AAPS PharmSciTech (© 2016)
DOI: 10.1208/s12249-016-0609-9

Research Article

Improvement of the Ocular Bioavailability of Econazole Nitrate upon Complexation with Cyclodextrins

Abd El-Gawad Helmy Abd El-Gawad,[1,2] Osama A. Soliman,[1] Marwa S. El-Dahan,[1] and Saeed A. S. Al-Zuhairy[1]

Received 16 May 2016; accepted 1 August 2016

Abstract. Econazole nitrate (EC) is an active, imidazole antifungal agent. However, low aqueous solubility and dissolution rate of EC has discouraged its usage for the treatment of ophthalmic fungal infection. In this study, inclusion complexes of EC with cyclodextrins were prepared to enhance its solubility, dissolution, and ocular bioavailability. To achieve this goal, EC was complexed with β-CyD/HP-β-CyD using kneading, co-precipitation, and freeze-drying techniques. Phase-solubility studies were performed to investigate the complexes in the liquid form. Additionally, the complexes in the solid form were characterized with Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), and transmission electron microscopy (TEM). Furthermore, different eye drops containing EC-CyD complexes were prepared using different polymers and then characterized regarding their drug contents, pH, viscosity, mucoadhesive strength, and *in vitro* release characteristics. The results showed that stable EC-CyD complexes were formed in 1:1 molar ratio as designated by B_S-type diagram. Econazole nitrate water solubility was significantly increased in about three- and fourfold for β-CyD and HP-β-CyD, respectively. The results showed that the prepared complexes were spherical in shape having an average particle diameter from 110 to 288.33 nm with entrapment efficiency ranging from 64.24 to 95.27%. DSC investigations showed the formation of real inclusion complexes obtained with co-precipitation technique. From the *in vitro* studies, all eye drops containing co-precipitate complexes exhibited higher release rate than that of other complexes and followed the diffusion-controlled mechanism. *In vivo* study proved that eye drops containing EC-CyD complexes showed higher ocular bioavailability than EC alone which indicated by higher AUC, C_{max}, and relative bioavailability values.

KEY WORDS: cyclodextrins; econazole nitrate; inclusion complexes; ocular bioavailability.

INTRODUCTION

Ophthalmic drug delivery is one of the greatest interesting endeavors facing the pharmaceutical scientist. Topical application of medications is the most advantageous route for ocular drug delivery to treat eye diseases affecting the anterior segment because it avoids systemic absorption and serves to extend the drug effect in target tissues [1]. Moreover, eye drops are a very well tolerated means of ocular therapy for various eye disorders [2].

Econazole nitrate is halogenated aromatic compound structurally related to miconazole that has antifungal properties [3]. It is an antifungal agent having topical efficacy against several mycotic infections in mucous membranes, the skin, and the hair [4, 5]. The inhibitory and bactericidal properties of many azole antifungal compounds (including EC) against mycobacterium smegmatis has been examined [6]. The lower bioavailability of EC might be owing to poor aqueous solubility which slower its dissolution rate. In order to improve the dissolution characteristics of EC and enhance its solubility, complexation with oligosaccharide derivatives cyclodextrins (CyD) appears to be one of the most effective methods [5].

Cyclodextrins are cyclic oligosaccharides consisted of seven units of α-D-glucopyranose that form a rigid cone-shaped arrangement. It have a hydrophilic outer surface and hydrophobic inner cavity that function as molecular cages to entrap a variety of hydrophobic drugs of appropriate sizes and shapes, thus leading to formation of inclusion complexes through non-covalent bonds [7, 8]. Thus, they increase the aqueous solubility of hydrophobic compounds without changing their molecular structure [9, 10]. Cyclodextrins are prevalent for their ability to form inclusion complex and have been broadly utilized to improve the aqueous solubility, stability, and dissolution characteristics of poorly aqueous-

[1] Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, 35516, Mansoura, Egypt.
[2] To whom correspondence should be addressed. (e-mail: abdelgawadhelmy64@gmail.com;)

Published online: 09 November 2016

soluble drugs [11]. β-Cyclodextrins (β-CyDs) are most commonly used because of lower toxicities than other CyDs, and their internal cavity is suitable for a variety of guest molecules. However, with no substituent on the glucopyranose unit, β-CyD has low water solubility and is parenterally unsafe due to its nephrotoxicity [12]. Recently, hydroxypropyl-β-cyclodextrin (HP-β-CyD), synthetically modified β-CyDs, has been made to progress the physicochemical properties and inclusion capacity of parent CyDs. They can keeping hydrophobic molecules in solution form and transport them through biomembranes.

Some approaches suggest that CyDs improve drug permeability through biological membranes such as the eye cornea by disrupting the membrane, either *via* permeating into the membrane or *via* complexing with certain hydrophobic components like phospholipids and cholesterol from the membrane [13]. But, the correct mechanism is when CyD acts as a carrier by keeping the lipophilic drug molecules in solution and delivers them through the mucin layer to the ocular barrier (cornea or conjunctiva) where they pass into the barrier without disturbing the function of the barrier. Furthermore, CyD complexation is able to improve drug stability in aqueous solutions and also decrease drug irritation to the eye after topical administration [10]. Regarding ophthalmic formulations, ideal bioavailability of the active constituent is attained in appropriate concentration of CyDs (<15%) in eye drops [14]. Previous researches showed that both dissolution characteristics and, consequently, microbiological activity of EC could be enhanced through complexation with natural CyDs, particularly with β-CyD [15].

The objective of this study was to prepare and characterize EC inclusion complexes with β-CyD or HP-β-CyD by kneading, co-precipitation, and freeze-drying techniques. Both the complex preparation method and the nature of the particular CyD play important roles in the performance of a drug–CyD formulation application [16]. The complexation of EC with β-CyD/HP-β-CyD was characterized in the liquid form through phase-solubility diagrams study. Solid-state complexation was investigated with Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and transmission electron microscopy (TEM). The resulting inclusion complexes were evaluated for their entrapment efficiency, yield, drug loading, and particle size. Formulation of these complexes in ophthalmic drops using different polymers was of prime interest. Moreover, all the formulations were investigated for their physical properties and *in vitro* release characteristics. In addition, the pharmacokinetic parameters and the ocular bioavailability of EC from the tested formulations based on acceptable physical characteristics and *in vitro* release profiles were studied.

MATERIALS AND METHODS

Materials

Econazole nitrate (EC), ibuprofen (IB), β-cyclodextrin (β-CyD) (M_w=1135.12), hydroxypropyl-β-cyclodextrin (HP-β-CyD) (M_w=1375.35), and hydroxypropyl-methylcellulose (HPMC) were purchased by Provizer Pharma, India. Carbopol 940 (CP 940), methylcellulose (MC), and triethanolamine (TEA) were provided by LoBa Chemie, India. Spectra/Pore® dialysis membrane (12000–14000 M_w cutoff) was provided from Spectrum Laboratories Inc., Rancho Dominguez, Canada. The HPLC grade solvents (acetonitrile and 1-octanesulfonic acid sodium salt) were provided from Fisher scientific, UK. All other chemicals were of analytical grade; freshly double-distilled water was utilized during the study.

Phase-Solubility Study

The phase-solubility study was conducted in water as stated by Higuchi and Connors [17]. Econazole nitrate in excess amount (10 mg) was added to 5 mL of water containing increasing concentrations of CyDs (β-CyD/HP-β-CyD) (0.25×10^{-3}–2×10^{-3}M/L) in screw-capped glass bottles. Samples were shaken at 37°C±0.5°C in a thermostatically controlled water bath (Grant instrument Cambridge Ltd., England) at 50 rpm for 3 days. After equilibrium was attained, the contents were filtered through millipore membrane filter (0.45-μm pore size and 47-mm diameter, Gelman GN-6 Metricel membrane filter, USA) and assayed spectrophotometrically at 231 nm against blank prepared using the same concentrations of CyDs using an UV/VIS spectrophotometer (V-550, Jasco, Japan). Each experiment was done in three runs. The apparent stability constants of the complexes (Ks) were estimated from the slope of linear portion of the phase-solubility diagram according to Eq. (1):

$$Ks = Slope/S_o(1-slope) \quad (1)$$

where S_o is EC water solubility.

Formulation of Solid Econazole Nitrate-Cyclodextrin Complexes

As described in our published method, 1:1 molar ratio inclusion complexes of EC with CyDs (β-CyD/HP-β-CyD) were prepared by the kneading, co-precipitation, and freeze-drying methods [18]. Physical mixtures (PMs) were prepared for comparative study, where EC and different CyDs were mixed in a mortar for 20 min and used directly for evaluation study. The three different methods utilized for solid complexes preparation are described as follows:

Kneading Method (Kn)

A weighed quantity of EC and CyDs (β-CyD/HP-β-CyD) was mixed and kneaded in a mortar with an adequate amount (5 mL) of ethanol-water mixture (1:1) for 45 min and kept overnight in a dark place. The resulted masses were dried under reduced pressure then sieved and stored in desiccators, until use [19].

Co-precipitation Method

Econazole nitrate inclusion complexes with CyDs (β-CyD/HP-β-CyD) were obtained by the co-precipitation

method (Co) [20]. A weighed quantity of EC (500 mg) were dissolved in 5 mL of acetone and then added dropwise to CyDs aqueous solution (weighed quantity of β-CyD/HP-β-CyD in 10 mL water). The mixture was continuously stirred for 6 h, and then, a rotary evaporator was used to remove the solvent. The obtained product was dried at 45–50°C for 2 days and stored in airtight vessels for additional measurements.

