γCD/HPγCD Mixtures as Solubilizer: Solid-State Characterization and Sample Dexamethasone Eye Drop Suspension

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ABSTRACT - Purpose. Study the complexation of dexamethasone in combinations of γ-cyclodextrin (γCD) and 2-hydroxypropyl-γ-cyclodextrin (HPγCD) with emphasis on solid characterization and development of aqueous dexamethasone eye drop suspension for drug delivery through sclera.

Methods. Dexamethasone/cyclodextrin (dexamethasone/CD) solid complex systems were prepared and characterized by Fourier transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), X-ray diffractometry (XRD), and by in vitro drug dissolution testing. Sample eye drop suspensions were prepared applying solubilizer/suspender consisting of γCD/HPγCD mixtures, poloxamer 407 (P407) and polyvinylpyrrolidone. The eye drop suspension was characterized by its physicochemical properties.

Results. The solid characterization techniques applied suggested that solid complexes were being formed. The results indicated that dexamethasone formed non-inclusion or micelle-like aggregates with HPγCD and the γCD/HPγCD mixture. The dissolution and dexamethasone release from the solid dexamethasone/γCD/HPγCD complexes was much faster than from the solid dexamethasone/γCD and dexamethasone/HPγCD complexes. The diameter of the solid particles in the dexamethasone eye drop suspension formulations were in all cases less than 10 μm with a mean diameter from 2.5 to 5.8 μm. The particle size decreased with increasing amount of P407. Permeation studies through semipermeable membrane and porcine sclera showed that increasing the amount HPγCD could enhance drug transport through the membrane barriers and this was related to enhanced drug solubility. The permeation rates were, however, decreased compared to formulation containing γCD alone due to larger hydrodynamic diameter of dexamethasone/γCD/HPγCD complex aggregates. All formulations were both chemically stable for at least 8 months at 25°C and 40°C.

Conclusions. Combination of γCD and HPγCD, i.e., formation of dexamethasone/γCD/HPγCD complexes, resulted in synergistic effect. That is the mixture had greater solubilizing effect than the individual CD, resulted in enhanced dissolution and drug delivery through membranes. Furthermore, it is possible to control the drug release rate by adjusting the γCD:HPγCD ratio in the solid dexamethasone/γCD/HPγCD complexes.

INTRODUCTION

Cyclodextrins (CDs) are water-soluble oligosaccharides which are able to solubilize water-insoluble drugs through complexation (1, 2). The complexation does not affect the lipophilicity of the drug molecules and, thus, the CD complexation does not change the intrinsic properties of the drug molecules and their ability to permeate lipophilic barriers, such as the cornea, or interact with drug receptors within the body (3). It is well-documented that CDs can form inclusion complexes and non-inclusion complexes with drugs as well as water soluble CD aggregates (4-12). When used in pharmaceutical formulations, CDs can improve the aqueous solubility, stability, dissolution rate, bioavailability and/or local tolerance of drugs and thus they offer numerous possibilities in ophthalmic drug delivery (13). Addition of small amounts of water soluble polymers to the complexation media enhances the complexation efficacy (CE) and bioavailability of drugs from CD containing formulations (14-17). It is believed that the water soluble polymers interact with the drug-CD complexes in a similar way as with the micelles, forming drug-CD-polymer aggregates.

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that are more readily adsorbed onto biological membranes than the individual drug-CD complexes, resulting in a more effective drug delivery to the membrane surface (10). Polymers such as polyvinyl alcohol, hydroxypropyl methylcellulose and polyvinylpyrrolidone are commonly used in ophthalmic preparations (18-20). In this present study poloxamer 407 is used in aqueous eye drop formulations. The non-toxic properties and stability of poloxamers have been evaluated as an ophthalmic vehicle in various animal models (21-25).

