higher than that of co-evaporation method in both media. The larger stability constant was observed with kneading method meaning itis the most suitable method for complexation of ECZ. Also, the Complexation Efficiency CE was highest for kneading method.

Table 2: Stability constants Ks at (1:1) and (1:2) stoichiometry and complexation efficiency CE values of the inclusion complex of the two complexation methods and the initial solubility S0, in both medias

Complexation method	Media	Type of diagram	S0	Slope	CE	K 1:1	K 1:2 *
Kneading	Isotonic Saline	Ap	0.5667±0.05	0.1914	0.236± 0.007	0.417	2.051
	DW	Ap	0.5667±0.07	0.0817	0.088± 0.083	0.156	1.148
Co-evaporation	Isotonic Saline	Ap	0.443±0.065	0.1526	0.180± 0.091	0.406	2.214
	DW	Ap	0.443±0.008	0.0521	0.054± 0.0089	0.124	0.713

*measured at βCD concentration of 0.88 x 10^{-3}M (mean±SD, n=3)

Physical characters of the prepared gels

According to the results obtained from solubility study, the complexes prepared by kneading method were used to prepare different ophthalmic gel formulas since this method enhanced the solubility of ECZ as mentioned previously.

CB and SCMC combination was used to get the desired consistency, viscosity and appearance. Using two molar ratios of βCD and three different concentrations of CB were examined to achieve the desired release and viscosity. All the prepared gels were white soft gels. This may be attributed to the higher hygroscopicity of SCMC that give soft jelly nature in comparison to highly viscous gel prepared by CB alone [49].

pH

All the prepared gels had a pH range from 6.2-6.9.

Drug content

ECZ content was estimated in the range of 91.5%-96.33%. The drug content was found to be uniform in all gel dispersion and is in good agreement with the theoretical drug content.

The release study of EZC from ophthalmic gels

The cumulative percent of ECZ release as a function of time from a various ophthalmic gel containing ECZ: βCD complex system prepared by kneading method are shown in fig. 2 and 3 and the corresponding release kinetics data analysis is shown in table 3.

Three concentrations of CB where used to study their effect on the release of ECZ from the gel base. It was seen that increasing the concentration of CB from 0.5% to 1% the amount of ECZ released decreased for both βCD concentrations. The retardation of the release could be explained by increasing the overall gel viscosity with increasing concentration of CB polymer, [50] also the gels prepared using higher βCD concentration showed decrease in the fraction of the drug released this may be related to the capability of cyclodextrin cavities to retain the drug in the network, as previously reported for cyclodextrin-based hydrogel loaded with the hydrophobic hormone estradiol [51].

Concerning the best equation to fit the release kinetics; the n value of Korsmeyer Peppas equation at less than 60% release [52] of formulas tested, showed Super Case II transport, attributed to CB stress induced relaxation due to water sorption and swelling which takes place at the outer swollen shell of hydrophilic glassy polymer [53], except for the first formula in which the release was anomalous at lower concentration of CB and βCD so the stress induced by swelling is minimal and the release is due to couple effect of diffusion and polymer relaxation [53]. The results of Peppas–Sahlin equation [54] showed typical initial Fickian diffusion for formulas of CB 0.5 and 1%, while formulas with 0.75% CB exhibited super case II release in both steps, in which polymer swelling propagates elasticity into the vicinity of adjacent polymers [55]; thus polymer relaxation is the prominent step in the release of ECZ. In contrast to the second step of release of 0.5% and 1% CB, which showed anomalous and Fickian release.

The results indicate that gel containing 0.75% CB using 0.88(10^{-3}) M βCD is the more efficient formula for drug release and may be considered as a good candidate for ophthalmic gel dosage form since it has a suitable drug release profile in which about 50% of ECZ release is within 30 min and the best fit Super Case II transport with correlation coefficient r^2 of 0.99.

Table 3: Release kinetics data and correlation coefficient

	βCD	**0.88(10^{-3}) M**			**1.76(10^{-3}) M**		
	CB %	**0.5**	**0.75**	**1**	**0.5**	**0.75**	**1**
	t 50% (min)	**21.934**	**30.256**	**38.921**	**29.580**	**48.414**	**39.301**
Zero	K_0	2.155	2.052	1.056	1.671	1.011	1.003
Order	r^2	0.9881	0.8839	0.9628	0.9565	0.8851	0.8555
Korsmeyer–	K_{kp} *	3.811	0.456	0.162	0.393	0.172	0.687
Peppas	n *	0.834	1.377	1.566	1.430	1.462	1.168
	r^2 *	0.9949	0.9982	0.9868	0.9581	0.9945	0.9162
	n	Anomalous	Supercase II transport	Supercase II transport	Supercase II transport	Supercase II transport	Supercase II transport
Peppas-Sahlin	K_1	-25.351	0.250	-40.077	-120.035	0.120	-5058.177
	K_2	20.857	0.001	24.621	83.482	0.000	5017.452
	m	0.268	1.445	0.235	0.171	1.464	0.004
	2m	0.536	2.89	0.47	0.342	2.928	0.008
	r^2	0.9979	0.9916	1.0000	0.9951	0.9930	0.9390
	m	Fickian	Supercase II transport	Fickian	Fickian	Supercase II transport	Fickian
	2m	anomalous	Supercase II transport	anomalous	Fickian	Supercase II transport	Fickian