Freeze-Drying Method (FD)

A 1:1 molar ratio inclusion complex of EC and β-CyD/HP-β-CyD were dissolved in methanol and water, respectively. The resulting solutions were mixed for 24 h at 25°C using a magnetic stirrer. The final product was frozen at −70°C, and subsequently freeze-dried at −50°C for 48 h using a freeze-dryer (SIM, FD8-8 T controller, SIM international, USA). Finally, the lyophilized powder was sieved and kept in a sealed glass vial.

Characterization of Inclusion Complexes

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared spectroscopy (FT-IR) spectra of EC, β-CyD, HP-β-CyD, PMs, and its complexes were recorded using FT-IR (Thermo Fisher Scientific, Inc., USA). Each sample was mixed with potassium bromide (KBr) and pulverized into fine powder then pressed into KBr disks. Each KBr disk was scanned over a wavenumber region of 500–4000 cm^{-1} at ambient temperature and the resolution was 4 cm^{-1}.

Differential Scanning Calorimetry

The thermal behavior of EC, β-CyD, HP-β-CyD, PMs, and its complexes were analyzed using differential scanning calorimetry (DSC) (Perkin Elmer DSC7, USA). The grinded sample (5 mg) was sealed using aluminum pans and scanned at 10°C/min rate between 50 and 320°C under a stream of nitrogen.

Powder X-ray Diffraction

The powder X-ray diffraction (PXRD) patterns of EC, β-CyD, HP-β-CyD, PMs and the prepared complexes were recorded using X-ray diffractometer (Rigaku Denki, Rint-2500 VL, Tokyo, Japan). The samples were conducted using Cu-kα radiation at scanning rate 1°/min and analyzed between ranges of 5°–30° (2θ). The current and voltage used were 40 mA and 40 kV, respectively.

Drug Loading Assessment

To determine the actual amount of EC incorporated into inclusion complexes, a weighed quantity of each inclusion complex (0.1 g) was dissolved in 25 mL phosphate buffer pH 7.4. The obtained solution was assayed spectrophotometrically at 231 nm. The experiment was repeated thrice. The entrapment efficiency (%EE), the yield percent (%Y), and drug loading percent (%DL) were calculated according to Eqs. (2–4), respectively [21].

$$\%EE = \frac{m1}{m2} \times 100 \tag{2}$$

$$\%Y = \frac{m4}{m2 + m3} \times 100 \tag{3}$$

$$\%DL = \frac{m1}{m4} \times 100 \tag{4}$$

where m1 is the weight of EC entrapped within the inclusion complex, m2 is the weight of EC used in the preparation of inclusion complex, m3 is the weight of CyD used in the preparation of inclusion complex, and m4 is the weight of inclusion complex obtained finally.

Measurement of Particle Size

The particle size of PMs and inclusion complexes were determined using transmission electron microscopy (TEM) (JEOL-JTEM, 2100 CX, Japan). Each sample was dispersed in deionized water, and then, a drop of the diluted dispersion was applied to a carbon-coated 300 mesh copper grid covered with Formvar film. After complete drying, the particle size measured by TEM.

Preparation of Eye Drops

Econazole nitrate (0.2%, *w/v*) or its equivalent weights of EC-β-CyD/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 (Table I). Methyl- and propylparabens were used as preservatives [22]. In case of CP 940, 5 μL of TEA was added to enhance viscosity since CP 940 (polyacrylic acid) undergoes a sol–gel transition in aqueous solution at pH above its pKa 5.5 [23].

Physicochemical Characterization of Eye Drops

Determination of the Drug Content

From each formulation, 1 g was accurately weighed and dissolved in 100 mL phosphate buffer pH7.4 and shaken for 30 min in thermostatically controlled water bath at 37±0.5°C. Then, the solution was filtered using 0.45-μm membrane filters and assayed at 231 nm.

Determination of the Formulations pH

From each formulation, 1 g was disseminated in 20 mL of distilled water, and then, the pH was measured using a pH meter (Beckman Instruments Fullerton, Germany).

Table I. Composition of Eye Drops Containing EC-CyDs Complexes

Type of complex	Formulation code	Polymer % (*w/v*)		
		MC	HPMC	CP 940
Control	CT			
EC-β-CyD_R	Dβ 1	0.2		
EC-HP-β-CyD_R	DH-β 11	0.2		
EC-β-CyD_R	Dβ 2		0.3	
EC-HP-β-CyD_R	DH-β 12		0.3	
EC-β-CyD_R	Dβ 3			0.1
EC-HP-β-CyD_R	DH-β 13			0.1

All formulae contain 0.2%*w/v* EC or its equivalent weights of complexes, 20% propylene glycol, 0.05%*w/v* methylparaben, and 0.01%*w/v* propylparaben. R is (K) kneading method, (Co) co-precipitation method, or (FD) freeze-drying method
MC methylcellulose, *HPMC* hydroxypropyl-methylcellulose, *CP 940* Carbopol 940, *-β-CyD* β-cyclodextrin, *HP-β-CyD* hydroxypropyl-β-cyclodextrin, *EC* econazole nitrate

Determination of the Formulations Viscosity

The viscosity of eye drops was measured using a cone and plate rotary viscometer (Haake Inc., Germany). From each formulation, 1 g was placed on the stationary plate and allowed to equilibrate for 5 min to attain the running temperature. The rotary viscometer was thermostatically controlled at 37°C±0.5°C [24]. Then, the viscosity values were calculated according to Eq. (5):

$$\eta = \mathrm{G.S/N} \tag{5}$$

where η is the viscosity in mPa.s (mPa.s=1 centipoise, cP), G is the instrumental factor=14,200 (mPa.s/scalagrad.min), S is the torque (scale grad), and N is the speed (rpm).

Determination of In Vitro Mucoadhesive Strength

The mucoadhesive strength of the ophthalmic formulations was measured using a modified two-arm balance [25]. One metal holder was used to suspend the water-collecting beaker to the balance and another holder to suspend a glass vial to the other side of the balance as shown in Fig. 1. A corneal tissue of the rabbit was separated and washed with physiological saline. The mucoadhesive strength of the formulations was determined as follows: the corneal tissues were fixed with mucosal side out onto each glass vials using rubber band. The first vial with membrane was connected to the balance in an inverted position while the second vial was placed on a height-adjustable pan. The balance was made balanced. Thirty microliters of the formulation was added onto the second vial. Then the height of the second vial was so adjusted that surface of both vials come in intimate contact. A preload of 5 g was placed over vials for 2 min (preload time) to establish adhesion bonding between sample and rabbit corneal tissue. The preload weight and preload time were kept constant for all formulations. After completion of the preload time, preload weight was removed, and water was then added into the beaker from the burette with a constant flow rate. The addition of water was stopped when sample was detached from corneal tissues. Mucoadhesive strength was determined from the minimal weights of water that detached the sample from corneal tissues. The rabbit corneal tissues were changed for each measurement. All measurements were performed in triplicate (n=3).

In Vitro Dissolution Study

The dissolution experiment was performed according to the method adapted by Levy and Benita [26], using the dialysis method. Spectra/Pore® dialysis membrane was soaked in phosphate buffer overnight before the experiment. The membrane was spread over the open-end glass tube (3 cm diameter) and was wrapped with a rubber band. Two grams of each formulation were accurately weighed and thoroughly spread on the membrane. To each tube, 1.5 mL of the buffer solution was added. Then, the tube was immersed upside-down in a vessel containing 30 mL phosphate buffer pH 7.4 which is maintained at 37°C±0.5°C using thermostatically controlled water bath shaker (50 rpm). The tube height was adjusted, so that the dialysis membrane was just below the release medium surface. The samples of dissolution medium were taken at time intervals up to 12 h and replaced by fresh dissolution medium. Each sample was diluted, filtered using millipore filter, and assayed spectrophotometrically at 231 nm.

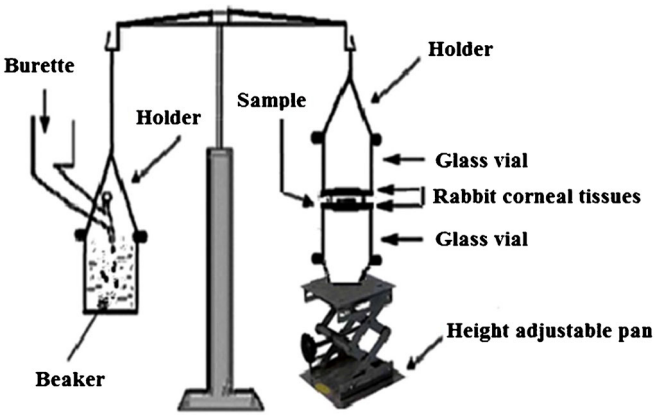

Fig. 1. Mucoadhesive strength measurement device

Kinetics of Release Data

The different release kinetics is supposed to reveal various release mechanisms. The release data were fitted to three kinetic models: zero-order, first-order, and Higuchi model [27], and the kinetic modeling of drug release was determined. The Korsmeyer–Peppas model was used for more analysis, where the value of the release exponent (*n*) is governed by the release mechanism, so could be used to describe it [28].

EC-CyD Ocular Bioavailability

For this study, two formulations were selected in comparison with the control (drug suspension in water). The two selected formulae are Dβ 3 and DH-β 13 (Table I) based on their acceptable physical and *in vitro* release characteristics.