Drugs can be delivered to the posterior segment of the eye via four different routes, i.e., topical, systemic, periorcular and intraocular route (26). In general, conventional aqueous eye drop formulations are unable to deliver therapeutic drug dosages to the posterior segment of the eye. However, in theory therapeutic levels should be achievable by transcleral drug diffusion. In vitro permeation studies through sclera and Bruch’s membrane-choroid show that the drug permeability decreased with increasing molecular radius, molecular weight and lipophilicity (27). Our previous studies have shown that CD-based formulations are able to deliver drugs into the posterior segment (13, 28-30). Furthermore it was shown that combinations of parent CD with its derivative (i.e., γ-CD and HPγCD) have synergistic effect on drug solubilization. Also, some excipients such as benzalkonium chloride, hydroxypropyl methylcellulose and some drugs such as amphotericin B are able to enhance dexamethasone solubility in aqueous cyclodextrin solutions (31, 32). The purpose of this present study is to investigate further the complexation of dexamethasone with mixtures of γCD and HPγCD focusing on characterization of solid dexamethasone/CD complexes and to develop dexamethasone eye drop suspension that is able to deliver dexamethasone to the posterior segment of the eye.

METHODS

Materials

Dexamethasone was purchased from Fagron group (Amsterdam, Netherlands), γ-cyclodextrin (γCD) and 2-hydroxypropyl-γ-cyclodextrin (HPγCD) molecular substitution (MS) 0.6 (MW 1576 Da) from Wacker Chemie (Munich, Germany), disodium edetate dihydrate (EDTA) and sodium chloride (NaCl) from Merck (Darmstadt, Germany), benzalkonium chloride and polyvinylpyrrolidone (PVP) from Sigma (St. Louis, MO, USA), poloxamer 407 from BASF (Lutrol F-127, Ludwigshafen, Germany), semipermeable cellophane membrane (SpectaPor, molecular weight cut-off (MWCO) 12-14000) from Spectrum Europe (Breda, Netherlands). All other chemicals used were of analytical reagent grade purity. Milli-Q (Millipore, Bedford, MA, USA) water was used for the preparation of all solutions.

Preparation and characterization of drug/CD inclusion complex

Sample preparations

1:1 and 1:2 molar ratio (m:n; DmCDn where m and n represents the total moles of drug and CD, respectively) of drug and individual CD (γCD or HPγCD) or their mixtures (γCD/HPγCD, weight ratio 80/20 or 20/80) were prepared by freeze-drying an aqueous solution containing dexamethasone and various CDs. The filtrated solution was frozen and then freeze-dried at -52°C for 48 h, using a Freeze-dryer (Snijders Scientific LY-3-TT, Tilburg, Netherlands), yielding a solid complex powder (FD). Identical physical mixtures (PM) were prepared by careful blending drug and individual CD or their mixtures in a mortar with pestle.

Fourier transform infra-red spectroscopy (FT-IR)

The FT-IR spectra of dexamethasone, CDs and the complexes obtained were measured as potassium bromide discs on AVATAR 370 FT-IR (Thermo Nicolet Corporation, Madison, USA). The data was obtained in the range of 400-4000 cm⁻¹ for each sample. Analyses were performed at room temperature.

Differential scanning calorimetry (DSC)

DSC thermograms were determined in a scanning calorimeter (DSC822° Model, Mettler Toledo, USA). The instrument was calibrated using Indium as a standard. Samples (3-5 mg) were heated (10°C/min) in sealed aluminium pans under nitrogen. The temperature range was from 30-350°C.
X-ray diffraction studies (XRD)

The powder X-ray diffraction patterns were recorded using an X-ray diffractometer (Siemens, Karlsruhe Germany) equipped with CuKα radiation at 40 kV and 20 mA. X-ray diffractogram was scanned with diffraction angle increasing from 3° to 40°, 2θ angle, with a step angle of 0.04° and count time of 1.5 second.

