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EFFECT OF HYDROXYPROPYL-β-CYCLODEXTRIN ON THE SOLUBILITY, STABILITY AND *IN-VITRO* RELEASE OF CIPROFLOXACIN FOR OCULAR DRUG DELIVERY

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Abstract: Eye drops in the form of an aqueous solution with a lower viscosity are preferred for local administrations in ophthalmology. In ophthalmic formulations, cyclodextrins (CDs) are frequently used in recent years in order to increase water solubility, stability and bioavailability of an active substance and decrease an irritation to the eye. The scope of the present study was to investigate the influence of hydroxypropyl-β-cyclodextrin (HPCD) on the solubility, stability and *in vitro* release of ciprofloxacin (CIP). According to the phase solubility studies, A_L type solubility curve was obtained. HPCD improved the solubility of CIP 3 times at pH 5.5 and 2 times at pH 7.4. The two month stability studies indicated that CIP was more stable at pH 5.5 than at pH 7.4 and the stability of CIP was significantly increased by HPCD. The stability constant of the HPCD:CIP complex was increased further by addition of 0.1% (w/v) polymer (HPMC and PVP) to the aqueous medium including HPCD. Stability constant of solutions prepared in an ultrasonic water bath was higher than solutions prepared by heating in an autoclave. The results indicated that the CIP:HPCD complex increased *in vitro* release of CIP and the addition of polymer promoted this increase even more.

Keywords: ciprofloxacin, hydroxypropyl-β-cyclodextrin, ophthalmic solution, solubility, stability

Eye drops in the form of an aqueous solution with a lower viscosity are preferred for local administrations in ophthalmology. Other locally administered formulations, such as suspensions, oily drops, gels, ointments and solid inserts are frequently associated with unwanted side effects (e.g., eye irritation and blurred vision) (1). An active substance of a successful aqueous eye drop solution should be sufficiently hydrophilic to be able to cross the hydrous external surface of the eye and should be sufficiently lipophilic to be able to cross the ocular barriers in the eye (2).

In ophthalmic formulations, cyclodextrins (CDs) are frequently used in recent years in order to increase water solubility, stability and bioavailability of an active substance and decrease an irritation to the eye (1, 3–5). It has been pointed out that cyclodextrins can enhance the aqueous solubility of lipophilic drugs without affecting their chemical structure or ability to permeate biological membranes (1, 5, 6). Due to their nearly infinite water solubility and the lower toxicity, particularly hydroxypropyl derivatives of cyclodextrins are used to increase solubility, stability and bioavailability of the active substances (5, 6). HPCD is more significant cyclodextrin in aqueous eye drop formulations (5, 7). In many *in vitro* (8–10) and *in vivo* [11– 13) studies, a complex of the poorly water soluble active substances with HPCD has been found to increase water solubility, stability, corneal permeability and ophthalmic bioavailability. In addition, some studies have investigated the effects of water soluble polymers on the drug-CD complex (14–17).

In our study, ciprofloxacin (CIP), an agent with antimicrobial activity practically insoluble in water and commonly used to treat intraocular infections in ophthalmology, was chosen as the model active substance (18–22). Ciprofloxacin is sensitive to the sunlight and loses its antibacterial activity. Our aim in the present study was to investigate characteristics of the CIP's complex with HPCD at pH 5.5 and 7.4, and to increase water solubility and stability of CIP with the CIP:HPCD complex. In addition, the effects of a water soluble polymer addition (HPMC,

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PVP) on solubility and stability constant of the CIP:HPCD complex and on the CIP's *in vitro* release were also investigated.

This study differs from other studies for the following reasons; the inclusion complex was prepared in a liquid medium, solubility and stability studies were performed at two different pH values (pH 5.5 and 7.4) and results were compared, formulations were prepared with two different polymers (HPMC, PVP) and by two different methods (ultrasonic water bath, autoclave).

MATERIALS AND METHODS

Materials

CIP base is supplied by Bayer Drug Company (Istanbul, Turkey). Hydroxypropyl-β-cyclodextrin is purchased from Aldrich Company (molar substitution 0.8 and average m.w. 1460, HPCD). All other compounds and solvents used in this study were of reagent grade.

Solubility studies

A complex of CIP with HPCD was determined by using the phase-solubility method of Higuchi and Connors [23]. An excess amount of CIP was added to phosphate buffer solutions (pH 5.5 and 7.4) containing from 0 to 137 mM of HPCD. The suspensions were shaken at 25°C for 24 h. After equilibration, the suspensions were filtered through 0.45 mm membrane filters (Millipore, USA), diluted with distilled water and analyzed spectrophotometrically at 275 nm with a Shimadzu 1601 UV-visible spectrophotometer.