Ocular Bioavailability Studies

Ocular bioavailability of the selected formulations was achieved using male New Zealand albino rabbits (each 2–2.5 kg). All rabbits were healthy with no clinical observable abnormalities. Animals were retained individually in cages, in a light-controlled room (12-h light and 12-h dark cycles) at 20–24°C, with no restriction to water or food. The animal experimental procedures conform to the ethical principles of the scientific committee of the Pharmacy Faculty, Mansoura University, Egypt (code number, 2016–9). Animals were divided into three groups, each of 12. Each animal was received 30 μL of eye drops which instilled into the center of the lower lid (cul-de-sac) of the right animal eyes, while the left eyes were served as control by application of the plain formulation. The lower eyelid was carefully 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 h. 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°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 using 4 mL acetonitrile for 24 h at 25°C to extract the drug from different eye tissues and aqueous humor. These solutions were filtered using 0.45-μm nylon membrane filter. The tissue extracts were spiked with 20 μL of ibuprofen (IB) as an internal standard (50 μg/mL). Each mixture was mixed using vortex mixer (Snijders Scientific Tilburg-Holland) for 1 min then filtered using 0.45 μm nylon membrane filter, and then, 20 μL of the solution was injected into HPLC system. EC concentration in each tissue was measured by HPLC assay as reported by Medendrop *et al.* [29] with slight modification. 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-18column, 5 μm, 4.6×250 mm, phenomenex, USA). The mobile phase system, consisting of 70% acetonitrile and 25 mM potassium dihydrogen orthophosphate with 0.65gm/L of 1-octanesulfonic acid sodium salt 30%, was filtered under vacuum through a 0.45-μm nylon membrane filter was pumped at a flow rate 1.2 mL/min. The UV detector was adjusted at 213 nm. The retention time of IB and EC was 4.6 and 5.7 min, respectively. The concentration of EC was expressed as nanograms of drug/milligram of tissue.

Pharmacokinetic Parameters

The pharmacokinetic parameters were calculated for each rabbit according to the Cheruvu *et al.* [30] method. The maximum drug concentration in the eye tissues (C_{max}) and the time at which it was attained (T_{max}) were determined directly from the eye tissue concentration-time curves. Also, the elimination rate constant (K_e) was estimated from the terminal linear portion of eye tissue concentration-time profile. The elimination half-life ($T_{1/2}$) was estimated at $0.693/K_e$. Additionally, area under eye tissue concentration-time curve from 0 to 7 h ($AUC_{0–7}$) was estimated by using trapezoidal rule. The AUC was extrapolated to infinity ($AUC_{0-\infty}$) and calculated according to Eq. (6):

$$AUC_{0-\alpha} = AUC_{0-7} + C_{last}/K_e \quad (6)$$

where C_{last} is the last measurable concentration of the drug after 7 h.

The relative bioavailability of EC was determined as the ratio between $AUC_{0-\infty}$ of the tested formulation to that of control.

Statistical Analysis

The resulting data of *in vitro* dissolution study are presented as mean±SD, while all results of ocular bioavailability studies are presented as mean±SEM. Multiple groups comparisons was conducted using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test, and pairs of groups were compared by performing one-tailed student's *t* test at $p<0.05$ with Instate Graphpad prism software (version 5.00; Graphpad software, San Diego, CA, USA) [31].

RESULTS AND DISCUSSION

Phase-Solubility Study

Figure 2 shows the phase-solubility diagrams of EC and CyDs. The phase-solubility diagram of EC-CyDs resulted in B_S-type Higuchi phase-solubility diagrams, where the initial rising portions are followed by plateau regions and then the total EC concentration decreased which may be due to the precipitation of microcrystalline complexes. EC aqueous solubility was initially

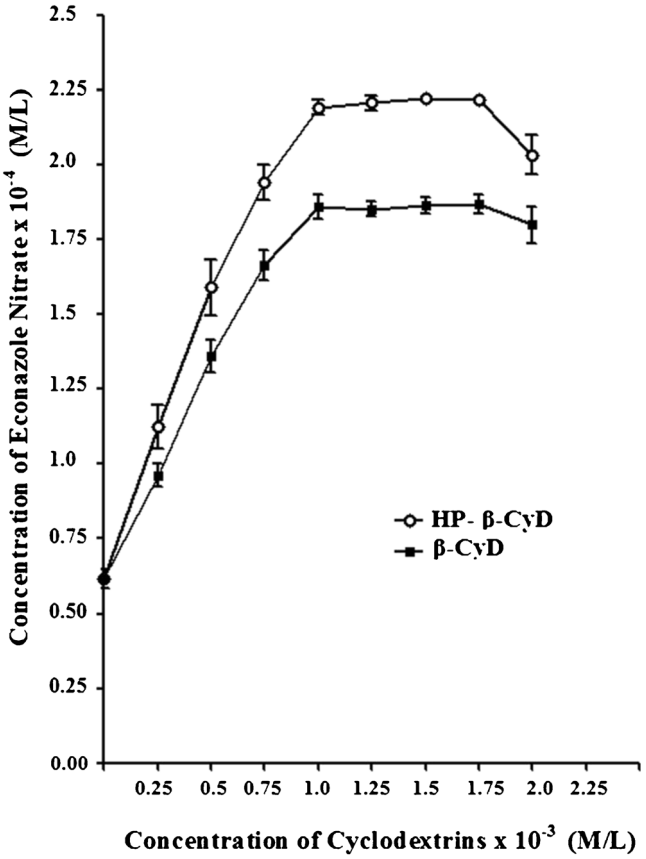

Fig. 2. Phase-solubility diagram of EC in the presence of β-CyD and HP-β-CyD

increased linearly as a function of the concentration of β-CyD/HP-β-CyD with a slope <1 showing that the increase in the solubility was because of 1:1 M complex formation. The stability constant (Ks) of 1:1 complex can be calculated from the slop and intercept of the initial straight line portion of the solubility curve (Table II). The value of Ks was greater with HP-β-CyD (811.38 M^{-1}) than that of β-CyD (597.95 M^{-1}) at $p<0.05$. These values of Ks, ranged between 200 and 5000 M^{-1}, indicate that complexes formed are quite stable and that they seem suitable for enhancement the solubility, dissolution rate, and stability of low aqueous solubility drugs [32]. It was proposed that the spatial correlation between host and guest molecules that responsible for their interaction [7, 17, 33]. The highest Ks value exhibited by HP-β-CyD could be ascribed to the presence of 2-hydroxypropylated substituents on the CyD molecule [34].

In our study, EC solubility in the presence of HP-β-CyD increased more than in the presence of β-CyD may be attributable to the larger cavity size of HP-β-CyD [35], which is optimal for entrapment of the drug molecules, thus providing a greater solubilization effect compared to β-CyD. The performance difference of CyDs can be related to Ks values, which is an empirical factor that describes increasing in solubility of the drug in the existence of CyDs [17, 36]. Collectively, EC water solubility was significantly increased in about three- and fourfold for β-CyD and HP-β-CyD, respectively with 1×10^{-3}M/L.

Characterization of Inclusion Complexes

Econazole nitrate inclusion complexes with CyDs were prepared and characterized in the solid state; FT-IR, DSC, PXRD, and TEM definite the existence of EC-CyD complex in the solid state.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FT-IR) spectroscopy was performed for assessing the interaction between EC and CyD in solid form. Figure 3 illustrates the FT-IR spectra of EC, β-CyD, HP-β-CyD, inclusion complexes, and their PMs. The FT-IR spectrum of EC shows evident several characteristics bands at ~3430 cm^{-1} (N-H stretching), ~3174, and 3107 cm^{-1} (two bands for aromatic C-H stretching), ~1586 cm^{-1} (aromatic C=C stretching), ~1448 cm^{-1} (C=N stretching), ~1087 cm^{-1} (C-O-C stretching), and ~860 cm^{-1} (aromatic C-CL stretching). 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 C-H and CH_2 groups appear at 2800–3000 cm^{-1} regions, whereas a broad band appeared at 1634 cm^{-1} due to adsorbed water. Similar observations were observed and explained previously [5]. While that of HP-β-CyD shows prominent absorption bands at 3438 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 [37].

The FT-IR spectra of both PMs were found to be the summation of the IR spectra of the drug and CyDs. The 3430 cm^{-1} bands of N-H stretching for EC were shifted at 3350 cm^{-1}. The 3174 and 3107 cm^{-1} bands of aromatic C-H stretching were disappeared. In addition, the intensity of aromatic C=C stretching band at 1586 cm^{-1} was reduced with β-CyD and disappeared with HP-β-CyD. These changes suggest that the drug were partially entrapped in CyD cavities during physical mixing. On contrast, the spectrum of the inclusion complexes exhibits relevant changes in widths and intensities of the characteristic absorption bands, revealing formation of new drug–CyD chemical bond, especially with the co-precipitation method [38]. In the FT-IR spectra of both co-precipitate complexes, aromatic C-H, C=C, and C-CL

Table II. Parameters of the Phase-Solubility Study

Type of cyclodextrins	So (M/L)	S/So	Type of phase-solubility curve	Ks (M^{-1})
β-CyD	$0.617\times10^{-4}\pm0.031$	576.64±21.94	B_S-type	597.95±23.87
HP-β-CyD	$0.617\times10^{-4}\pm0.031$	772.61±84.43	B_S-type	811.38±91.29