In vitro dissolution studies

1:4, 1:10 and 1:20 (m:n) molar ratio of drug and individual CD or their mixtures (γCD, HPγCD or γCD/HPγCD mixtures, weight ratio 80/20 and 20/80) were prepared according to the method described in the sample preparation section. All solid complexes were sieved prior to study and the 75-150 μm fraction used for the dissolution studies. Dissolution studies of 1:4, 1:10 and 1:20 dexamethasone/CD complexes were performed using transparent gelatin capsules No. 00 (Parke-Davis, USA) containing the composition to be tested corresponding to 10 mg of dexamethasone. Samples were hanged with a stainless steel wire 2 cm from the top of the cylindrical vessel containing 10 ml of phosphate buffer saline (PBS, pH 7.4). The vessel is 6.3 cm high, its inside diameter 1.5 cm, and its nominal capacity is 12 ml. The experiments were performed at ambient temperature (22-23°C) and under constant magnetic stirring (300 rpm). At fixed time intervals, samples were withdrawn and replaced with an equal volume of fresh medium. Quantitative determination of dexamethasone was analyzed by HPLC. The dissolution experiments were conducted in triplicate.

Quantitative determinations

The quantitative determinations of dexamethasone were performed on a reversed-phase HPLC component system from Hewlett Packard Series 1100, consisting of a G132A binary pump with a G1379A solvent degasser, a G13658 multiple wavelength detectors, a G1313A auto sampler, and Phenomenex Luna 5μ C18 reverse-phase column (150 x 4.6 mm). The HPLC conditions were as follows. Mobile phase: 33% (v/v) acetonitrile and 1% (v/v) tetrahydrofuran in pure water; UV wavelength: 241 nm; flow rate: 1.5 ml/min; injection volume: 20 μl; and column oven temperature: 25°C.

Dexamethasone eye drop formulation

The aqueous 1.5% (w/v) dexamethasone eye drop suspensions were prepared by suspending 1.5 g of dexamethasone in 50 ml of water containing benzalkonium chloride (20 mg) and EDTA (100 mg) in solution and various types of CD (γCD, and γCD/HPγCD 80/20 and 60/40 mixtures) (18 g), and sufficient sodium chloride to make the final solutions isotonic. Then, solution of poloxamer 407 and PVP (125 mg) in 25 ml sterile water, that had been allowed to equilibrate over night at 4°C, was added under stirring until homogenously suspensions were obtained. Then the suspensions were heated in an autoclave in sealed containers (121°C for 20 min). The suspensions were allowed to cool to room temperature and then the volume was adjusted to 100 ml with sterile water. The pH of the formulations was adjusted to 7.40±0.05 with concentrated sodium hydroxide solution and allowed to equilibrate at room temperature under constant agitation for 7 days. Compositions of dexamethasone eye drop suspensions are given in Table 1. Each formulation contained 1.5% w/v dexamethasone, 18% CD, 0.02% benzalkonium chloride, 0.1% EDTA, and 0.125% PVP. Total dexamethasone content was determined by diluting 120-μl sample with 5 ml of the mobile phase. For the amount of dissolved dexamethasone in solution, the sample was centrifuge at 3000 rpm, 20°C for 10 min (Model Rotina 35R, Hettich, Germany), then the supernatant diluted further with the mobile phase and analyze by HPLC.

Table 1. Composition of dexamethasone eye drop formulations

| Formulation | γCD (%) | HPγCD (%) | Poloxamer 407 (%) |
|-------------|---------|-----------|-------------------|
| F1          | 14.4    | 3.6       | 2.5               |
| F2          | 14.4    | 3.6       | 5                 |
| F3          | 10.8    | 7.2       | 2.5               |
| F4          | 10.8    | 7.2       | 5                 |

Each formulation consisted of 1.5% dexamethasone, 0.02% benzalkonium chloride, 0.1% disodium edetate, 0.125% PVP and 0.5% sodium chloride to obtain isotonicity, all % w/v.

Determination of physico-chemical parameters

The pH values of preparations were determined at room temperature with Thermo Orion Star Series pH meter (USA). The osmolality of preparations

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was measured at room temperature using Knauer K-700 vapor pressure osmometer (Knauer, Germany). The viscosity measurement were performed with a viscometer (Brookfield model DV-I’, USA) equipped with a thermostated water bath at 25°C (Polystat model, USA). Surface tensions of formulations were also measured in quadruplicate at 35°C by De Nouy ring method with digital tensiometer K10 (Kruss GmbH, Germany).