Phase-solubility studies were carried out in duplicate. The association constant (K_c) for the complex formed was calculated from the slope of the phase-solubility profiles and the aqueous solubility of CIP (S_o) according to equation (23):

$$K_{1:1} = \text{Slope} \times [(S_o)\,(1-\text{Slope})]^{-1} \qquad \text{Eq. 1}$$

where $K_{1:1}$ is stability constant of the complex and $[S_o]$ = CIP's solubility in absence of HPCD.

Stability studies

The effects of HPCD on the aqueous stability of CIP were studied at pH 5.5 and 7.4. Previous studies had shown that cyclodextrins were used in the 1–20% (w/v) concentration range for ophthalmic formulations (7, 10, 24, 25). Two HPCD concentrations were studied between this concentration range. The studies were performed at elevated temperatures (40 and 60°C) as a function of HPCD (8 and 16% w/v) concentration. In addition, photostability studies were performed under anaerobic condition at 25°C as a function of HPCD concentration 8–16% and were further exposed to the fluorescent light

Table 1. Aqueous eye drop formulations (w/v %).

Ingredients	F1	F2	F3	F4	F5	F6
CIP	0.2	0.2	0.2	0.2	0.2	0.2
HPMC	–	0.1	–	–	0.1	–
PVP	–	–	0.1	–	–	0.1
HPCD	–	–	–	20	20	20
Sodium edetate	0.05	0.05	0.05	0.05	0.05	0.05
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
PBS (pH 5.5) made up to the volume	100	100	100	100	100	100

Table 2. pH and viscosity of formulations (n = 3).

Formulations	pH	Viscosity (mPa.s)
F1	5.50 ± 0.012	1.07 ± 0.01
F2	5.51 ± 0.015	3.45 ± 0.01
F3	5.52 ± 0.011	2.67 ± 0.0058
F4	5.51 ± 0.010	1.26 ± 0.015
F5	5.52 ± 0.015	3.93 ± 0.0058
F6	5.52 ± 0.013	2.97 ± 0.0058

Data are presented as the means ± SD.

with the source at a distance of 30 cm from the samples. The remaining CIP in the solution was assayed with ultraviolet spectrophotometry.

Preparation of an aqueous ophthalmic formulation

In order to determine the complex formation ratio of CIP with HPCD in the presence of polymer and the solubility diagram, serial solutions containing 27–137 mM of HPCD were prepared in pH 5.5 medium with addition of 0.1% (w/v) PVP and HPMC. Formulations were prepared at pH 5.5 due to the higher solubility and stability of CIP at this pH. An excess amount of CIP was added to the vials and the vials were stoppered. The solutions were divided into two parts. In order to investigate the effect of likely to be used production methods, one part was heated at 120°C for 20 min in an autoclave and was allowed to equilibrate for 24 h at 25°C and the other part was kept in an ultrasonic bath at 25°C for 4 h and was allowed to equilibrate at 25°C for 24 h.

After equilibration, the suspensions were filtered through 0.45 μm membrane filters (Millipore, USA), diluted with distilled water and analyzed spectrophotometrically. The association constant (K_c) for the complex formed was calculated according to Eq. (1).

For preparing the eye drop solutions, CIP (0.2% w/v) was dissolved in an aqueous vehicle. HPCD (20% w/v), HPMC (0.1% w/v) and PVP (0.1% w/v) were included in different formulations (Tab. 1). Benzalkonium chloride (0.01% w/v), sodium EDTA (0.05% w/v) and appropriate amount of sodium chloride for adjusting the isotonicity were added. Formulations were sterilized by filtration through a 0.22 μm membrane filter. pH and viscosity of formulations are shown in Table 2.

***In vitro* release studies**

Release studies were carried out with a Franz cell apparatus at 32°C and at 300 rpm stirring rate (Ildam Instruments, Turkey). Hundred microliters of the test formulation was loaded into a donor cell and 30 mL phosphate buffer (pH 7.4) was used as the diffusion medium in the receptor cell. Cellulose acetate membrane with the pore size of 0.45 μm (Millipore, USA) was used. Membrane area available for diffusion was 1.15 cm^2. Half milliliter of samples was taken from the receptor cell at each pre-determined time intervals and CIP concentration was measured by ultraviolet spectrophotometry.

RESULTS AND DISCUSSION

Solubility studies

When different HPCD concentrations *versus* dissolved CIP concentrations were plotted, the solubility values obtained were found to comply with A_L type solubility diagram (Fig. 1). By using the stochiometric calculations developed for A_L type, the complex stability constant was calculated according to Eq. 1 for the complex formation ratio 1:1 ($K_{1:1}$).