So EC water solubility

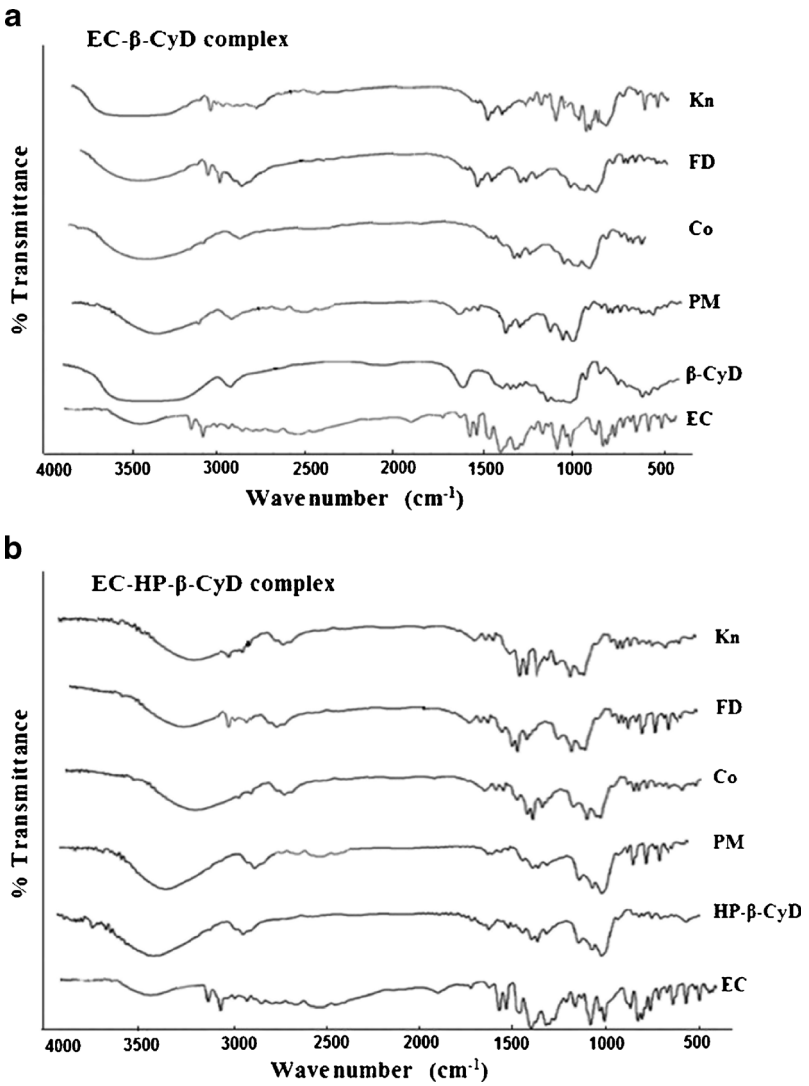

Fig. 3. The Fourier transform infrared (FT-IR) spectra of EC, β-CyD or HP-β-CyD, physical mixtures (PM), co-precipitate (Co), freeze-dried (FD), and kneaded complexes (Kn)

stretching vibration bands were disappeared. Also, in the FT-IR spectra of EC-HP-β-CyD co-precipitate complex, O-H stretching vibration bands of HP-β-CyD were shifted to 3119 and 2645 cm^{-1}. The intensity of C-O-C stretching band at 1087 cm^{-1} was reduced for both co-precipitate complexes. EC-β-CyD and EC-HP-β-CyD co-precipitate complexes show no peaks similar to the drug alone. This is may be due to the formation of real inclusion complex between EC and CyDs.

Differential Scanning Calorimetry

The thermal analysis was an important method to recognize and characterize CyDs complexes. The melting, boiling, and sublimating points of guest molecule generally shifted to different temperatures or disappeared when it was embedded into the CyDs cavities [39]. The thermal behavior of EC-β-CyD and EC-HP-β-CyD complexes was conducted using differential scanning calorimetry (DSC) to confirm the solid complex formation. DSC thermograms of EC, β-CyD, HP-β-CyD, PMs, and its complexes are illustrated in Fig. 4. The thermogram of pure EC revealed a sharp endothermic peak at 165°C, attributable to the melting process of the anhydrous crystalline form of the drug, followed by a large and irregular exothermic peak at 199°C, corresponding to its thermal decomposition. Similar results were observed previously [34]. The thermogram of β-CyD showed two peaks: one broad endothermic peak at 105°C, corresponding to water release from β-CyD, and second less broad endothermic peak above 300°C, corresponding to β-CyD decomposition. Similar observations were observed and explained previously [40]. While the thermogram of HP-β-CyD showed regular broad endothermic peak between 58 and 118°C, which attained a maximum at 84°C that might be corresponding to dehydration process [41].

The thermograms of PMs of EC with β-CyD or HP-β-CyD were found to be the summation of those the drug and CyDs. Also, it was found that the peaks that are characteristic of EC even though some size reduction of endothermic peak and broadening of exothermic peak was obtained, indicating

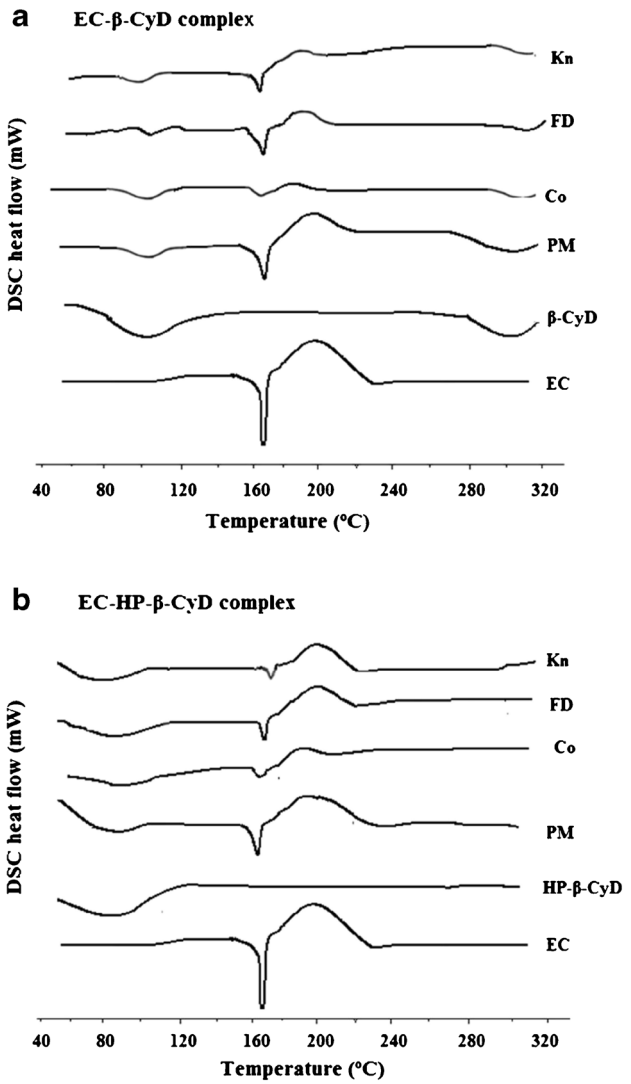

Fig. 4. Differential scanning calorimetry thermograms of EC, β-CyD or HP-β-CyD, physical mixtures (PM), co-precipitate (Co), freeze-dried (FD), and kneaded complexes (Kn)

that there was partial interaction in physical mixture between the drug and CyDs. For the co-precipitate EC-β-CyD complex, the endothermic and exothermic peaks of EC were shifted and lowered to 162 and 194°C, respectively. Also, there was no sharp endothermic peak produced. While kneaded and freeze-dried complexes showed slight endothermic and exothermic peaks, which is indicative of some drug–CyD interaction resulting in a loss of EC crystallinity and suggests that this methods did not produce a complete inclusion complex. Similar results were observed for EC-HP-β-CyD complex prepared by co-precipitation technique, where the shift in the endothermic and exothermic peaks of EC was observed at 163 and 196°C, respectively. Also, the intensity of both peaks was decreased in both complexes. These findings indicate that co-precipitate inclusion complexes exist in the new solid state, which confirms that co-precipitation method was the best technique for inclusion complexes preparation. Additionally, the increase in the dissolution rate of EC-CyDs complexes which could be correlated with the reduction in melting point of the drug as previously outlined [42].

Powder X-Ray Diffraction

The diffractograms of EC, β-CyD, HP-β-CyD, PMs, and its complexes of EC-β-CyD and EC-HP-β-CyD are depicted in Fig. 5. The diffraction pattern corresponding to EC is typical of crystalline materials, as it is characterized by numerous distinct peaks at 2θ angles 10.8, 17.2, 22.1, 26.9, and 29.3° [34]. Characteristic peaks of β-CyD appeared at 2θ equal to 14.3, 20.8, 36.8, and 38.6°, while the diffractogram of HP-β-CyD showed one broad peak at 2θ of 19.8°, which is a characteristic of an amorphous material. Physical mixture of EC with β-CyD/HP-β-CyD exhibited the identifiable peaks of EC in their spectra. As shown in Fig. 5, the PMs diffractograms correspond to the superposition of the pure components peaks, with lower intensities compared with the powder X-ray diffraction (PXRD) pattern of pure EC. This could be explained by the particle size reduction during physical mixing and some interaction between EC and β-CyD/HP-β-CyD resulting in reduction in crystalline nature of EC [43].

