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Formulation and Evaluation of Ophthalmic Preparations Containing Econazole Nitrate-Cyclodextrin Complexes

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ABSTRACT

Improvement of ocular delivery systems properties remains a major challenge to achieve a continued therapeutic effect of drugs. The aim of the present study was to improve the solubility, dissolution rate and hence the ocular bioavailability of econazole nitrate (EC). To achieve this goal, econazole nitrate was complexed with β-cyclodextrin (β-CyD) and hydroxypropyl-β-cyclodextrin (HP-β-CyD) using co-precipitation method. The influence of different CyDs on EC complexation was investigated by different methods as fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). The present work includes the preparation of eye gels and ocuserts containing econazole nitrate-cyclodextrins complexes using different polymers. Physicochemical properties (pH, viscosity, uniformity of drug content, *in-vitro* release) of the preparations were studied by the appropriate methods. The obtained results revealed that, EC solubility increased as a function of CyDs concentration. All formulations possessed uniform drug content with pH and viscosity compatible with the eye. *In-vitro* release data of ophthalmic formulations showed a sustained release with the diffusion-controlled mechanism (Higuchi model). Ocular bioavailability of EC-CyD complexes from the selected formulations was significantly ($p<0.05$) improved compared to that of the drug alone. Collectively, the improvement of the solubility, dissolution rate and ocular bioavailability of EC was more pronounced with HP-β-CyD.

Keywords: Econazole nitrate; cyclodextrins; complexation; ophthalmic preparations; ocular bioavailability.

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INTRODUCTION

Topical drug delivery in ocular therapeutics is the most advantageous route for the treatment of eye diseases affecting the anterior segment because it avoids systemic absorption and serves to extend the drug effect in target tissues[1]. The poor bioavailability of drugs from ocular dosage forms is mainly due to efficient protective mechanisms of the eye including; blinking, the rapid turnover of lachrymal fluid, short pre-corneal residence time, and the relative impermeability of the corneal epithelial membrane[2, 3]. In recent years, numerous strategies were developed to increase the bioavailability of ophthalmic drugs by prolonging its contact time with eye tissues. Different approaches, like viscosity enhancement, use of mucoadhesive drug delivery, particulate drug delivery, vesicular drug delivery, and other controlled systems, like ocuserts, are being explored[4, 5].

Eye infections can be produced by many organisms, including bacteria, viruses, and fungi. Eye infections caused by fungi are extremely rare, but they are very serious. Fungal infections can affect different parts of the eye. Keratitis is an infection of the clear, front layer of the eye (the cornea) while endophthalmitis is an infection obtained inside the eye (the vitreous and/or aqueous humor)[6]. The azoles represent a class of versatile antifungal agents. In general, they are effective against most fungi that cause superficial infections of the skin and mucous membranes[7]. Econazole nitrate (EC) is an imidazole and halogenated aromatic compound structurally related to miconazole that has antifungal properties. EC is a potent broad-spectrum antifungal agent topically in the treatment of many mycotic infections of skin, hair and mucous membranes[7, 8]. However, the poor aqueous solubility of this drug has limited its use for the treatment of ophthalmic fungal infection[8]. Therefore, it is desirable to formulate EC in a suitable system that could deliver the drug in an efficient concentration to the eye.

Multiple materials such as cyclodextrins (CyDs) have been used to increase the aqueous solubility of poorly soluble drugs. Cyclodextrins are cyclic oligosaccharides that have a hydrophilic outer surface and a hydrophobic inner cavity that function as molecular cages to entrap a variety of hydrophobic guest molecules of appropriate sizes and shapes, thus leading to the formation of inclusion complexes through non-covalent bonds[9, 10]. Thus CyDs increase the aqueous solubility of hydrophobic compounds without changing their molecular structure[11, 12]. CyDs enable keeping hydrophobic drugs in solution and transport them to biomembranes surface[13]. Previous studies showed that both dissolution properties and consequently, microbiological activity of EC can be improved by complexation with natural cyclodextrins,

particularly with β-CyD[14]. The objective of this study was designed to prepare and characterize the inclusion complexes of EC with β-CyD or HP-β-CyD by co-precipitation method. Characterization of the complexes will be performed using FT-IR, PXRD and DSC. Formulation of EC-CyD complexes in different ophthalmic preparations was of prime interest. Moreover, all the formulations were investigated for their physical properties and *in*-vitro release characteristics. In addition, the ocular bioavailability and the pharmacokinetic parameters of the drug from the selected formulations on the basis of an acceptable physical characteristics and *in-vitro* release profiles were studied.

MATERIALS AND METHOD

Econazole nitrate (EC), Ibuprofen (IB), beta cyclodextrin (β-CyD) (Molecular weight = 1135.12), hydroxypropyl-beta cyclodextrin (HP-β-CyD) (Molecular weight = 1375.35) and hydroxy propyl methylcellulose (HPMC) were provided by Provizer Pharma, India. Methylcellulose (MC) was provided by Winlab, India. Carbopol 940 (CP 940) was provided by LoBa chemie, India. n-octanol was purchased from Prolabo, Chemicals, Paris, France. Spectra / Pore® dialysis membrane (12000–14000 molecular weight cutoff) was purchased from Spectrum Laboratories Inc., Rancho Dominguez, Canada. The HPLC grade solvents (acetonitrile and 1-octane sulfonic acid sodium salt) were purchased from Fisher scientific, UK. All other chemicals and solvents were of analytical reagent grade.

Phase Solubility Study of Econazole Nitrate

An excess amount of EC (10 mg) was added to aqueous solutions of CyDs (β-CyD or HP-β-CyD) at various concentrations ($0.25x10^{-3}$ – $2x10^{-3}$ M/L) in screw-capped glass bottles. Samples were shaken on thermostatically controlled water bath (Grant instrument Cambridge Ltd., Barrington Cambridge, B2, 5002, England) at the temperature of 37 ± 0.5°C at 50 rpm. After equilibrium was attained (3 days), the solutions were filtered through 0.45 µm membrane filter and then analyzed spectrophotometrically (UV spectrophotometer; Shimadzu, UV-150-02, sersakusho, Ltd, Kyoto, Japan) at 231 nm for total drug content. The experiments were done in triplicate, the mean and SD were calculated. The apparent 1:1 stability constants of the complexes (K_c) were calculated, by using the slope of the initial straight portion of the phase solubility diagram and EC solubility in distilled water in the absence of CyD ($\mathbf{S_o}$) [15]:

$$\mathbf{K_c = Slope / S_o (1 - slope)}$$

Preparation of EC-CyDs Complexes by Co-precipitation Method

Inclusion complexes of EC with CyDs (β-CyD or HP-β-CyD) were prepared by the co-

precipitation method[16]. A weighed quantity of EC were dissolved in 5 ml of acetone and then added dropwise to the aqueous solution of CyDs (weighed quantity of β-CyD or HP- β-CyD in 10 ml of water). The contents were continuously stirred for 6 hrs and then the precipitates were filtered, dried at 45-50°C for 48 hrs, collected, and stored in airtight containers until use.

Preparation of EC-CyDs Physical Mixture

The calculated amounts of EC and CyDs corresponding to 1: 1 molar ratios were thoroughly mixed, and used directly for evaluation study.