Degree of flocculation

The physical stability of the dexamethasone eye drop suspensions was evaluated by determining their sedimentation volume (F) and degree of flocculation (β). Each preparation (10 ml) was stored in 10 ml-measuring cylinder for 7 days at room temperature (22-23°C). Observations were made at every hour for 7 hours and then every 24 hour for 7 days. β was then calculated using the following equation below (33).

Re-dispersion time

Dexamethasone (1.5% w/v) eye drop suspensions were filled in a 5-ml colorless glass container. The time required for re-dispersion of the suspensions was determined after standing the containers in an upright position for 5 days at 25°C from the precipitated conditions at the bottom of the container, when the container rolled in a horizontal position using mechanical shaker. The measurements were done in triplicate and the results are the mean values ± standard deviation (S.D.).

Particle size analysis

A laser diffraction particle sizer (Mastersize, Mavern, UK) was used to determine the particle size of dexamethasone eye drop suspension. Particle size distribution was analyzed by plotting a curve between particle diameters versus percentage volume of particles. Cumulative frequency of volume diameter was calculated, and the diameter of particles at 50% volume percentile and uniformity were determined. The particle size was also measured by light microscopy (Model BHT, Olympus, Japan). The sample was diluted with water. One drop was placed on slide and measured the particle size (50-100 particles) by using ocular micrometer which calibrated with stage micrometer before analysis. The magnification x400 was used in all experiments. The acceptable criteria for particle size of eye drop suspension followed by the section eye preparations, European Pharmacopoeia (34).

Scanning Electron Microscopy (SEM)

The morphology of the dexamethasone eye drop suspension was analyzed using a scanning electron microscope (SEM). Briefly, an aqueous eye drop suspension was layered on the slide, and the sample was allowed to dry overnight in desicator at room temperature. Subsequently, this layer was coated with gold under argon atmosphere at room temperature. Samples were then observed for their surface morphology with a SEM (Model JEM-1230, JEOL, Japan).

Chemical stability

Chemical stability of dexamethasone eye drop formulations was determined at 25±1°C and 40±1°C in thermostated incubators (Venticell, MMM Medcenter Einrichtungen GmbH, Germany). Each of the four formulations were divided into three samples and transferred into glass vials and sealed with rubber stopper and aluminium cap after preparation. Aliquots (120 μl) were withdrawn at 0, 2, 3, 4, 6 and 8 months.

\[
F = \frac{\text{Final volume of sediment}}{\text{Original volume of suspension}} \quad (1)
\]

\[
\beta = \frac{\text{Sedimentation volume (F)}}{\text{Sedimentation volume of the most deflocculated suspension (Fa)}} \quad (2)
\]

where the F and Fa are the ultimate sedimentation volume in flocculated suspension and deflocculated suspension, respectively.
All samples were diluted with the mobile phase and analyzed by HPLC as described under the quantitative determinations section.

**In vitro permeation studies**

To study the effect of CD mixtures and poloxamer 407 on dexamethasone permeability through semipermeable membrane, the dexamethasone eye drop formulations containing pure γCD were used as reference. The permeability of dexamethasone eye drop preparations were carried out using Franz diffusion cell apparatus consisting of a donor and a receptor chamber (FDC 400 15FF, Vangard International, Neptune, USA). The donor chamber and the receptor chamber were separated by a cellophane membrane (MWCO 12-14,000). The membrane was soaked overnight in the receptor phase, i.e., pH 7.4 phosphate buffer saline containing 2.5% (w/v) γCD/HPγCD (weight ratio 50/50). CD was added to the receptor phase to allow sink condition. The receptor phase was sonicated under vacuum to remove dissolved air before it was placed in the receptor chamber. The study was conducted at 22-23°C and during the study the receptor phase was continuously stirred by a magnetic stirring bar. A 150-µl aliquot of receptor medium was withdrawn at 30, 60, 120, 180, 240 and 360 minutes intervals and replaced immediately with an equal volume of fresh receptor medium. Dexamethasone concentration in the samples was determined by HPLC. The cumulative amount of drug release in to the receptor phase was calculated. All measurements were done in triplicate. The flux (J) was calculated from the linear part of each permeability profile, i.e., cumulative amount of drug versus time plot. The apparent permeation coefficient (P_{app}) in units of centimeters per second, defined by the expression:

\[
J = \frac{dq}{A \cdot dt} = P_{app} \cdot C_d \quad (3)
\]

where A is the surface area of the mounted membrane, taken to be 1.77 cm² and C_d is the initial concentration of the drug in the donor chamber. The steady state flux (J) was the linear slope of plots of amount of drug in the receptor chamber (q) versus time (t).

**Ex-vivo permeability studies**

Ex-vivo method was used in an effort to predict drug permeation from the eye drop suspensions into the eye. The permeation studies were performed across sclera isolated from porcine eyes obtained from slaughterhouse. In this study, sclera that was dissected from porcine eyes which were obtained within 4 hr after the death of the animals and replaced the semipermeable cellophane membrane in the previously described in vitro permeation studies. Each formulation was conducted at least in quadruplicate.

**RESULTS**

**FT-IR Spectra**

Fourier transform infra-red spectroscopy was applied to verify presence of both guest and host components in the inclusion complex. The FT-IR spectra of dexamethasone, γCD, HPγCD and the γCD/HPγCD mixtures as well as of the dexamethasone/CD complexes prepared by the freeze-drying are reported in Figures 1A and 1B, respectively. Pure dexamethasone spectrum exhibited bands around 1600-1700 cm⁻¹ that correspond to C=O and C-O stretching vibrations, whereas γCD and HPγCD were characterized by bands at 3370 cm⁻¹ due to the symmetric and antisymmetric O-H stretching mode, a band at 2930 cm⁻¹ that can be related to the C-H stretching vibrations, and other bands at lower frequencies. In the 1:1 dexamethasone/CD samples the intense bands of dexamethasone were still present but were in all cases shifted to higher frequencies. The results indicated that in these samples dexamethasone might be present both in its free form and as a dexamethasone/CD complex. However, when the 1:1 and 1:2 dexamethasone/CD samples are compared it can be seen that the intense band at 1704 cm⁻¹ that can be observed in the 1:1 samples disappears in the dexamethasone / γCD and dexamethasone / (γCD/HPγCD) (80/20) 1:2 samples but could still be found in dexamethasone/HPγCD and dexamethasone / (γCD/HPγCD) (20/80) 1:2 samples but it was shifted to 1710 cm⁻¹ and 1712 cm⁻¹, respectively.
Figure 1. Fourier Transform-Infra Red (FT-IR) Spectra. Pure compounds (A), and freeze-dried complexes (FD) containing 1:1 and 1:2 products (dexamethasone:CD molar ratios) (B).

DSC Thermograms

The thermograms of pure material in the melting range of the drug and dehydration and decomposition of the carriers are shown in Figure 2A. The DSC thermogram of dexamethasone was a typical of a crystalline anhydrous substance with a sharp endothermic peak at 272.1°C, corresponding to the melting point with decomposition of the drug. Thermal profile of γCD showed a broader endothermal effect at 106.5°C and 316.3°C which corresponded to its dehydration and decomposition, respectively. Similar behavior was observed for HPγCD, the broad endothermic peak at 87.2°C indicated dehydration associated with water loss from solid HPγCD. Figure 2B shows the DSC curves of the complexes of dexamethasone with pure γCD and HPγCD as well as with their mixtures. The dexamethasone/CD molar ratio was 1:1. The dexamethasone endothermic peak was shifted to higher temperature in binary dexamethasone/HPγCD and dexamethasone/({γCD/HPγCD)} (20/80) complexes. The disappearance of the dexamethasone endothermic peak in the binary FD products which used dexamethasone/γCD and dexamethasone/({γCD/HPγCD)} (80/20) as carriers is a strong evidence of the formation of amorphous entities and/or complexes.