The diffractograms of EC-β-CyD complex showed diffuse peaks with low intensities, indicating that the drug

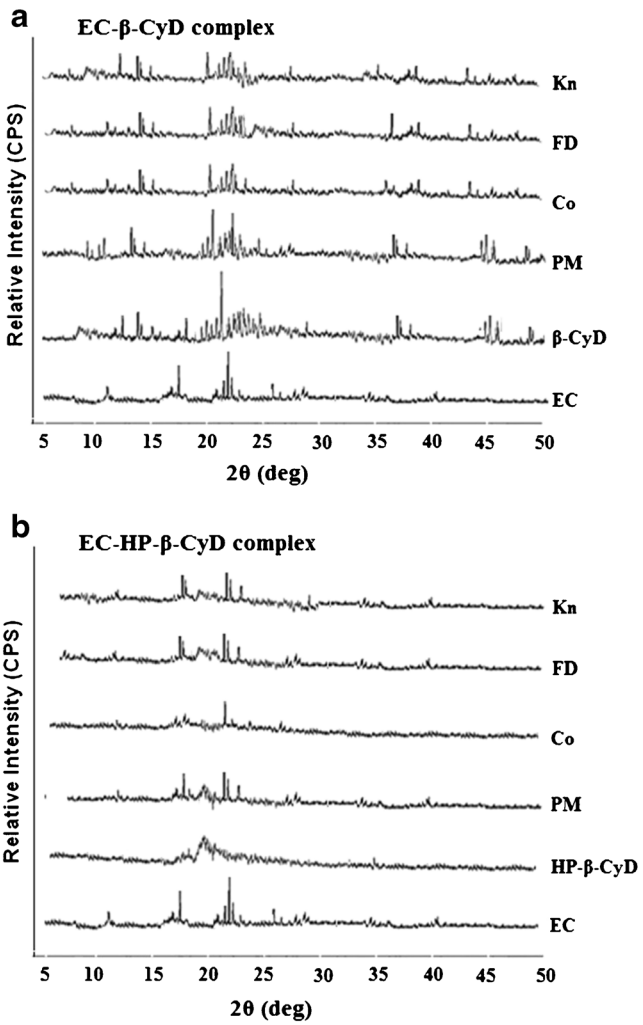

Fig. 5. Powder X-ray diffraction patterns of EC, β-CyD or HP-β-CyD, physical mixtures (PM), co-precipitate (Co), freeze-dried (FD), and kneaded complexes (Kn)

crystallinity was remarkably reduced, leading to the formation of a new solid state due to inclusion complex formation between EC and β-CyD. Similar finding has been reported by other authors [44]. The diffractograms 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. In the co-precipitate complex, the EC crystallinity was reduced to a greater extent, in EC inclusion complexes with HP-β-CyD more than with β-CyD which evidenced by complete disappearance of intense of EC peaks. It also differs much from the diffractograms of the corresponding PMs indicating that EC and β-CyD/HP-β-CyD form true inclusion complexes in solid state [45]. Based on these findings, a decrease in the drug crystallinity with subsequent increase in the drug surface area exposed to the dissolution medium might be responsible for the improved dissolution rate of EC.

Econazole Nitrate Entrapment Efficiency

Table III represents the entrapment efficiency of EC, the complex yield and the drug loading in EC-β-CyD and EC-HP-β-CyD inclusion complexes that prepared by different methods. The obtained results showed that the entrapment efficiency of EC was very high (82.14 and 95.27% for EC-β-CyD and EC-HP-β-CyD co-precipitate inclusion complexes, respectively) with a final drug loading 24.03 and 24.60% for EC-β-CyD and EC-HP-β-CyD co-precipitate inclusion complexes, respectively. These findings indicate that the co-precipitation method was the best technique for inclusion complex preparation of EC.

Particle Size Analysis

The reduced sizes of the inclusion complexes are very interesting in view of their potential application in ophthalmic drops formulations. Figure 6 shows the TEM micrographs of PMs and EC-CyDs co-precipitate inclusion complexes. All the resulting EC-CyDs inclusion complexes ranged in diameter from 110 to 288.33 nm (Table III). The minimum diameters were obtained with inclusion complexes prepared by co-precipitation method (110 and 168.33 nm for EC-β-CyD and EC-HP-β-CyD inclusion complexes, respectively). These finding indicate that the co-precipitation method was the best technique for inclusion complexes preparation. The maximum diameters were obtained with PMs; 564.33±14.01 and 711.67 ±10.41 nm for β-CyD and HP-β-CyD, respectively. The TEM images of PMs show the characteristics EC crystals adhered to CyD surface or partially entrapped inside their cavities. In contrast, a drastic change in the shape of the drug particle were observed in inclusion complexes, the drug completely entrapped inside CyD cavities, revealing an apparent interaction between the drug and CyD. The size of particles in ophthalmic dosage forms apart from influencing bioavailability plays an important role in the irritation potential of the formulation; hence, it is recommended that particles of ophthalmic solution should be less than 10 μm to minimize irritation to the eye [46].

Physicochemical Characterization of Eye Drops

The results of FT-IR, DSC, PXRD, TEM, and entrapment efficiency revealed the superiority of co-precipitation technique due to the combined effect of complexation and crystallinity reduction; hence, the systems prepared by this method with either carrier were further evaluated. Table IV represents the average values of percentage drug content, pH, viscosity, and mucoadhesive strength of the prepared eye drops containing either untreated EC or its co-precipitate complexes with each of β-CyD/HP-β-CyD.

Drug Content

Table IV represents the actual EC content of the formulated eye drops ranged from 96.76±4.35% to 99.09 ±4.55%. The obtained results showed that, the drug content deviation is less than ±4%, which complies with the pharmacopeal limits ranging from 90 to 110% of the label claim (47).

Formulations pH

The eye can tolerate the ophthalmic formulations with a wide pH range (3.5–8.5), because of eye tears natural buffering capacity (pH 7.4). Because the ideal ophthalmic dose is only one drop, the tear film can be rapidly restored its

Table III. Characterization of Econazole Nitrate-Cyclodextrins Inclusion Complexes

Parameters	Inclusion complex formation method					
	Kneading (Kn)		Co-precipitation (Co)		Freeze drying (FD)	
	EC-β-CyD	EC-HP-β-CyD	EC-β-CyD	EC-HP-β-CyD	EC-β-CyD	EC-HP-β-CyD
%EE	68.09±3.59	80.83±3.09	82.14±3.57	95.27±5.26	64.24±2.43	75.89±1.71
%Y	89.10±1.91	89.68±3.83	95.83±2.48	96.67±1.92	85.5±3.07	86.54±1.42
%DL	21.34±0.18	22.07±0.31	24.03±0.42	24.60±0.88	21.09±0.59	21.44±0.75
Particle size (nm)	219.67±9.5	269.33±9.02	110.0±10.0	168.33±7.64	245.33±7.64	288.33±7.64

Each value are expressed as mean±SD (n=3)
$\%EE$ percentage entrapment efficiency, $\%Y$ percentage inclusion complex yield, $\%DL$ percentage drug loading

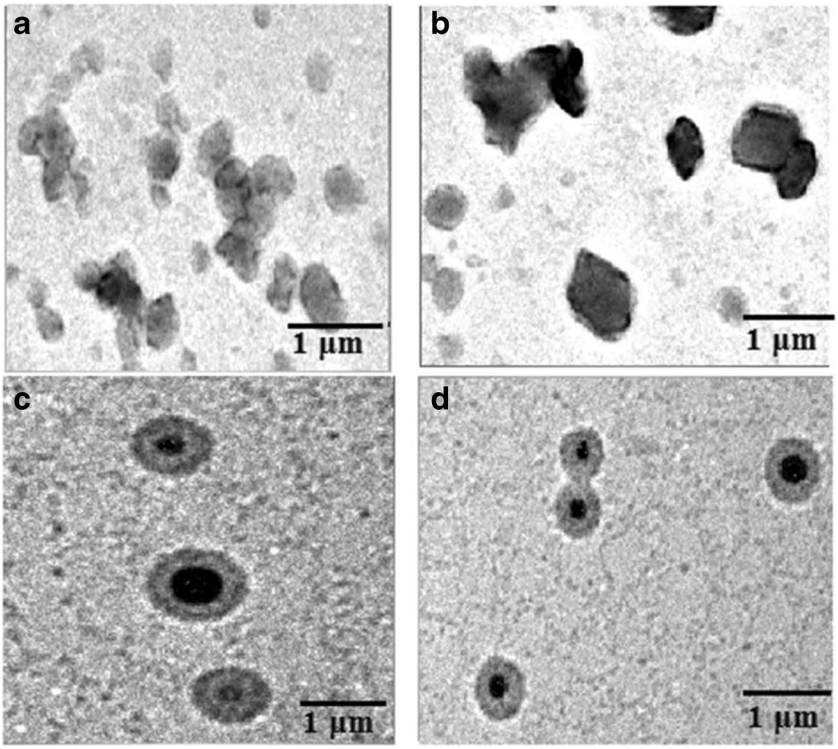

Fig. 6. Transmission electron microphotographs of **a** EC and HP-β-CyD physical mixture, **b** EC and β-CyD physical mixture, **c** EC-HP-β-CyD co-precipitate inclusion complex, and **d** EC-β-CyD co-precipitate inclusion complex

neutral pH [47]. The results showed that the pH values of the prepared eye drops ranged from 6.1±0.95 to 7.1±0.98, which can be easily tolerated by eye without discomfort or irritation (Table IV).

Formulations Viscosity

Generally, eye drops containing EC-β-CyD or EC-HP-β-CyD co-precipitate complex exhibited low viscosity values ranged from 169.56±20.2 to 220.3±25.1 cP, probably due to polymer chains and cyclodextrin hydrophobic interaction, thus polymer swelling properties decreased [48]. The viscosity of the eye drops can be arranged in the following order: HPMC>MC>CP 940 (Table IV). These outcomes can be explained on the basis that the viscosity of eye drops can be related to the polymer concentration used that was in the same order of viscosity.