Characterization of Inclusion Complexes

Fourier Transform Infrared Spectroscopy (FT-IR) Studies

FT-IR spectra of EC, β-CyD, HP-β-CyD, physical mixtures and the prepared complexes were determined using Fourier transform infrared spectroscopy (FT-IR; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Each sample was mixed with potassium bromide (KBr). These mixtures were ground into fine powder and then compressed into KBr discs using a hydraulic press. Each KBr disc was scanned over a wavenumber region of 500–4000 cm^{-1} and the resolution was 4 cm^{-1}. Characteristic bands were determined for tested samples.

Differential Scanning Calorimetric (DSC) Studies

The thermal behavior of EC, β-CyD, HP-β-CyD, physical mixtures and the prepared complexes were analyzed using differential scanning calorimetry (DSC; Perkin Elmer DSC 7, USA with a data analysis system). The powdered sample (5 mg) was filled in aluminum pans and covered by lids and scanned at a rate of 10^{o}C/min over the temperature range of 50-320^{o}C under a stream of nitrogen.

Powder X-ray Diffraction (PXRD) Studies

Powder X-ray diffraction patterns of EC, β-CyD, HP-β-CyD, physical mixtures and the prepared complexes were performed using X-ray diffractometer (Rigaku Denki, Rint-2500 VL, Tokyo, Japan) over the interval of 5-30^{o} (2θ) and operated at the conditions of Ni-filtered Cu-kα radiation. The voltage and current used were 40 KV and 40 mA, respectively. The time constant was 1.25 Sec, and a scanning speed was adjusted to 1^{o} / min.

Preparation of EC Formulations:

Preparation of Eye Gels

Econazole nitrate (0.2 w/v %) or its equivalent weights of EC-β-CyD or EC-HP-β-CyD complexes were dissolved in 20 % propylene glycol and added to the aqueous solution of different polymers namely MC, HPMC and CP 940 in concentration of 3, 5 and 3% w/v, respectively (Table 1). Methyl and propyl parabens were used as preservatives in concentration

of 0.05 and 0.01 % w/v, respectively[17]. In case of CP 940 gels, two drops of triethanolamine (TEA) was added to form gel since CP 940 is a polyacrylic acid which undergoes a sol – gel transition in aqueous solution at pH above its pKa 5.5[18].

Preparation of Ocuserts

Ocuserts containing EC or its complexes were prepared according to the film-casting method[19]. Econazole nitrate (0.2 % w/v) or its equivalent weights of EC-β-CyD or EC-HP-β-CyD complexes were dissolved in 10 ml of propylene glycol which employed as a plasticizer to aid the formation of flexible films as well as to protect the polymeric inserts from being brittle upon storage[20]. Then, the solution was added to the different polymeric solutions as shown in Table 1. Methyl and propyl parabens were used as preservatives in concentration of 0.05 and 0.01 % w/v, respectively[17]. All of the prepared polymeric solutions were then sonicated for 2 hrs in an ultrasonic water bath (Sonix IV, Saris Ultrasonic Bath, USA) to exclude entrapped air and then stored for 24 hrs at ambient temperature to ensure total hydration of the polymers. Then, equal volumes of the prepared solutions were transferred into teflon plate. The solvent was permitted to evaporate for 72 hrs at ambient temperature. The formed films were weighed and transferred to a desiccators containing silica gel, where it was stored for another 24 hrs before use. The prepared ocuserts (0.4-0.5 mm thickness) were cut in the form of circular discs, 5 mm in diameter. Then they were sealed individually in foil sachets until use.

Table 1: The composition of different formulations containing EC-CyDs complexes

Type of formulations	Polymer Concentration % (w/v), type	Type of complex	Formulation code
Control			CT
Eye gels	3 % MC	EC- β-CyD	Gβ 1
		EC-HP- β-CyD	GH-β 11
	5 % HPMC	EC- β-CyD	Gβ 2
		EC-HP- β-CyD	GH-β 12
	3 % CP 940	EC- β-CyD	Gβ 3
		EC-HP- β-CyD	GH-β 13
Ocuserts	2 % MC + 0.1 % CP 940	EC- β-CyD	Oβ 4
		EC-HP- β-CyD	OH-β 14
	1 % HPMC + 0.1 % CP 940	EC- β-CyD	Oβ 5
		EC-HP- β-CyD	OH-β 15
	2 % CP 940	EC- β-CyD	Oβ 6
		EC-HP- β-CyD	OH-β 16

Physicochemical Evaluation of Different Formulations

Determination of Drug Content

One gram of each formulation was accurately weighed and dissolved in 100 ml phosphate buffer pH 7.4, and shaken in thermostatically controlled water bath at 37± 0.5^{o}C for 30 min. Then, the solution was filtered using 0.45 µm membrane filters and measured spectrophotometrically at 231 nm for drug content. The experiments were triplicated and the results were calculated as mean ± SD.

Measurement of the Formulations pH

One gram of each formula was dispersed in 20 ml of distilled water, and then the pH was measured using pH-meter (Beckman Instruments Fullerton, CA 92634, Germany).

Determination of the Eye Gel Viscosity

The viscosity of eye gels was determined using a cone and plate rotary viscometer (Haake Inc., Germany). One gram of each formula was placed on the stationary plate of the viscometer and allowed to equilibrate for 5 min to reach the running temperature before each measurement. The rotary viscometer was thermostatically controlled at 37± 0.5^{o}C. Then, the viscosity values were calculated using the following equation[21];

$$\eta = G.\ S / N$$

Where; (η) viscosity in mPa.s (mPa.s = 1 centipoise, cP), (G) Instrumental factor = 14200 (mPa.s /scalagrad. min), (S) torque (scale grad) and (N) speed (rpm).

***In-vitro* Release Study of EC from Different Formulations**

The drug release from eye gels and ocuserts, in phosphate buffer solution of pH 7.4 was carried out, using the dialysis method[22]. Spectra / Pore® dialysis membrane (12000–14000 molecular weight cutoffs) was soaked in phosphate buffer pH 7.4 for 24 hrs before the experiment. The membrane was stretched over the open end of 3 cm diameter glass tube and was made water tight by a rubber band. Two grams of each formulation were accurately weighed and thoroughly spreaded on the membrane. To each tube, 1.5 ml of the buffer solution pH 7.4 was added. The tube was then immersed upside- down in a beaker containing 30 ml phosphate buffer pH 7.4 which is preheated and maintained at 37 ± 0.5^{o}C using thermostatically controlled water bath. The tube height was adjusted, so that the membrane was just below the surface of the release medium. The rotary shaker was adjusted to a rate of 25 strokes / min. At predetermined time intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hrs, aliquots of 1 ml were withdrawn from the release medium and replaced by an equivalent volume of the buffer solution. The released amounts of the drug were analyzed spectrophotometrically at 231 nm. The experiments were repeated three times and the results were calculated as mean ± SD. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple

comparisons test at a level of significance of $p<0.05$.[23] Statistical calculations were carried out using Instate Graphpad prism software (version 5.00 Graphpad software, San Diego, CA, USA).

Kinetics of Drug Release

To investigate the release mechanism of the kinetic model that better describes the pattern of drug release, the *in-vitro* release data were analyzed mathematically according to the following models: zero-order (as the cumulative amount of drug release versus time), first-order (as the log cumulative percentage of drug remaining versus time) and Higuchi diffusion models (as the cumulative percentage of drug released versus square root of time). The preference for a certain model was based on the correlation coefficient, where the highest correlation coefficient was preferred for the selection of the release mechanism[24]. The Korsmeyer–Peppas semi empirical model, as the log cumulative percentage of drug release versus log time, was used for further in depth analysis, where the value of the release exponent (n) depends on the release mechanism and thus could be used to characterize it[25].