XRD Patterns

Power X-ray diffractograms of pure dexamethasone, γCD, HPγCD and different dexamethasone/CD complexes prepared by FD and PM in 1:1 molar ratio are shown in Figure 3. In all cases, some diffraction peaks attributed to dexamethasone crystals are still detectable in PM system, whereas they are completely absent in the FD system. Thus, it was concluded that the FD products obtained from dexamethasone with individual CDs or their mixtures are completely amorphous while the physical mixtures show some degree of drug crystallinity. These observations were in accordance with the results of the DSC studies. However, the diffractograms cannot be used as a confirmation of inclusion or non-inclusion complex formation.
Figure 2. DSC thermograms. Pure compounds (A), 1:1 (dexamethasone:CD) molar ratio of physical mixture (PM) and freeze-dried product (FD) of dexamethasone in γCD, HPγCD and γCD/HPγCD mixture (80/20) and (20/80) (B).

Figure 3. X-ray diffraction spectra. Pure compounds (A), 1:1 (dexamethasone:CD) molar ratio of physical mixture (PM) and freeze-dried product (FD) of dexamethasone in γCD and γCD/HPγCD mixture (80/20) (B), 1:1 molar ratio of physical mixture (PM) and freeze-dried product (FD) of dexamethasone in HPγCD and γCD/HPγCD mixture (20/80) (C).
In vitro dissolution studies

Figure 4 shows the dexamethasone dissolution profiles from 3 different dexamethasone/CD binary systems, with molar ratio of 1:4, 1:10 and 1:20, where the binary γCD, HPγCD complexes are compared to the ternary γCD/HPγCD 80/20 and 20/80 complexes. Dissolution efficiency (DE₁₈₀) values based on the dissolution profiles were calculated (35), and the time needed to dissolve 50% of drug (t₅₀%) together with dissolution rate constant (K₁) were calculated from the slopes of the first order linear plots of the dissolution profiles (Table 2). One-way analysis of variance was used to evaluate the statistical significance of difference between binary and ternary dexamethasone/CD complexes. Significance of difference in the means was tested using Scheffe at 95% confidence. In all cases the drug/HPγCD complex exhibited higher rates of dissolution than those of γCD. Interestingly, the ternary γCD/HPγCD 20/80 complexes displayed significantly higher DE₁₈₀ than the corresponding binary HPγCD (p < 0.05). DE₁₈₀ of dexamethasone/HPγCD and dexamethasone/(γCD/HPγCD) 20/80 displayed significant decrease with increasing drug:CD molar ratio, i.e., when the molar ratio of the FD complexes was increased from 1:4 to 1:20 (Table 2) (p < 0.05). Dexamethasone/γCD and dexamethasone/(γCD/HPγCD) 80/20 had the lowest dissolution rate. The dissolution rate constants were increased by as much as 67% when pure γCD was replaced by a γCD/HPγCD (80/20) mixture.

Determination of physico-chemical parameters

The pH value, osmolality, viscosity, degree of flocculation, redispersion time, surface activity and particle size of each of the four formulations are presented in Table 3. The pH was initially adjusted to 7.4 but it was shifted to 5.0-6.5 during production of the eye drop suspensions. Osmolalities of these formulations increased with increasing poloxamer and HPγCD concentrations. Acceptable values were obtained for formulations F1 and F2 (within the 260-330 mOsm/kg range) while formulations F3 and F4 were hypertonic. The degree of flocculation was related to the redispersion time, i.e., the less degree of flocculation the less time was needed to regenerate homogenous suspensions. The surface tension values of these formulations were within the physiological value of the lachrymal fluid’s surface tension (40-50 mN/m) in humans (36). The particle sizes of formulations containing 2.5% or 5% poloxamer 407 without CD, as determined by laser diffraction (LD) and microscopic methods, were approximately 7.5 μm (data not shown). The particle size decreased to about 2.5 to 3.3 μm upon addition CD.