In Vitro Mucoadhesive Strength of the Formulations

The mucoadhesive strength values of the prepared eye drops were influenced by the nature of the bioadhesive polymers and showed in order of CP 940>HPMC>MC (Table IV). The mucoadhesive strength of CP 940 formulations was significantly higher ($p<0.05$) than those of the corresponding HPMC and MC formulations. The highest mucoadhesive strength was obtained with CP 940 formulations probably due to the numerous proton-donating carboxylic groups in CP forming hydrogen bonds with the negatively charged mucus gel [49]. In addition, formation of intermolecular complexes of CP 940 with the glycoprotein mucin could explain its high mucoadhesive strength [50]. The lowest mucoadhesive strength was observed with HPMC and MC polymers probably due to more neutral cellulose groups, and thus,

Table IV. Physical Evaluation of Different Eye Drops

Formulae	pH	Drug content (% *w/v*)	Viscosity (cP)	Mucoadhesion (g)
Dβ 1	6.1±0.95	99.03±3.87	199.34±22.3	4.17±0.57
DH-β 11	6.8±0.66	98.02±4..07	180.46±19.4	4.43±0.67
Dβ 2	7.0±0.49	98.11±3.53	220.3±25.1	4.47±0.55
DH-β 12	6.9±0.52	99.09±4.55	202.65±27.6	4.70±0.53
Dβ 3	7.1±0.98	98.57±2.61	177.64±18.9	7.17±0.32
DH-β 13	6.7±0.72	96.76±4.35	169.56±20.2	7.33±0.60

All values are expressed as means±SD (n=3)

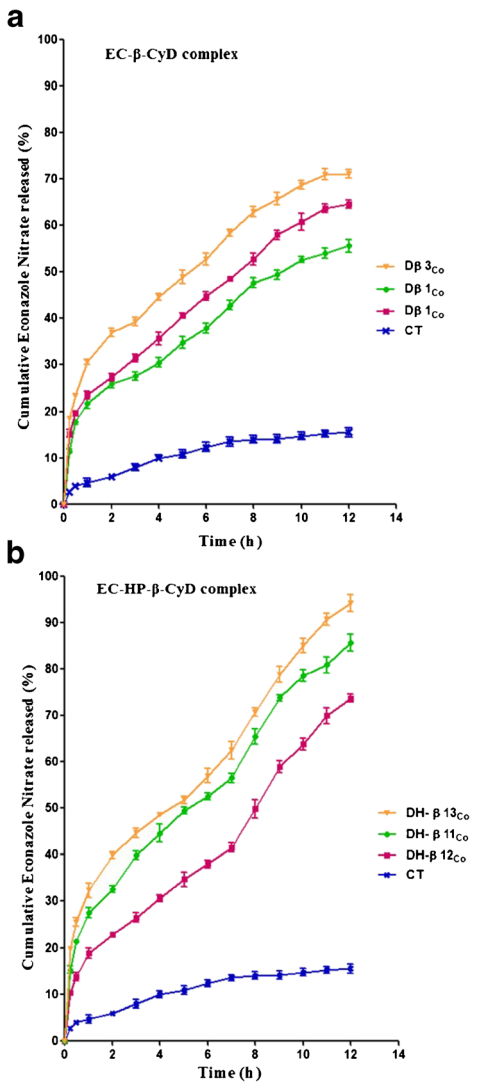

Fig. 7. *In vitro* dissolution profiles of EC-CyDs inclusion complex (co-precipitation method) from different eye drops in phosphate buffer (pH 7.4) at 37°C

fewer hydrogen bonds with glycoprotein mucin leading to weaker mucoadhesive forces [25].

In Vitro Dissolution Study

The *in vitro* release results of EC in phosphate buffer pH 7.4 from eye drops containing EC complexes prepared by different methods indicated the superiority of co-precipitation technique. Thus, only release data for the prepared eye drops containing EC-β-CyD or EC-HP-β-CyD 1:1 co-precipitate complex were represented in Fig. 7. It was found that no complete dissolution was obtained for EC alone, only 15.51% even after 12 h was released. This may be referred to the hydrophobic nature of EC which prohibited its contact with the release medium and consequently hindering its dissolution. It can be observed that dissolution rate was significantly improved by the complexation of EC with β-CyD/HP-β-CyD. The extent of dissolution rate enhancement was dependent on the preparation method, since co-precipitate complexes of both β-CyD and HP-β-CyD showed higher dissolution rate of the drug compared with the other methods indicating a better interaction of the drug with CyD by this method and soluble complexes formation in the solid form with reduction in EC crystallinity [51], as confirmed by DSC, FT-IR, and PXRD studies. The lower dissolution rates exhibited by the kneaded and freeze-dried systems (data not shown) could be explained by the partial formation of true inclusion complex by these methods. Additionally, the HP-β-CyD co-precipitate complex showed high dissolution than that of β-CyD, this indicates a higher solubilizing effect of HP-β-CyD than β-CyD.

Figure 7 illustrates the *in vitro* release behavior of EC-CyD complexes from different eye drops. It is found that, the incorporation of complexed drug into a gel vehicle resulted in a significant ($p<0.05$) higher drug release compared with control possibly because of greater hydrophilicity, higher wetting effect, decrease of drug crystallinity which increased the drug–carrier contact surface and ability to form stable complex of the β-CyD/HP-β-CyD [52]. The hydrophilic polymers nature affected the release of EC either complexed with β-CyD or HP-β-CyD from the prepared formulations. Also, it is clear that EC-CyD complexs release was significantly ($p<0.05$) higher in case of CP 940 drops compared with other drops after 12 h. This might be owing to the dissimilarity in their viscosities when exposure to the release conditions, as the greater the viscosity, the delaying drug release rate [53]. The higher release of EC from CP 940 drops

Table V. Kinetic Analysis of the Drug Release Data

Formulations	Correlation coefficient (r^2)			Korsmeyer–Peppas		Drug transport mechanism
	Zero	First	Higuchi	n	r^2	
CT	0.9103	0.9207	0.8988	0.4312	0.9872	Fickian
EC-β-CyD complex						
Dβ 1_{Co}	0.9269	0.9690	0.9829	0.4858	0.9729	Non-Fickian
Dβ 2_{Co}	0.9396	0.9816	0.9856	0.4764	0.9694	Non-Fickian
Dβ 3_{Co}	0.8958	0.9717	0.9840	0.4510	0.9870	Non-Fickian
EC-HP-β-CyD complex						
DH-β 11_{Co}	0.9563	0.9637	0.9813	0.4598	0.9779	Non-Fickian
DH-β 12_{Co}	0.9372	0.9502	0.9734	0.4932	0.9543	Non-Fickian
DH-β 13_{Co}	0.9481	0.9063	0.9717	0.4898	0.9645	Non-Fickian

n release exponent

may be also due to enhancing effect of TEA on CyDs solubilizing power for the poorly water soluble drugs [54].

Kinetics of Drug Release

The kinetic analyses of the release data (Table V) showed that EC released from all eye drops followed the Higuchi model, suggesting that the mechanism of release is diffusion, while the control drop followed first-order kinetics that suggesting dissolution of EC from its complexes. Further examination using the Korsmeyer–Peppas equation exhibited that (n) values for all eye drops between 0.45 and 0.89 that indicated exhibition of non-Fickian (anomalous) diffusion. So, the drug release from eye drops was a combination of diffusion from the complex and polymers erosion. The (n) value for control drop was 0.4312, which indicates Fickian mechanism suggesting that the release depends only on dissolution of EC from complex [55].

Ocular Bioavailability of EC-CyDs

Gross examination of the rabbit eyes during *in vivo* study showed no signs of abnormal lachrymation or increased blinking upon instillation of ophthalmic formulations. No irritation, redness, or allergic complications with formulations was observed. No ocular damage or abnormal clinical signs to the cornea, conjunctiva, or iris were visible. Previous studies demonstrated that *in vitro* cytotoxicity is indicative of irritation potential [56]. Berry *et al.* [57] who found that, econazole, miconazole, and clotrimazole appeared least toxic in all *in vitro* cytotoxicity study over chloramphenicol, gentamicin, and methicillin upon using cultured human corneal cells. In addition, azoles are usually well tolerated by the corneal epithelium even after weeks-long therapy. Many studies for determining the acute toxicity of natural CyDs on rats give very high LD50 values in various administration routs. Saarinen-Savolainen *et al.* [58] studied

Table VI. Pharmacokinetic Parameters of Eye Drops Containing EC-CyDs Complexes

Parameters	Formulation		
	Dβ 3_{Co}	DH-β 13_{Co}	CT
Cornea			
C_{max} (ng/mg)	128.44±4.436*	192±3.977*,a	75.97±1.530
T_{max} (h)	3*	3*	1
K_e (h^{-1})	0.657±0.026	0.605±0.034	0.721±0.059
$T_{1/2}$ (h)	1.06±0.041	1.15±0.064	0.974±0.077
AUC_{0-7} (ng h/mg)	442.8±16.580*	643.1±16.041*,a	258.3±1.184
$AUC_{0-\alpha}$ (ng h/mg)	457.3±18.280*	659.6±18.470*,a	271.9±4.848
Relative bioavailability	1.68±0.039	2.42±0.026a	–
Conjunctiva			
C_{max} (ng/mg)	75.27±2.957*	100.83±3.196*,a	57.26±1.908
T_{max} (h)	3	3	1
K_e (h^{-1})	0.531±0.021*	0.544±0.019*	0.773±0.024
$T_{1/2}$ (h)	1.31±0.049*	1.27±0.047*	0.897±0.027
AUC_{0-7} (ng h/mg)	309.9±14.780*	424.9±17.360*,a	219.7±8.850
$AUC_{0-\alpha}$ (ng hr/mg)	327.3±17.510*	446.5±20.290*,a	226.7±9.706
Relative bioavailability	1.44±0.024	1.97±0.033a	–
Iris-ciliary body			
C_{max} (ng/mg)	33.36±1.701*	41.64±2.099*	15.37±1.207
T_{max} (h)	3	3	1
K_e (h^{-1})	0.424±0.023*	0.441±0.021	0.572±0.049
$T_{1/2}$ (h)	1.64±0.094*	1.58±0.069	1.23±0.099
AUC_{0-7} (ng h/mg)	124.6±8.130*	148.6±6.431*	64.17±3.534
$AUC_{0-\alpha}$ (ng h/mg)	139.6±11.120*	165.3±9.222*	80.32±3.534
Relative bioavailability	1.77±0.099	2.11±0.109	–
Aqueous humor			
C_{max} (ng/mg)	20.42±0.703*	23.87±1.157*	15.25±1.559
T_{max} (h)	3	3	1
K_e (h^{-1})	0.429±0.036*	0.42±0.028*	0.583±0.027
$T_{1/2}$ (h)	1.62±0.147	1.66±0.115	1.19±0.052
AUC_{0-7} (ng h/mg)	77.91±3.145*	93.74±1.050*,a	60.46±4.335
$AUC_{0-\alpha}$ (ng h/mg)	86.9±1.806*	104.7±1.045*,a	64.28±4.480
Relative bioavailability	1.36±0.095	1.64±0.110	–

All values are expressed as means±SEM (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 to 7 h, $AUC_{0-\infty}$ the area under eye tissue concentration-time curve from 0–∞
*Considered significant compared to control (p<0.05)
[a] Considered significant compared to Dβ 3_{Co} (p<0.05)

the *in vitro* cytotoxicity of cyclodextrins on viability of immortalized human corneal epithelial cell, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and propidium iodide assay. They found that topically applied HP-β-CyD seem to be relatively safe on the corneal epithelium.