Ocular Bioavailability of EC from Selected Formulations

Selection of Formulations for Bioavailability Study

The selected formulations for bioavailability study were; eye gels and ocuserts containing EC-β-CyD or EC-HP-β-CyD and EC suspension in water (control), Table 2. These formulations were selected according to their physical acceptable characteristics and sustained release profiles.

Table 2: The composition of selected formulations for ocular bioavailability study

Type of formulations	Polymer Concentration % (w/v), type	Formulation code
Control		CT
Eye gels	3 % CP 940	Gβ 3
		GH-β 13
Ocuserts	1 % HPMC + 0.1 % CP 940	Oβ 5
		OH-β 15

Ocular Bioavailability Studies

Ocular bioavailability of the selected formulations was performed on male New Zealand albino rabbits, each weighing 2-2.5 kg. All animals were healthy and free of clinically observable abnormalities. Animals were housed singly in standard cages, in a light controlled room (12-hrs light and 12-hrs dark cycles) at 20–24°C, with no restriction to food or water. The experimental procedures conform to the ethical principles of the scientific committee of the Faculty of Pharmacy, Mansoura University, Egypt for the use of experimental animals. The rabbits were divided into five groups, each of 12 rabbits. Each animal was received 30 µg of eye gels or one ocusert which instilled into the center of the lower lid (cul-de-sac) of the right eyes of the

animals, while the left eyes were served as control by application of the plain formulation. The lower eyelid was gently moved to spread the dose on corneal surface during application. All rabbits were kept in up-right position in restraining boxes. Three rabbits were sacrificed for each formulation at each time intervals of 1, 3, 5, and 7 hrs. Both eyes were enucleated and dissected while fresh to separate different eye tissues (cornea, conjunctiva, iris-ciliary body and aqueous humor) which were kept frozen at -80^{o}C until subjected for further analysis. The amount of the drug disposed in different eye tissues and aqueous humor at each time interval was determined.

HPLC Assay

At each time interval, each eye tissue and aqueous humor were separated immediately, then each eye tissue rinsed with isotonic saline solution, weighed and grinded with powdered glass. The grinded tissues were extracted with 4 ml acetonitrile for 24 hrs at 25^{o}C to extract the drug from different eye tissues and aqueous humor. These solutions were filtered using 0.45µm nylon membrane filter. The tissue extracts were spiked with 20 µl of ibuprofen (IB) as an internal standard (50µg/ml). The mixture was mixed using vortex mixer (Snijders Scientific Tilburg-Holland) for 1 min, then filtered through 0.45µm nylon membrane filter and 20 µl of the solution was injected into HPLC system. The concentration of EC in each tissue was determined by HPLC assay with slight modification[26]. The quantitative analysis of EC was performed by a reverse phase HPLC system consisting of a pump (LC -20 AD), degasser (DGU-20A5), CBM-20A interface, UV-Vis spectrophotometric detector (SPD-20A UV-Vis detector) and a reverse phase column (C-18 column, 5 µm, 4.6 x 250 mm, phenomenex, USA). For data processing and acquisition, LC solution software version 1.3 from shimadzu, Japan was used. The mobile phase, consisting of 70 % acetonitrile and 25 mM potassium dihydrogen orthophosphate with 0.65 gm/L of 1-octane sulfonic acid sodium salt 30 %, was filtered under vacuum through a 0.45µm nylon membrane filter. The flow rate was adjusted at 1.2 ml/ min and the detection wavelength was 213 nm. The retention time of the internal standard and EC was 4.6 min and 5.7 min, respectively. The concentration of EC was expressed as ng of drug / mg of tissue weight.

Pharmacokinetic Parameters

The pharmacokinetic parameters were calculated for each rabbit[27]. The maximum drug concentration in eye tissues (C_{max}) and the time required to reach the maximum eye tissue concentration (T_{max}) were directly determined from the eye tissue concentration-time curves. Also, the elimination rate constant (K_e) was estimated from the terminal linear portion of the eye tissue concentration-time profile by linear regression analysis. The elimination half life ($T_{1/2}$) was calculated as 0.693/K_e. In addition, the area under eye tissue concentration-time curve from

0-7 hrs (AUC_{0-7}) was calculated using the linear trapezoidal methods. The AUC was extrapolated to infinity ($AUC_{0-\infty}$) by adding AUC_{0-7} to C_{last}/ K_e, where C_{last} is the last measurable concentration of the drug after 7 hrs. The relative bioavailability (Rel. Bio.) of EC was determined as the ratio between $AUC_{0-\infty}$ of the tested formulation to that of control.

Statistical Analysis

The data are expressed as mean ± SD. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test at a level of significance of $p<0.05$[23] Statistical calculations were carried out using Instate Graphpad prism software (version 5.00 Graph pad software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Phase Solubility Study of Econazole Nitrate

Figure 1 shows the phase solubility diagrams of EC with β-CyD and HP-β-CyD in distilled water at37 ± 0.5°C. The data revealed that, the phase solubility diagram of the drug can be classified as B_S type solubility curves, where the initial rising portions are followed by plateau regions and then the total concentration of EC decreased which may be due to the precipitation of microcrystalline complexes. In addition, EC has higher affinity for HP-β-CyD than β-CyD. This may be due to the larger cavity size of HP-β-CyD[28], which is optimal for entrapment of the drug molecules, thus providing a greater solubilization effect compared to β-CyD[29].

A further characterization of EC complexation with tested CyDs was performed by determining K_c of the respective complexes, calculated from the initial linear segment of the phase solubility curves. Assuming that a 1:1 complex is initially formed, the HP-β-CyD stability constant value (4.178 x102 M^{-1}) was significantly ($p < 0.05$) superior to that of β-CyD (2.629 x102 M^{-1}). From the obtained results, it was found that the stability constant values between 200 and 5000 M^{-1} demonstrate an improvement in the solubility and stability of poorly water-soluble drugs[30].

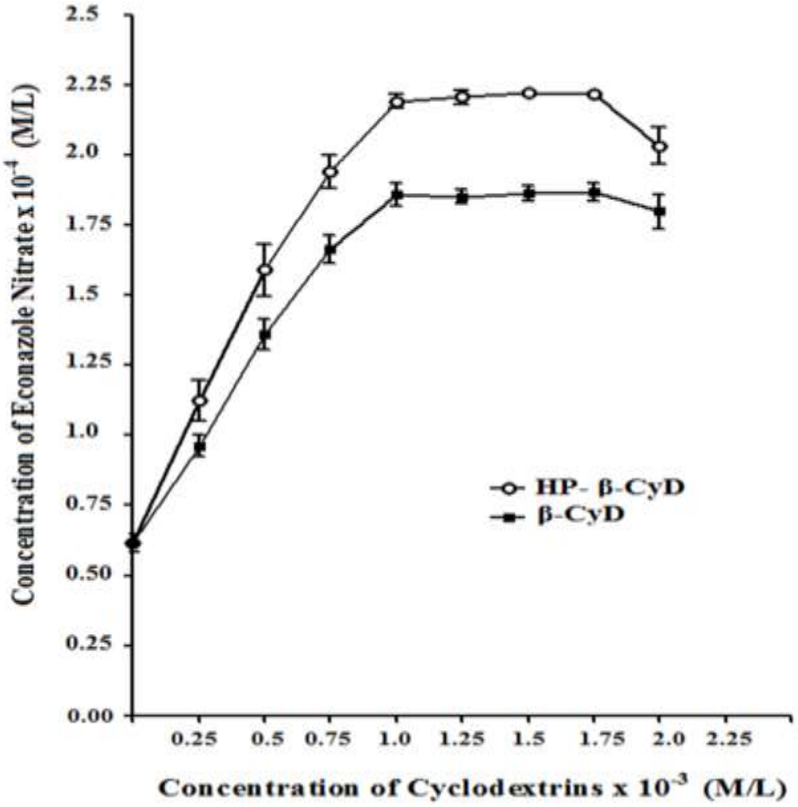

Figure 1: Phase solubility diagram of EC in the presence of β-CyD and HP-β-CyD.