Solubility of CIP at pH 5.5 and pH 7.4 of phosphate buffers at 25°C is 5.84 ± 0.072 mg/L (the mean ± SE, n = 3) and 2.93 ± 0.033 mg/L (the mean ± SE, n = 3), respectively. These values were used to calculate stability constants. The stability constants of the complex in phosphate buffers at pH 5.5 and pH 7.4 were 175 M^{-1} and 83 M^{-1}, respectively. The complex stability constant is a function of the slope. If the slope is smaller, the interaction between an active substance and CD is smaller, and if the slope is higher, the interaction between the active substance and CD is higher. High $K_{1:1}$ value indicates that the complex is highly resistant. In case of the overresistant complex formation, solubility would increase but since the active substance is not released, absorption and bioavailability would decrease (26). The calculated $K_{1:1}$ values indicate that the overresistant complex was not formed between CIP and HPCD, and A_L type solubility diagram indicated the formation of a soluble complex (27, 28).

Table 3. Observed shelf lives ($t_{90\%}$) for overall degradation of CIP at various temperatures in buffer solutions without HPCD and with 8% and 16% HPCD.

Temperature	pH 5.5 ($t_{90\%}$ days)			pH 7.4 ($t_{90\%}$ days)		
	Without HPCD	8% HPCD	16% HPCD	Without HPCD	8% HPCD	16% HPCD
Under light (25°C)	7.6	20.4	42.3	5.2	15.3	30.7
40°C	12.7	38.1	77.8	11.1	32.7	65.8
60°C	8.1	24.7	49.3	6.7	19.6	41.6
25°C	18.5	54.8	114.0	17.0	50.2	96.6
4°C	33.6	97.2	208.4	33.1	96.9	177.1

Table 4. First-order rate constants as 10^4 k [day^{-1}] and Arrhenius energy of activation in kJ/mol for CIP buffer solutions.

Temperature	pH 5.5			pH 7.4		
	Without HPCD	8% HPCD	16% HPCD	Without HPCD	8% HPCD	16% HPCD
k_c 4°C	31.39	10.84	5.06	31.88	11.66	5.95
k_c 25°C	56.91	19.23	9.24	62.16	20.98	10.91
k_c 40°C	82.98	27.66	13.55	94.94	32.23	16.02
k_c 60°C	130.1	42.67	21.38	157.30	53.77	25.33
Ea (kJ/mol)	19.84	21.87	22.17	18.77	19.48	19.75

Table 5. Effect of addition of HPMC and PVP to aqueous HPCD solution on complex stability constants (n = 3).

Complexes	In autoclave [$K_c(M^{-1})$]	In ultrasonic water bath [$K_c(M^{-1})$]
CIP:HPCD	22.0 ± 0.087	181.3 ± 0.112
CIP:HPCD:HPMC	30.1 ± 0.126	219.0 ± 0.097
CIP:HPCD:PVP	31.2 ± 0.101	233.5 ± 0.120

Data are presented as the means ± SD.

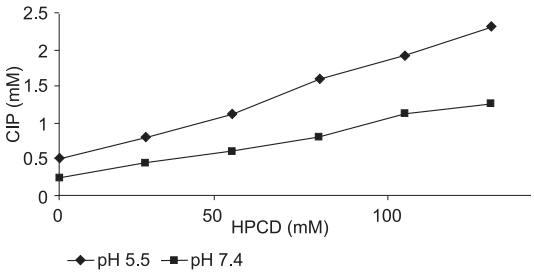

Figure 1. Phase solubility profile of CIP with HPCD at pH 5.5 and pH 7.4, at 25°C (n = 2)

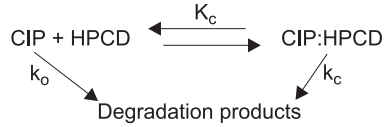

Figure 2. The degradation kinetics of a drug (CIP) forming inclusion complex with HPCD.
K_c: binding coefficient for CIP:HPCD complex; k_0: degredation rate constant of the active substance that did not form any complex; k_c: degredation rate constant of the active substance in the complex

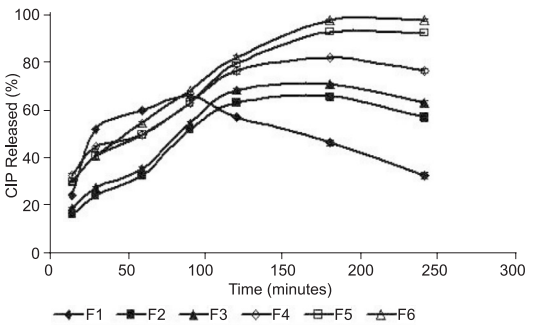

Figure 3. The *in vitro* release profiles of CIP from six different formulations

Stability studies

CIP's degradation in the presence and absence of HPCD at different temperatures (40, 60°C – in dark environment) and in light environment was examined for 60 days (Tab. 3). CIP's stability at the lower temperatures (25 and 4°C) was calculated according to Arrhenius equation by using its degradation values at 40 and 60°C. CIP's chemical and photochemical degradation in different environments was found to follow the first-order degradation. The stabilizing effect of cyclodextrins depends on the grade of the complex formation (K_c) and the degradation rate of the drug (k_c) in the complex (Fig. 2).