The eye tissues and aqueous humor concentrations of EC after a single application of selected formulations or control to rabbits were studied. The pharmacokinetic parameters of EC are illustrated in Table VI and Fig. 8. It is clear that, the ocular bioavailability of EC was improved in all eye tissues from the selected eye drops. This improvement was indicated by the higher C_{max}, $AUC_{0–7}$, and $AUC_{0-\infty}$ of the tested formulations than control. Also, the tested formulations extended the duration of EC which indicated by the higher T_{max}, $T_{1/2}$, and lower K_e than that of the control. From the obtained results it is clear that, EC ocular bioavailability are in the arrangement 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 findings are in agreement with those obtained by Yamaguchi *et al.* [59] who found that, the higher difluprednate concentration in cornea than in aqueous humor.

The obtained results revealed that, the higher C_{max}, T_{max}, $AUC_{0–7}$, and $AUC_{0-\infty}$ values in different eye tissues obtained after application of DH-β13 drops. Regarding T_{max} values, the tested formulations gave extended T_{max} values which reached up to 3 h for selected eye drops in different eye tissues. Thus, the results revealed that there was about threefold higher in the time required to achieve the maximum eye tissue concentration with tested eye drops than control. This can be explained by the mucoadhesive properties of the polymer used, and hence, retain it in the eye for longer period, so, the sustained effect of selected eye drops was expected to be stronger than that control. It is worth noting that, the C_{max}, T_{max}, $AUC_{0–7}$, and $AUC_{0-\infty}$ of selected eye drops were significantly ($p<0.05$) superior to that of control in all eye tissues and aqueous humor. The EC elimination half-life ($T_{1/2}$) from the selected eye drops was more than that of

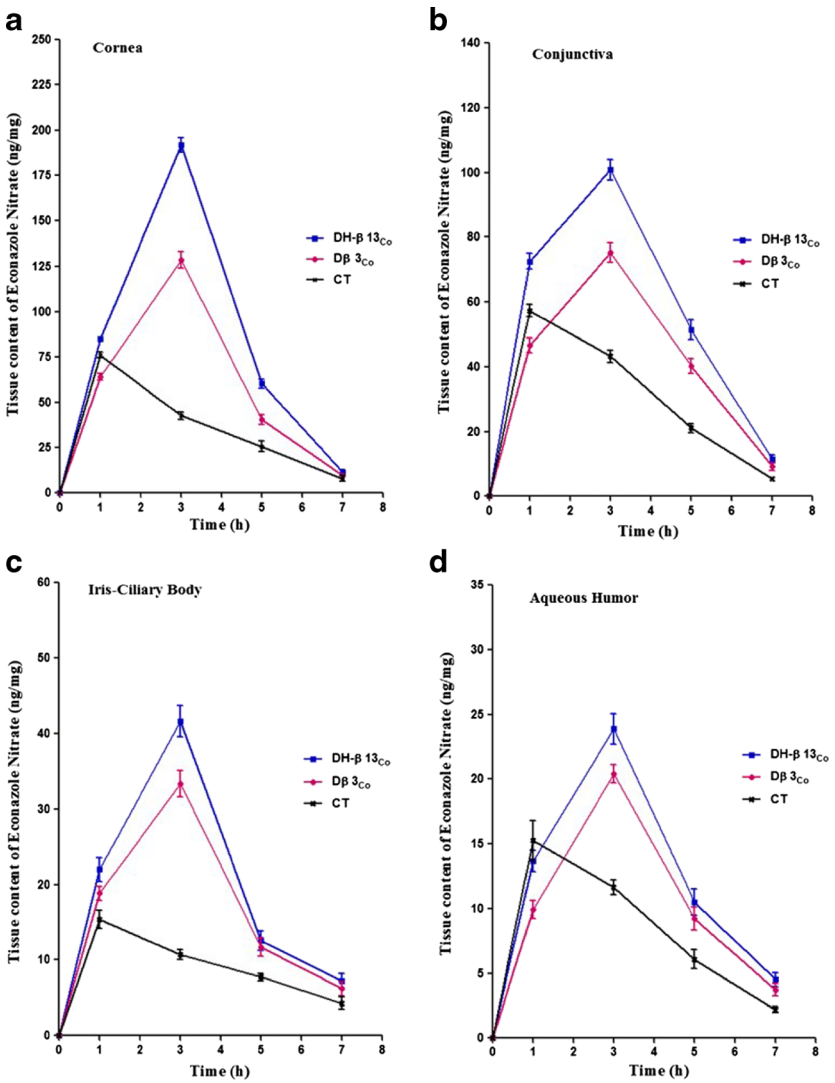

Fig. 8. The eye tissue concentration-time profiles of EC-CyDs inclusion complex following topical application of selected eye drops

control indicating that EC was eliminated from the eye slowly, which in turn, was supported by low K_e values of EC-CyDs in selected formulations in comparison with control. EC-CyDs in selected eye drops 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 enhanced EC ocular bioavailability from the inclusion complex in comparison with control. This finding could be owing to improved EC solubility and dissolution rate from the prepared complex.

CONCLUSION

Inclusion complexes of EC with β-CyD/HP-β-CyD have been successfully prepared. The co-precipitation method yielded the higher degree of amorphous entities suggesting the formation of true EC-β-CyD/EC-HP-β-CyD inclusion complexes. The dissolution profiles showed that eye drops containing EC-CyDs co-precipitate complexes exhibit higher dissolution rate than that of other complexes. Thus, preparing complexes method had an important role in improving the dissolution rate. Also, the EC ocular bioavailability was improved by complexation of EC with HP-β-CyD more than β-CyD. Eye drops 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.

COMPLIANCE WITH ETHICAL STANDARDS

The animal experimental procedures conform to the ethical principles of the scientific committee of the Pharmacy Faculty, Mansoura University, Egypt (code number, 2016–9).

Conflict of Interest The authors declare that they have no conflict of interest.