Characterization of Inclusion Complexes

Fourier Transform Infrared Spectroscopy (FT-IR) Studies

The FT-IR spectra of EC, β-CyD, HP-β-CyD, their physical mixtures and inclusion complexes were analyzed using FT-IR spectrophotometer for characteristic bands as shown in Fig. 2. The FT-IR spectrum of EC show evident several characteristics bands, stretching band at 3430 cm^{-1} for N-H; two bands characteristic for aromatic C-H stretching vibration bands at 3174 and 3107 cm^{-1}; aromatic C=C and C=N stretching vibration bands at 1586 and 1448 cm^{-1}, respectively; aromatic C-CL stretching vibration band at 860 cm^{-1} and C-O-C stretching vibration band at 1087 cm^{-1}. The FT-IR spectrum of β-CyD is characterized by intense bands at 3200-3600 cm^{-1} due to O-H stretching vibration bands. The vibration bands of the C-H and CH_2 groups appear in 2800-3000 cm^{-1} region, a broad band appeared at 1634 cm^{-1} due to adsorbed water. These results were in agreement with those obtained by Mahmoud et al.[8]. While that of HP-β-CyD, showed prominent absorption bands at 3438 cm^{-1} and 2930 cm^{-1} for O-H stretching vibrations. Also, two stretching vibration bands were obtained at 1414 and 1016 $cm^{-1}$for C-H and C-O, respectively[31]. The FT-IR spectra of the physical mixtures did not differ significantly from those of the individual components. In the FT-IR spectra of the prepared complexs, aromatic C-H, C=C and

C-CL stretching vibration bands were disappeared. Also, in the FT-IR spectra of EC-HP-β-CyD complex, O-H stretching vibration bands were shifted to 3119 $cm^{-1}$and 2645 cm^{-1}. Thus, the complexes show no peaks similar to the drug alone that may be due to the inclusion complexation of EC into the CyDs cavity.

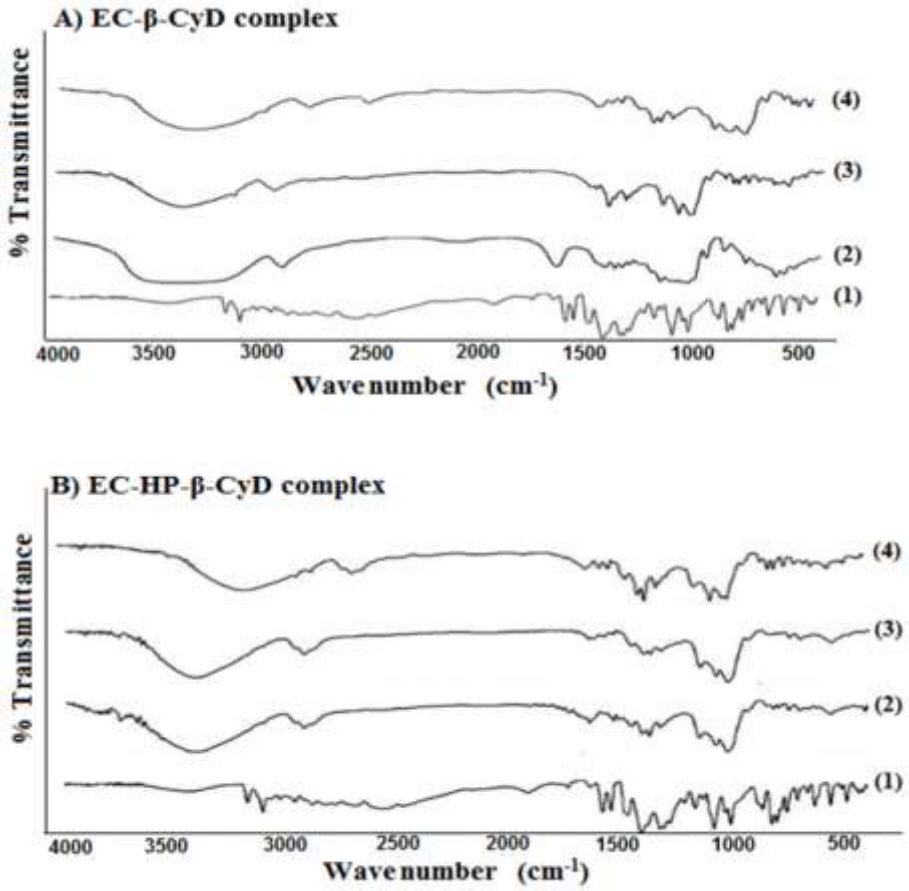

Figure 2: FT-IR spectra of (1) EC, (2) β-CyD or HP- β-CyD, (3) Physical mixture, (4) Prepared complex.

Differential Scanning Calorimetric (DSC) Studies

The thermal analysis was an important method to recognize and characterize CyDs complexes**.** When guest molecules were included inside CyDs cavities or in the crystal lattice, the peak corresponding to their melting point is generally shifted to another temperature. Also the peak intensity may be decreased or disappeared[32]. Figure 3 shows the DSC thermograms of EC, β-CyD, HP-β-CyD, their corresponding physical mixtures and inclusion complexes. EC exhibited a sharp exothermic peak at 169^{o}C followed by a large and irregular endothermic peak at 199^{o}C, attributable to the melting process of the anhydrous crystalline form of the drug, followed by its thermal decomposition. This result was in agreement with that obtained by Jug et al[33]**.** The

thermogram of β-CyD showed two peaks; one broad endothermic peak at 109°C, corresponding to the release of water from β-CyD, and another less broad endothermic peak above 290°C, corresponding to the decomposition of β-CyD[34]. While the thermogram of HP-β-CyD showed regular broad endothermic peak between 58°C and 110°C, which attained a maximum at 84°C that might be corresponding to dehydration process of CyD[35].