The CIP:HPCD complex containing 16% HPCD at pH 5.5 seems to provide maximum stabilization effect. The K_c, k_o and k_c values at this concentration were calculated as 169 M^{-1}, 56.91 × 10^{-4} h^{-1}, 9.24 × 10^{-4} h^{-1}, respectively. These results are consistent with a kinetic system where a free drug degrades at a higher rate than the complex form. Activation energy values of CIP at different pH val-

ues and temperatures were calculated with Arrhenius equation and it was found that CIP had the highest activation energy again at pH 5.5 and in solutions containing 16% HPCD (Tab. 4). When the Arrhenius graphs related to the solutions were investigated, it was found that CIP had the highest log k values at pH 5.5 and 7.4, and as HPCD concentration increased the log k values decreased, thus CIP's stability is greater.

***In vitro* release studies**

Solubility enhancement and complex formation effects of CDs are improved with the addition of water-soluble polymers into the medium (15). In our study, the effect of CIP:HPCD complex on solubility, complex formation and stability characteristics of CIP was also investigated by the addition of 0.1% (w/v) HPMC and PVP. In order to be able to make comparisons, the solutions were prepared by keeping one part of the solutions in an autoclave and the other part in an ultrasonic water bath. Complex stability constant values of the solutions prepared in an autoclave were observed to be lower (Tab. 5). Complex stability constant of the solutions were evaluated individually, among the solutions prepared in an autoclave, the stability constant of the solution containing CIP: HPCD was increased by 37 and 42% with the addition of HPMC and PVP, respectively. In contrast, among the solutions prepared in an ultrasonic water bath, the stability constant of the solution containing CIP:HPCD was increased by 21 and 29% with the addition of HPMC and PVP, respectively. According to these results, in contrast to the results of other authors (29), the stability constant values of the CD complexes of the solutions prepared in an ultrasonic water bath were found to be higher. This may be due to the decrease in ciprofloxacin stability with heating in an autoclave. Researchers showed that quinolones are very resistant to different heat treatments with maximum lose of concentration 12.71% for ciprofloxacin at 120°C and 20 min [30]. Therefore, *in vitro* release characteristics of formulations prepared by an ultrasonic water bath were investigated. The content of the formulations are presented in Table 1. *In vitro* release characteristics of six formulations containing the CIP:HPCD were evaluated by using modified Franz diffusion cell and cellulose acetate membrane. According to the release profiles obtained (Fig. 3), the CIP release was 64.66% at 90 min from F1 and started to decrease after 90 min. Release of the active substance from formulations F2, F3 and F4 started to decrease after 180 min and released CIP from these formulations were 65.65, 69.32 and 82.19%, respectively. Release of CIP from F5 and F6 formulations started to decrease after 240 min and released CIP from these formulations were 92.48 and 97.66%, respectively. The results indicated that the CIP:HPCD complex increased CIP's *in vitro* release values and duration, and that the addition of polymer promoted this increase even more. In addition, these results suggested that the increase in viscosity resulting from addition of polymer also had an impact on the duration of CIP release (15, 29). The greatest and longest increase was obtained with formulation F6.

PVP increased the solubilizing effect of HPCD by enhancing the apparent stability constant of the HPCD complex (31). The combined use of PVP and HPCD resulted in a synergistic increasing effect of the solubility of CIP. Similar to our results, Mura et. al. (32) found about 65% increase in the apparent stability constant of the Naproxen:HPCD complex in the presence of only 0.1% (w/v) PVP. The addition of HPMC was also increasing the CIP release, but *in vitro* CIP release increased even more with the addition of PVP.

It was concluded that for aqueous eye drops that contain CIP, a hydrophobic active substance, the HPCD complex, could increase solubility, stability and *in vitro* permeability properties of CIP, and that these properties could be optimized even more by adding in the medium a water soluble polymer as well as PVP. Assuming that the results of *in vitro* release studies are a preliminary stage for the *in vivo* studies to be conducted, it can be suggested that bioavailability of CIP ophthalmic solutions will be increased in the presence of HPCD and polymer.

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Declaration of interest

The authors report no declarations of interest.

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