REFERENCES

1. Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK. Polymeric nanoparticulate system: a potential approach for ocular drug delivery. J Control Release. 2009;136:2–13.
2. Brayfield A. Martindale (the complete drug reference). 38th ed., The Pharmaceutical Press, Lambeth High Street, London SE1 7JN, UK; 2014. P. 531.
3. Pijls RT, Sonderkamp T, Daube GW, Krebber R, Hanssen HH, Nuijts RM, *et al*. Studies on a new device for drug delivery to the eye. Eur J Pharm Biopharm. 2005;59:283–8.
4. Gaona-Galdos AA, García PL, Aurora-Prado MS, Santoro MI, Kedor-Hackmann ÉR. Simultaneous determination of econazole nitrate, main impurities and preservatives in cream formulation by high performance liquid chromatography. Talanta. 2008;77:673–8.
5. Mahmoud AA, El-Feky GS, Kamal R, Awad GEA. Chitosan/sufobutylether–β-cyclodextrin nanoparticles as a potential approach for ocular drug delivery. Int J Pharm. 2011;413:229–36.
6. Jackson CJ, Lamb DC, Kelly DE, Kelly SL. Bactericidal and inhibitory effects of azole antifungal compounds on Mycobacterium smegmatis. FEMS Microbiol Lett. 2000;192:159–62.
7. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci. 1996;85:1017–25.
8. Challa R, Ahuja A, Ali A, Khar RK. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech. 2005;6:E329–57.
9. Gaudana R, Jwala J, Boddu SHS, Mitra AK. Recent perspectives in ocular drug delivery. Pharm Res. 2009;26:1197–216.
10. Loftsson T, Stefánsson E. Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye. Acta Ophthalmol Scand. 2002;80:144–50.
11. Archontaki HA, Vertzoni MV, Athanassiou-Malaki MH. Study on the inclusion complexes of bromazepam with β-and β-hydroxypropyl-cyclodextrins. J Pharm Biomed Anal. 2002;28:761–9.
12. Patel RPM, Shah D. Application of cyclodextrin in drug delivery. Int J Pharm Wor Res. 2010;1:1–21.
13. Shimpi S, Chauhan B, Shimpi P. Cyclodextrins: application in different routes of drug administration. Acta Pharm. 2005;55:139–56.
14. Rajasekaran A, Kumaran KSGA, Preetha JP, Karthika K. A comparative review on conventional and advanced ocular drug delivery formulations. Int J Pharm Tech Res. 2010;2:668–74.
15. Fouda MMG, Knittel D, Hippler UC, Elsner P, Schollmeyer E. Antimycotic influence of beta-cyclodextrin complexes–in-vitro measurements using laser nephelometry in microtiter plates. Int J Pharm. 2006;311:113–21.
16. Mura P, Faucci MT, Parrini PL, Furlanetto S, Pinzauti S. Influence of the preparation method on the physicochemical properties of ketoprofen-cyclodextrin binary systems. Int J Pharm. 1999;179:117–28.
17. Higuchi T, Connors KA. Phase solubility techniques. Adv Anal Chem Instrum. 1965;4:117–22.
18. Abd El-Gawad AH, Soliman OA, El-Dahan MS, Al-Zuhairy SA. Formulation and evaluation of ophthalmic preparations containing econazole nitrate-cyclodextrin complexes. Am J Pharm Health Res. 2016;4:74–96.
19. Tsuruoka M, Hashimoto T, Seo H, Ichimasa S, Uenoo O, Fujinaga T, *et al*. Enhanced bioavailability of phenytoin by beta-cyclodextrin complexation. J Pharm Soc Jap Yakugakuzasshi. 1981;101:360–7.
20. Arias MJ, Moyano JR, Muñoz P, Ginés JM, Justo A, Giordano F. Study of Omeprazole-gamma-cyclodextrin complexation in the solid state. Drug Dev Ind Pharm. 2000;26:253–9.
21. Hui-Yun Zhou HY, Jiang LJ, Yan-Ping Zhang YP, Li JB. β-Cyclodextrin inclusion complex: preparation, characterization, and its aspirin release in-vitro. Front Mater Sci. 2012;6:259–67.
22. Epstein SP, Ahdoot M, Marcus E, Asbell PA. Comparative toxicity of preservatives on immortalized corneal and conjunctival epithelial cells. J Ocul Pharmacol Ther. 2009;25:113–9.
23. Davies NM, Fan SJ, Hadgraft J, Kellaway IW. Evaluation of mucoadhesive polymers in ocular drug delivery. I. Viscous solutions. Pharm Res. 1991;8:1039–43.
24. Ibrahim MM, Abd-Elgawad AE, Soliman OA, Jablonski MM. Nanoparticle-based topical ophthalmic formulations for sustained celecoxib release. J Pharm Sci. 2013;102:1036–53.
25. Choi MK, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK. Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm. 1998;165:33–44.
26. Levy MY, Benita S. Drug release from submicronized o/w emulsion: new *in-vitro* kinetic evaluation model. Int J Pharm. 1990;66:29–37.
27. Higuchi T. Mechanism of sustained action medication. J Pharm Sci. 1963;52:1145–9.
28. Korsmeyer RW, Gurny R, Docler E, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm. 1983;15:25–35.
29. Medendrop J, Yedluri J, Hammell DC, Ji T, Lodder RA, Stinchcomb AL. Near-infrared spectrometry for the quantification of dermal absorption of econazole nitrate and 4-cyanophenol. Pharm Res. 2006;23:835–43.
30. Cheruvu NPS, Amrite AC, Kompella UB. Effect of eye pigmentation on transscleral drug delivery. Invest Ophthalmol Vis Sci. 2008;49:333–41.

31. De Muth JE. Basic statistics and pharmaceutical statistical applications. 2nd ed. New York: Chapman & Hall/CRC, Taylor &Francis Group; 2006. p. 201–43.
32. Patel RP, Patel MM. Preparation and evaluation of inclusion complex of the lipid lowering drug lovastatin with β-cyclodextrin. Dhaka Univ J Pharm Sci. 2007;6:25–36.
33. Loftsson T, Jarho P, Másson M, Järvinen T. Cyclodextrins in drug delivery. Expert Opin Drug Deliv. 2005;2:335–51.
34. Jug M, Mennini N, Kövérc KE, Mura P. Comparative analysis of binary and ternary cyclodextrin complexes with econazole nitrate in solution and in solid state. J Pharm Biomed Anal. 2014;91:81–91.
35. Yadav VR, Suresh S, Devi K, Yadav S. Effect of cyclodextrin complexation of curcumin on its solubility and antiangiogenic and anti-inflammatory activity in rat colitis model. AAPS PharmSciTech. 2009;10:752–62.
36. Del Valle EMM. Cyclodextrins and their uses: a review. Process Biochem. 2004;39:1033–46.
37. Al-Omaria MM, Daraghmha MH, El-Barghouthib MI, Zughulc MB, Chowdhryd BZ, Leharned SA, *et al*. Novel inclusion complex of ibuprofen tromethamine with cyclodextrins: physico-chemical characterization. J Pharm Biomed Anal. 2009;50:449–58.
38. Semcheddine F, Guissi NE, Liu X, Wu Z, Wang B. Effects of the preparation method on the formation of true nimodipine SBE-β-CD/HP-β-CD inclusion complexes and their dissolution rates enhancement. AAPS PharmSciTech. 2015;16:704–15.
39. Liu LX, Zhu SY. A study on the supramolecular structure of inclusion complex of β-cyclodextrin with prazosin hydrochloride. Carbohydr Polym. 2007;68:472–6.
40. Kohata S, Jyodoi K, Ohyoshi A. Thermal decomposition of cyclodextrins (α-, β-, γ-, and modified β-CyD) and of metal-(β-CyD) complexes in the solid phase. Thermochim Acta. 1993;217:187–98.
41. Wang J, Cao Y, Sun B, Wang C. Characterization of inclusion complex of trans-ferulic acid and hydroxypropyl β-cyclodextrin. Food Chem. 2011;124:1069–75.
42. Jun SW, Kim MS, Kim JS, Park HJ, Lee S, Woo JS, *et al*. Preparation and characterization of simvastatin/ hydroxypropyl β-cyclodextrin inclusion complex using supercritical antisolvent (SAS) process. Eur J Pharm Biopharm. 2007;66:413–21.
43. Bekers O, Uijtendaal EV, Beijnen JH, Bult A, Underberg WJM. Cyclodextrins in the pharmaceutical field. Drug Dev Ind Pharm. 1991;17:1503–49.
44. Salústio PJ, Feio G, Figueirinhas JL, Pinto JF, Cabral Marques HM. The influence of the preparation methods on the inclusion of model drugs in a β-cyclodextrin cavity. Eur J Pharm Biopharm. 2009;71:377–86.
45. Batt DK, Garala KC. Preparation and evaluation of inclusion complexes of diacerein with β-cyclodextrin and hydroxypropyl β-cyclodextrin. J Incl Phenom Macrocycl Chem. 2013;77:471–81.
46. Aggarwal D, Garg A, Kaur IP. Development of topical niosomal preparation of acetazolamide: preparation and evaluation. J Pharm Pharmacol. 2004;56:1509–17.
47. USP 34-NF 29. "The United States Pharmacopeia", 34th, The National formulary 29th: The United States Pharmacopeial Convention, Twinbrook Parkway, Rockville, MD 2011, pp. 700–1.
48. Bilensoy E, Rouf MA, Vural I, Sen M, Hincal AA. Mucoadhesive, thermosensitive, prolonged-release vaginal gel for clotrimazole: β-cyclodextrin complex. AAPS Pharm Sci Tech. 2006;7:E1–7.
49. Gu JM, Robinson JR, Leung SHS. Binding of acrylic polymers to mucin/epithelial surface: structure property relationships. Crit Rev Ther Drug Carrier Syst. 1988;5:21–67.
50. Mortazavi SA, Smart JD. Factors influencing gel strengthening at the mucoadhesive mucus interface. J Pharm Pharmacol. 1994;46:86–90.
51. Dollo G, Le Corre P, Chollet M, Chevanne F, Bertault M, Burgot JL, *et al*. Improvement in solubility and dissolution rate of 1,2-dithiole-3-thiones upon complexation with β-cyclodextrin and its hydroxypropyl and sulfobutyl ether-7 derivatives. J Pharm Sci. 1999;88:889–95.
52. Carrier RL, Miller LA, Ahmed I. The utility of cyclodextrins for enhancing oral bioavailability. J Control Release. 2007;123:78–99.
53. Budai L, Hajdú M, Budai M, Gróf P, Béni S, Noszál B, *et al*. Gels and liposomes in optimized ocular drug delivery: studies on ciprofloxacin formulations. Int J Pharm. 2007;343:34–40.
54. Granero GE, Maitre MM, Garnero C, Longhi MR. Synthesis, characterization and in-vitro release studies of a new acetazolamide-HP-beta-CD-TEA inclusion complex. Eur J Med Chem. 2008;43:464–70.
55. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010;67:217–23.
56. North-Root H, Yackovich F, Demetrulias J, Gacula Jr M, Heinze JE, Gacula MJR. Prediction of the eye irritation potential of shampoos using the in vitro SIRC cell toxicity test. Food Chem Toxicol. 1985;23:271–3.
57. Berry M, Gurung A, Easty DL. Toxicity of antibiotics and antifungals on cultured human corneal cells: effect of mixing, exposure and concentration. Eye. 1995;9:110–5.
58. Saarinen-Savolainen P, Järvinen T, Araki-Sasaki K, Watanabe H, Urtti A. Evaluation of cytotoxicity of various ophthalmic drugs, eye drops excipients and cyclodextrins in an immortalized human corneal epithelial cell line. Pharm Res. 1998;15:1275–80.
59. Yamaguchi M, Yasueda SI, Isowaki A, Yamamoto M, Kimura M, Inada K, *et al*. Formulation of an ophthalmic lipid emulsion containing an anti-inflammatory steroidal drug, difluprednate. Int J Pharm. 2005;301:121–8.