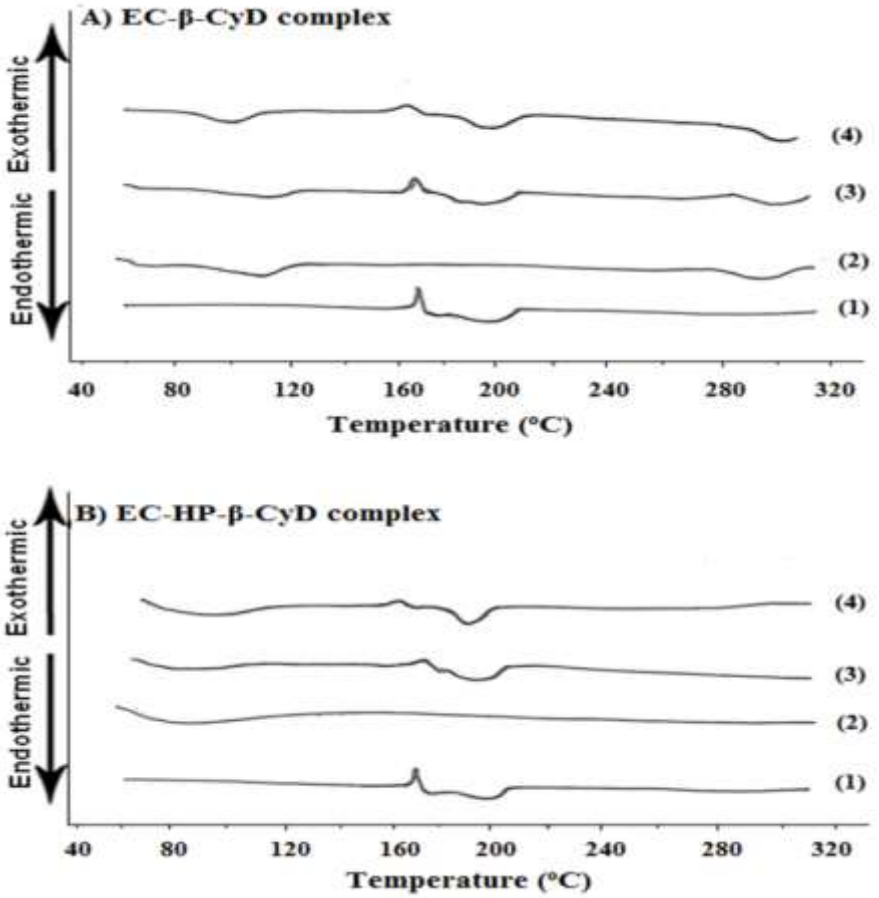

Figure 3: DSC thermograms of (1) EC, (2) β-CyD or HP-β-CyD, (3) Physical mixture, (4) Prepared complex.

The thermograms of physical mixtures of EC with β-CyD or HP-β-CyD were found to be the summation of those the drug and CyDs. In case of EC-β-CyD complex, shift in the exothermic and endothermic peaks of EC were observed, whereby the melting temperature of the drug was lowered to 162 and 194°C, respectively. While the thermogram of EC-HP-β-CyD complex, shift in the exothermic and endothermic peaks of EC were observed at 161 and 196 °C, respectively. Also, the intensity of both exothermic and endothermic peaks of the drug was decreased in both complexes. These results suggest that the increase in the dissolution rate of EC-CyDs complexes which could be correlated with the reduction in melting point of the drug[36].

Powder X-Ray Diffraction (PXRD) Studies

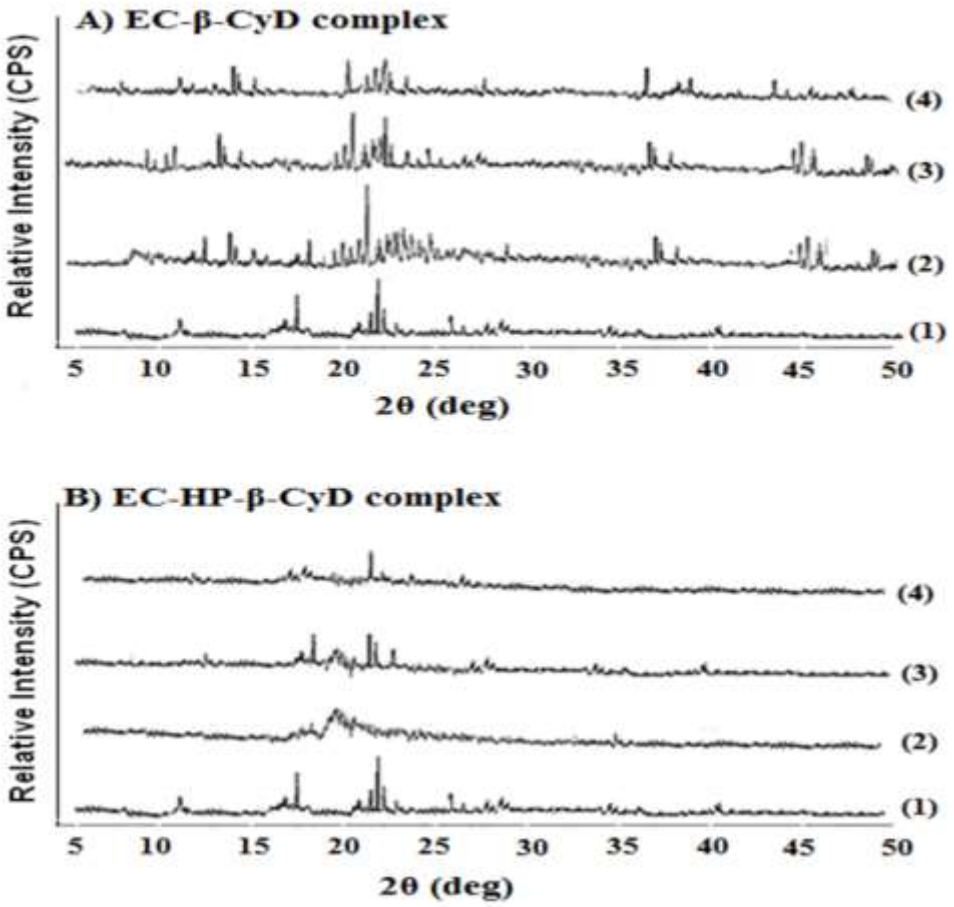

Figure 4: Powder X – ray diffraction patterns of (1) EC, (2) β-CyD or HP- β-CyD, (3) Physical mixture, (4) Prepared complex.

The X- ray diffraction patterns of EC, β-CyD, HP-β-CyD, their corresponding physical mixtures and inclusion complexes are illustrated in Fig. 4. The diffraction pattern of EC showed that the drug is crystalline in nature as demonstrated by numerous distinct peaks notably at 2θ angles 10.8°, 17.2°, 22.1°, 26.9° and 29.3°. Characteristic peaks of β-CyD appeared at 2θ equal to 14.3°, 20.8°, 36.8° and 38.6°. While the diffraction pattern of HP-β-CyD showed one broad peak at 2θ of 19.8°. The X- ray diffraction patterns of physical mixtures exhibited the identifiable peaks of EC, indicating no interaction occurring between the drug and CyDs. The X- ray diffraction patterns of EC-β-CyD complex showed diffuse peaks with low intensities, indicating that the crystallinity of the drug was remarkably reduced, leading to the formation of a new solid state due to inclusion complex formation between EC and β-CyD[37]. The X- ray diffraction patterns of EC-HP-β-CyD complex showed broad and diffuse peaks with low intensities, indicating an amorphous solid state was appeared due to inclusion complex formation between EC and HP-β-

CyD. Based on these findings, a decrease in the drug crystallinity with subsequent increase in the surface area of the drug exposed to the dissolution medium might be responsible for the improved dissolution of EC.

Physicochemical Evaluation

Drug Content in the Prepared Formulations

The drug content was determined for each formulation. It is obvious that, the percentage of EC content in all prepared formulations ranged from 96.76 ± 4.35 % to 101.3 ± 3.65 % which complies with pharmacopeal limits[38].