*Measured at less than 60% release, K_0, K_{KP}, and K_1, K_2 are the release constants for zero, Korsmeyer–Peppas and Peppas Sahlin equations, n,m is the release index for Korsmeyer–Peppas and Peppas Sahlin equations

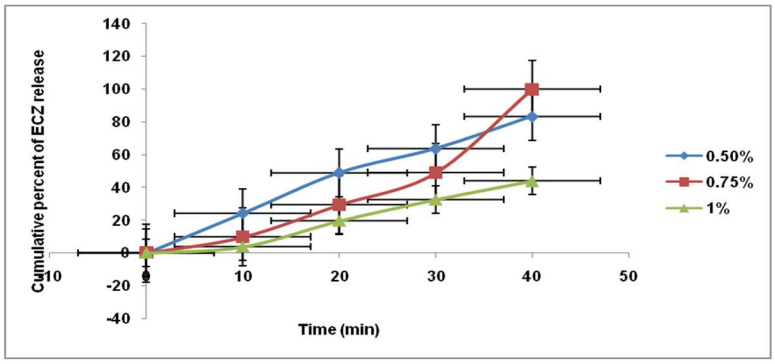

Fig. 2: The release of ECZ from ophthalmic gels at 0.88× 10^{-3}MβCD (mean±SD, n=3)

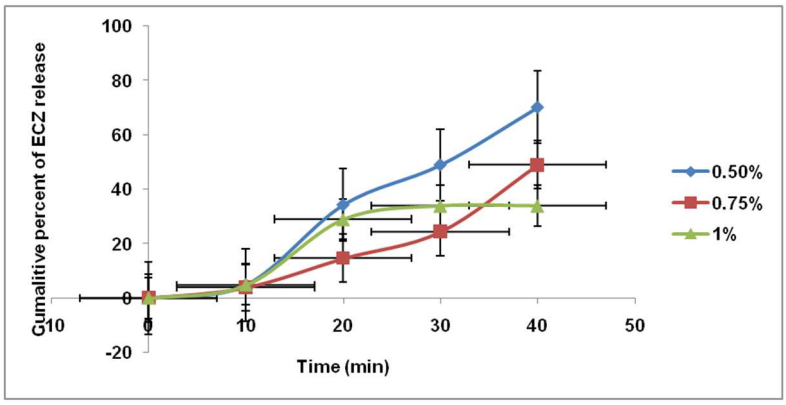

Fig. 3: The release of ECZ from ophthalmic gels at 1.76×10^{-3}MβCD (mean±SD, n=3

Rheological properties of ECZ ophthalmic gels

Ophthalmic ECZ gels viscosities were measured (fig. 4) and showed that increasing the concentration of CB resulted in an increase in viscosity of the gel, [56] since in gel system the viscosity depends on the ratio of the solid fraction which produces a structure to liquid. ECZ ophthalmic gels showed pseudo-plastic shear thinning with no thixotropy, on the rest they usually exhibit properties of the concentrated system due to the surface entanglement of the long chains between adjacent particles with each other. The profile showed that with increasing the shear rate the viscosity decreases. Applied stress will comb these entanglements out and align their long axes in direction of flow orientation reduce the internal resistance of the material and hence decrease the viscosity, with increasing shear rate [57, 58]. Pseudoplastic gels are preferred for ophthalmic application because blinking will lower the viscosity of the gel and allow the gel to spread on the cornea [59] offering comfortable application.

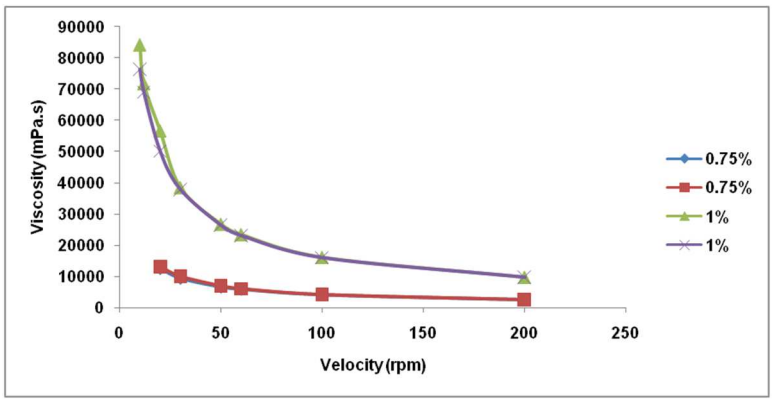

Fig. 4: The viscosity of ECZ ophthalmic gels using0.88×10^{-3}MβCD in ascending velocity and then descending (mean±SD, n=3)

CONCLUSION

The solubility of ECZ was enhanced by complexation with β cyclodextrin using kneading and co-evaporation, and both showed Ap type solubility diagram. The complex prepared by kneading method greatly enhanced the solubility of ECZ compared with the co-evaporation method. The ophthalmic gel formulated with CB and CMC using ECZ-βCD inclusion complex by kneading method showed that increasing the concentration of CB has a retardation effect on the *in vitro* release of the drug, and as the concentration of gelling agent increase the viscosity of the gel increase. The selected formula may be considered as a good candidate for ECZ ophthalmic gel dosage form, which showed Super case II transport release profile, and pseudo-plastic shear thinning behaviour.

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AUTHORS CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". We the authors conceived and designed the study, Halah T Sulaiman collected and analyzed the data, we both wrote the manuscript, Hanan J Kassab read, revised and approved the manuscript for publication

CONFLICT OF INTERESTS

No conflict of interest is associated with this work

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