PH of the Formulations

The pH of eye tears is 7.4 and because of their natural buffering capacity, the eye can tolerate the ophthalmic formulations with wide pH range (3.5 –10.5). Because the ideal ophthalmic dose is only one drop, the tear film can be rapidly restored neutral pH[38]. Results showed that the pH values of the prepared formulations ranged from 6.1 ± 0.98 to 7.2 ± 1.04, which are suitable values that can be easily tolerated by the eye without any irritation or discomfort.

The Viscosity of the Eye Gels

The viscosity values of the prepared eye gels were measured and the values were ranged from 1019 ± 89 to 1435.6 ± 102 cP. From the obtained results, it was found that the higher viscosity of eye gels, led to increase their contact time with the eye surface and prevented the rapid drainage of the formulations from the eye which in turn improved their bioavailability[2].

***In-vitro* Release of Econazole Nitrate from the Formulations**

Figure 5 illustrates the release characteristics of EC and its complexes from different formulations. The obtained results revealed that, the dissolution of EC increased when complexed with CyDs which may be due to increase the drug solubility by using CyDs, as indicated by solubility study. The enhancement of EC release was more pronounced with HP-β-CyD preparations compared to corresponding preparations of β-CyD. This may be attributed to the lower crystallinity imparted by co-precipitation with HP-β-CyD rather than β-CyD possibly due to the slightly crystalline nature of the later as indicated by PXRD data. The nature of hydrophilic polymers affected the release of EC either complexed with β-CyD or HP-β-CyD from the prepared formulations. The release of EC from eye gels was in the following order; CP 940 > HPMC > MC. The higher release of EC from CP 940 gels compared to HPMC and MC gels may be due to the enhancing effect of triethanolamine on CyDs solubilizing power for the poorly water soluble drugs[39]. While, the release of EC from ocuserts was in the following order; HPMC + CP 940 > CP 940 > MC + CP 940. After 12 hrs, it was found that, the release of EC-

CyDs complex from different formulations was significantly ($p<0.05$) compared to its release from control. Additionally, the obtained results revealed that, the dosage form vehicles played an important role in controlling the drug release rate, as we found that the release rates from eye gels were higher than that from ocuserts. This may be due to the difference in their viscosities upon exposure to the release conditions, as the higher the viscosity the slower drug release rate[40]. Also, ocuserts absorbed the solvent and will form the swelled gel layer around it with a longer diffusional path. So, lead to decrease in the effective diffusion coefficient of the drug and therefore a reduction in the drug release percentage[41].

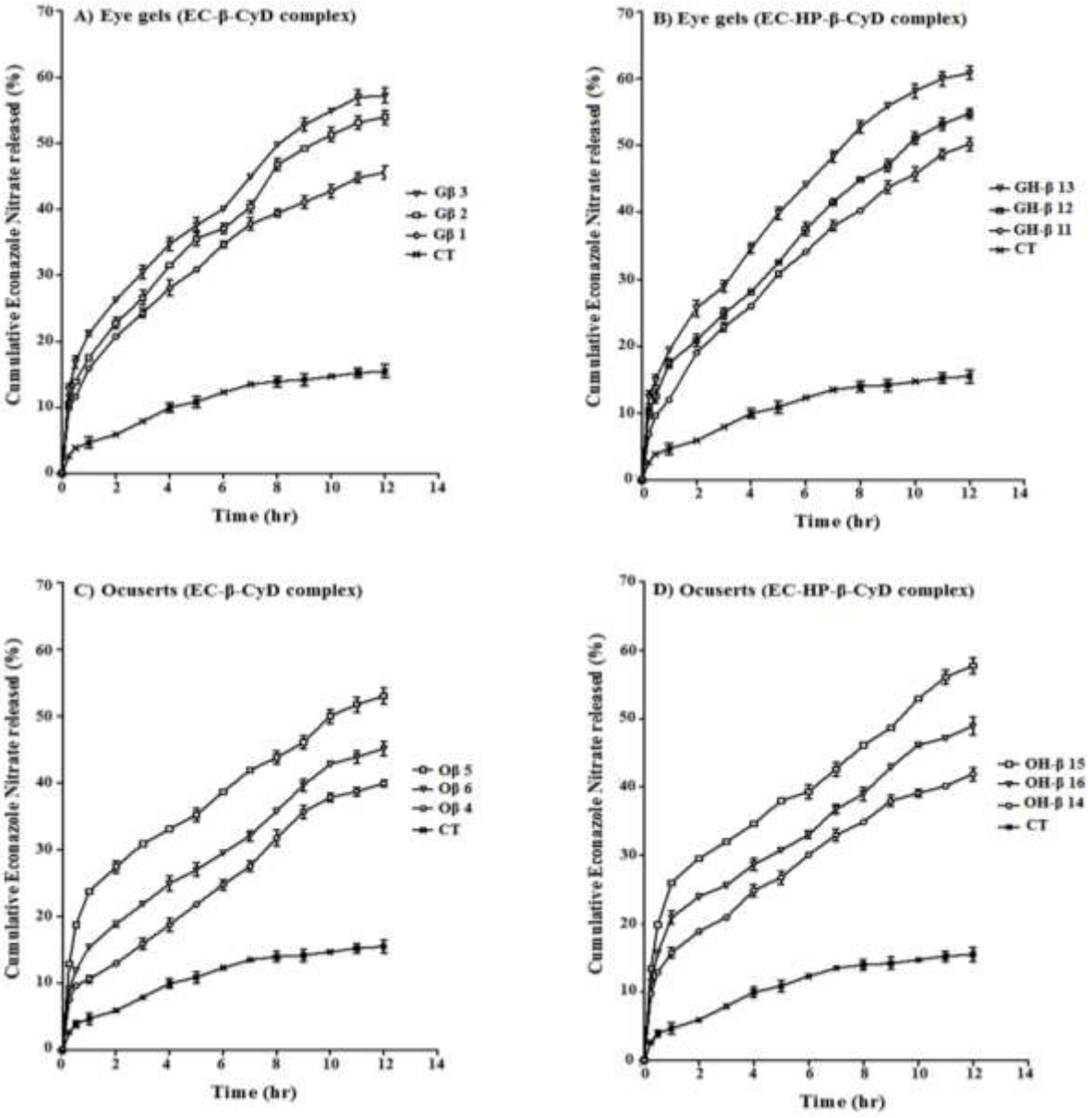

Figure 5: Dissolution profiles of EC-CyDs complex from different ophthalmic formulations in phosphate buffer (pH 7.4) at 37°C.

Kinetics of Drug Release

Table 3 summarizes the release kinetic parameters and correlation coefficients (r^2) calculated for the investigated formulations. The *in-vitro* release results showed that the release of EC-β-CyD and EC-HP-β-CyD complexs from different formulations is most fitted to diffusion- controlled mechanism (Higuchi model). This suggests that drug release is governed by a diffusion release mechanism. However, the control preparation followed the first-order kinetics that suggests dissolution of the drug from its complexes. Further analysis of the release data by the Korsmeyer–Peppas equation [25] showed that the release exponents (n) for eye gels were located between 0.45 and 0.89, which indicates that they exhibited a non-Fickian (anomalous diffusion) while (n) values for ocuserts and control were below 0.45, which suggests Fickian mechanism. These results indicate that no one model was able adequately to describe the release situation of these formulae. At least two mechanisms are present, in which one is more predominant than the other.

Table 3: Kinetic analysis of the release data of EC- CyDs complex from different formulations

Formulations		Correlation coefficient (r^2)			Korsmeyer – Peppas		Drug transport mechanism
		Zero	First	Higuchi	n	r^2	
Control	CT	0.9103	0.9207	0.8988	0.4312	0.9872	Fickian
		EC-β-CyD Complex					
Eye gels	Gβ 1	0.9149	0.9543	0.9957	0.4569	0.9948	Non-Fickian
	Gβ 2	0.9392	0.9758	0.9936	0.4513	0.9901	Non-Fickian
	Gβ 3	0.9234	0.9701	0.9913	0.4928	0.9901	Non-Fickian
Ocuserts	Oβ 4	0.9728	0.9832	0.9680	0.4490	0.9405	Fickian
	Oβ 5	0.8835	0.9429	0.9774	0.3385	0.9848	Fickian
	Oβ 6	0.9480	0.9737	0.9865	0.4108	0.9817	Fickian
		EC-HP-β-CyD Complex					
Eye gels	GH-β 11	0.9598	0.9870	0.9960	0.5271	0.9951	Non-Fickian
	GH-β 12	0.9573	0.9861	0.9906	0.449	0.9824	Non-Fickian
	GH-β 13	0.9397	0.9815	0.9942	0.4743	0.9866	Non-Fickian
Ocuserts	OH-β 14	0.9235	0.9576	0.9896	0.3750	0.9828	Fickian
	OH-β 15	0.8903	0.9487	0.9719	0.3389	0.9749	Fickian
	OH-β 16	0.9113	0.9539	0.9782	0.3520	0.9764	Fickian

Where; **(**n) is release exponent

Ocular Bioavailability of EC-CyDs from Selected Formulations

Table 4: Pharmacokinetic parameters of selected formulations containing EC-CyDs complexes

Parameters	Formulation				
	Gβ 3	GH-β 13	Oβ 5	OH-β 15	CT
A. Cornea					
C_{max} (ng/mg)	279.2±3.95*	296.9±4.87^{*,a}	233.97±2.34^{*,a,b}	237.9±2.29^{*,a,b}	75.97±2.7
T_{max} (hr)	3*	3*	3*	3*	1
K_e (hr^{-1})	0.74±0.04	0.71±0.03	0.78±0.06	0.75±0.04	0.82±0.11
$T_{1/2}$ (hr)	0.94±0.05	0.98±0.05	0.89±0.06	0.93±0.04	0.85±0.19
AUC_{0-7} (ng. hr /mg)	942.7±14.5*	1005±6.03^{*,a}	819.7±9.53^{*,a,b}	880.6±10.5^{*,a,b,c}	258.3±2.1
$AUC_{0-\alpha}$ (ng. hr /mg)	967±19.3*	1030±4.65^{*,a}	833.2±12.11^{*,a,b}	896.7±10.4^{*,a,b,c}	271.9±8.4
Rel. Bio.	3.56±0.05	3.79±0.11^{a}	3.06±0.11^{a,b}	3.29±0.08^{a,b}	----------
B. Conjunctiva					
C_{max} (ng/mg)	171.45±3.2*	183.91±3.5^{*,a}	117±2.7^{*,a,b}	127.56±3.4^{*,a,b,c}	57.26±3.3
T_{max} (hr)	3*	3*	3*	3*	1
K_e (hr^{-1})	0.65±0.05*	0.58±0.04*	0.47±0.03^{*,a}	0.56±0.03*	0.77±0.04
$T_{1/2}$ (hr)	1.07±0.09*	1.2±0.07*	1.47±0.1^{*,a,b}	1.24±0.08^{*,c}	0.89±0.05
AUC_{0-7} (ng. hr /mg)	504±13.69*	551±14.92^{*,a}	468.2±11.55^{*,a,b}	474±12.86^{*,b}	219.7±15.3
$AUC_{0-\alpha}$ (ng. hr /mg)	523.9±16.4*	582.8±19.39^{*,a}	506±18.83^{*,b}	498.7±18.34^{*,b}	226.7±16.8
Rel. Bio.	2.31±0.1	2.58±0.24	2.24±0.09	2.21±0.12	----------
C. Iris-Ciliary body					
C_{max} (ng/mg)	68.4±3.2*	47.85±2.6^{*,a}	55.23±3.1^{*,a,b}	59.61±1.85^{*,a,b}	15.37±2.1
T_{max} (hr)	3*	3*	3*	3*	1
K_e (hr^{-1})	0.51±0.05	0.44±0.05	0.44±0.05	0.43±0.05	0.54±0.14
$T_{1/2}$ (hr)	1.36±0.14*	1.59±0.18*	1.59±0.19*	1.62±0.19*	1.32±0.49
AUC_{0-7} (ng. hr /mg)	227.5±13.2*	167.9±8.05^{*,a}	218.1±4.31^{*,b}	247.7±9.89^{*,b,c}	74.17±6.12
$AUC_{0-\alpha}$ (ng. hr /mg)	245.3±12.3*	187.3±13.7^{*,a}	240.4±9.47^{*,b}	273.2±12.1^{*,b,c}	80.32±13.5
Rel. Bio.	3.18±0.77	2.42±0.48	3.08±0.45	3.54±0.81	----------
D. Aqueous Humor					
C_{max} (ng/mg)	29.16±2.57*	29.74±2.11*	33.83±2.79*	34.93±2.49*	15.25±2.7
T_{max} (hr)	3*	3*	3*	3*	1
K_e (hr^{-1})	0.41±0.03*	0.32±0.03^{*,a}	0.29±0.01^{*,a}	0.32±0.04^{*,a}	0.583±0.05
$T_{1/2}$ (hr)	1.69±0.11*	2.21±0.19^{*,a}	2.34±0.05^{*,a}	2.17±0.27^{*,a}	1.19±0.09
AUC_{0-7} (ng. hr /mg)	114.2±3.84*	120.2±7.76*	135.6±9.94^{*,a}	145.8±10.7^{*,a,b}	60.46±7.51
$AUC_{0-\alpha}$ (ng. hr /mg)	128±5.57*	147.2±14.34*	170±13.57^{*,a}	176±6.42^{*,a,b}	64.28±7.76
Rel. Bio.	2.0±0.16	2.3±0.11	2.69±0.57	2.76±0.34	----------

All values are expressed as means ± SD (n=3), C_{max} (the maximum concentration of drug in eye tissue); T_{max} (time required to reach the maximum eye tissue concentration); K_e (the elimination rate constant); $T_{1/2}$ (the biological half life); AUC_{0-7} (the area under eye tissue concentration time curve from 0-7 hrs) and $AUC_{0-\infty}$ (the area under eye tissue concentration time curve from 0-∞).

(*) considered significant compared to control (P< 0.05); (a) considered significant compared to Gβ 3 (P< 0.05); (b) considered significant compared to GH-β 13 (P< 0.05); (c) considered significant compared to Oβ 5 (P< 0.05).

The eye tissues and aqueous humor concentrations of EC after single application of selected formulations or control to rabbits were studied. The pharmacokinetic parameters of EC are illustrated in Table 4 and Figure 6. From the obtained results, it is obvious that, the selected formulations improved the bioavailability of EC in all eye tissues compared to the control. This improvement was indicated by the higher C_{max}, AUC_{0-7} and $AUC_{0-\infty}$ of the tested formulations than the control. Also, the tested formulations extended the duration of EC which indicated by the higher T_{max}, $T_{1/2}$ and the lower K_e of the tested formulations than the control. Regardless the formulations, from the obtained results it is clear that, EC bioavailability in eye tissues and aqueous humor are in the order of; cornea > conjunctiva > iris-ciliary body > aqueous humor which are indicated by the values of C_{max}, AUC_{0-7}, $AUC_{0-\infty}$ and the relative bioavailability. The higher EC bioavailability in cornea and conjunctiva may be attributed to the direct contact of these tissues with the tear pool which house the drug. These results are in agreement with those obtained by Yamaguchi *et al*[42], who reported that, the higher difluprednate concentration in cornea than in aqueous humor.

The obtained results revealed that, the higher C_{max} reached up to 296.9±4.9, 183.9±3.5, 68.4±3.2 and 34.93±2.49 ng/mg in cornea, conjunctiva, iris-ciliary body and aqueous humor for GH-β13, GH-β13, Gβ 3 and OH-β 15, respectively. In additions, the maximum AUC_{0-7} values are 1005±6.1, 551±14.9, 247.7±9.9 and 145.8±10.7 ng.hr/mg in cornea, conjunctiva, iris-ciliary body and aqueous humor for GH-β 13, GH-β 13, OH-β 15 and OH-β 15, respectively. Regarding T_{max} values, the tested formulations gave extended T_{max} values which reached up to 3 hrs for all formulations in different eye tissues. Thus, the results revealed that there was about 3 fold higher in the time required to reach the maximum eye tissue concentration (T_{max}) with all formulations than control. It is worth noting that, the C_{max}, T_{max}, AUC_{0-7} and $AUC_{0-\infty}$ of all formulations were significantly (p< 0.05) superior to that of control in all eye tissues and aqueous humor. The elimination half-life ($T_{1/2}$) of EC with all formulations was more than control indicating that the drug was getting eliminated from the eye slowly, which in turn was supported by low elimination rate constant values (K_e) of EC-CyDs in all formulations in comparison with control. EC-CyDs in all formulations showed a high AUC value indicating the greater extent of drug absorption from the inclusion complex. Thus, the higher T_{max}, $T_{1/2}$ and AUC values together indicated the improved bioavailability of EC from the inclusion complex in comparison with control. This

could be due to improved solubility and dissolution rate of the drug by the formation of inclusion complex with CyDs.. The higher bioavailability of EC from eye gels and ocuserts that contain CP 940 can be explained by the enhancing effect of triethanolamine on CyDs solubilizing power for the poorly water soluble drugs by forming drug-CyD-TEA multicomponent system improving the ocular delivery of the drug[39]. Also, the enhanced bioavailability of the eye gels and ocuserts containing EC-CyDs complexes may be due to the high viscosity and bioadhesiveness properties of the polymers. These properties prevented the rapid drainage of the formulations from the eye and so increased their contact time with the eye surface which in turn improved their bioavailability[2]. These results are in agreement with those obtained by Budai *et al* [24], who reported that the high viscosity and the bioadhesive properties of the gel formulations improved the ocular bioavailability of ciprofloxacin.

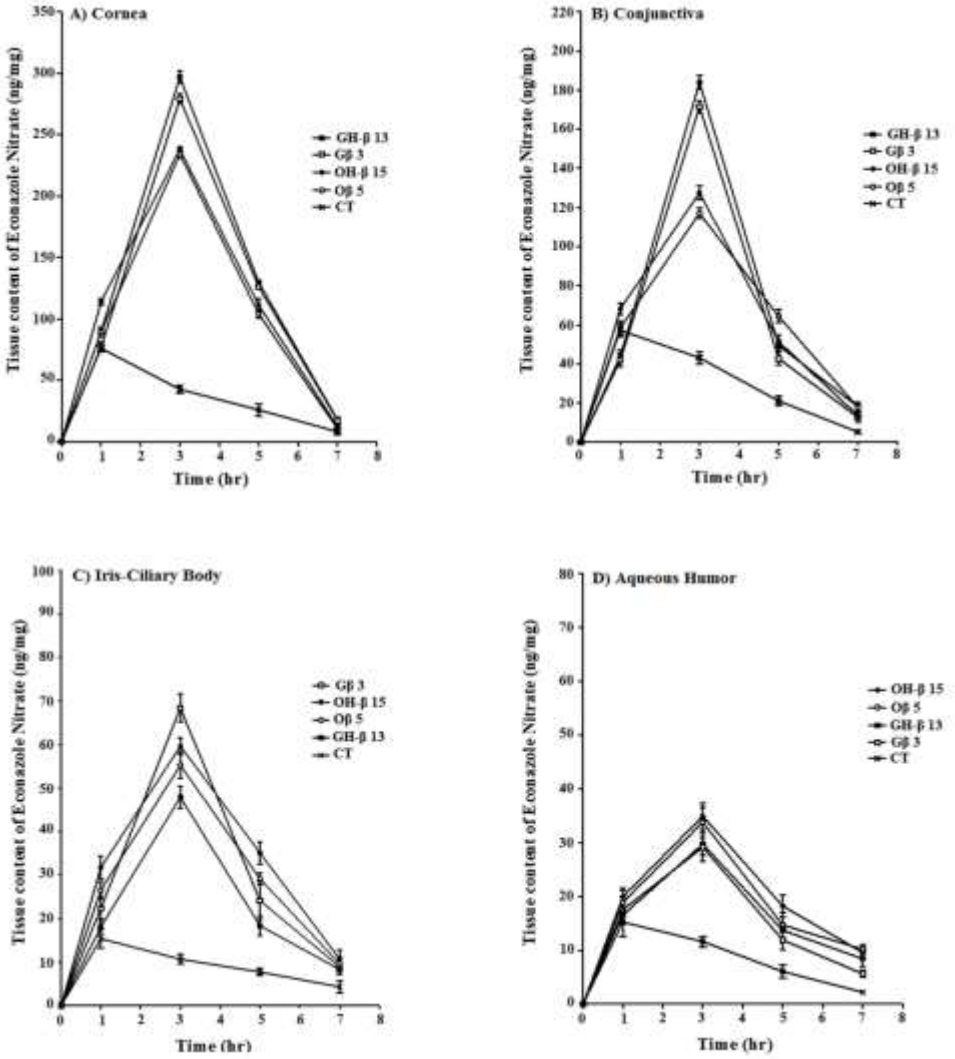

Figure 6: The eye tissue concentration-time profiles of econazole nitrate following topical application of different ophthalmic formulations.

CONCLUSION

This study demonstrated that, the solubility of EC was improved by the formation of inclusion complexes with β-CyD and HP-β-CyD using co-precipitation technique. The formed complexes responsible for a decrease in the drug crystallinity with subsequent increase in the surface area of the drug exposed to the dissolution medium which might be responsible for the improved dissolution and ocular bioavailability of EC. Moreover, HP-β-CyD has a higher solubilizing effect for EC than β-CyD. The drug release from different formulations is varied with the polymer types and dosage form, and found in order of eye gels > ocuserts. The ocular bioavailability of EC was improved by the complexation of the drug with HP-β-CyD more than β-CyD. Eye gels and ocuserts containing EC-CyDs have a higher bioavailability and more extended duration than control. On the basis of these results, the complexation of EC with CyDs provides a promising mean for enhancement the dissolution rate and ocular bioavailability of EC.

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