Table 2. Dissolution parameters of different dexamethasone/cyclodextrin solid complexes

| Sample | Ratio | DE₁₈₀ (Mean ± SD) | t₅₀% (h) | K₁ (min⁻¹) | Increase on K₁ (folds) |
|--------|-------|-------------------|---------|------------|-----------------------|
| 1:4    | 100:0 | 4.46 ± 0.46       | 18.8    | 0.046      | -                     |
| Dexamethasone/γCD/HPγCD | 80:20 | 5.58 ± 0.63       | 13.2    | 0.068      | 1.48                  |
| 0:100  |      | 71.5 ± 10.8       | 1.9     | 0.723      | -                     |
| 20:80  |      | 85.2 ± 6.0        | 1.7     | 0.861      | 1.19                  |
| 1:10   | 100:0 | 5.12 ± 0.58       | 12.9    | 0.070      | -                     |
| Dexamethasone/γCD/HPγCD | 80:20 | 6.72 ± 0.84       | 8.3     | 0.117      | 1.67                  |
| 0:100  |      | 29.7 ± 2.7        | 3.2     | 0.392      | -                     |
| 20:80  |      | 51.8 ± 2.9        | 2.3     | 0.581      | 1.48                  |
| 1:20   | 100:0 | 3.56 ± 0.06       | 23.7    | 0.036      | -                     |
| Dexamethasone/γCD/HPγCD | 80:20 | 4.34 ± 0.40       | 14.9    | 0.060      | 1.67                  |
| 0:100  |      | 20.9 ± 2.5        | 5.3     | 0.190      | -                     |
| 20:80  |      | 29.4 ± 5.4        | 3.9     | 0.272      | 1.43                  |

1Molar:molar ratio
2Ratio of γCD/HPγCD (w/w)
3Dissolution efficiency: area under the dissolution curve at 180 min is expressed as percent of the area of the triangle described by 100% dissolution in the same time.
4Time needed to dissolve 50% of the drug.
5First order dissolution rate constant.
6Ratio between K₁ of CD mixture complexes and K₁ of either pure γCD or HPγCD.

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The pH value, osmolality, viscosity, degree of flocculation, redispersion time, surface activity and particle size of each of the four formulations are presented in Table 3. The pH was initially adjusted to 7.4 but it was shifted to 5.0-6.5 during production of the eye drop suspensions. Osmolalities of these formulations increased with increasing poloxamer and HPγCD concentrations. Acceptable values were obtained for formulations F1 and F2 (within the 260-330 mOsm/kg range) while formulations F3 and F4 were hypertonic. The degree of flocculation was related to the redispersion time, i.e., the less degree of flocculation the less time was needed to regenerate homogenous suspensions. The surface tension values of these formulations were within the physiological value of the lachrymal fluid’s surface tension (40-50 mN/m) in humans (36). The particle sizes of formulations containing 2.5% or 5% poloxamer 407 without CD, as determined by laser diffraction (LD) and microscopic methods, were approximately 7.5 μm (data not shown). The particle size decreased to about 2.5 to 3.3 μm upon addition CD.
Figure 4. Dissolution profiles of dexamethasone:CD complexes in PBS pH 7.4 at ambient temperature (22-23°C); 1:4 (dexamethasone:CD) complexes (A); 1:10 (dexamethasone:CD) complexes (B); 1:20 (dexamethasone:CD) complexes (C); dexamethasone:γCD (◆); dexamethasone:HPγCD (■); dexamethasone:γCD/HPγCD (80/20) (◇); dexamethasone:γCD/HPγCD (20/80) (□).

Table 3. Some physicochemical characterizations of the dexamethasone eye drop formulations

| Formulation | Final pH | Osmolality (mOsm/kg) | Viscosity1 (cps) | Degree of Flocculation | Ease of Redispersion (sec) | Surface activity (mN/m) | Particle size LD (μm; Mean±S.D.) | Microscopy (μm; Mean±S.D.) |
|-------------|----------|----------------------|-----------------|------------------------|---------------------------|------------------------|----------------------------------|-------------------------------|
| F1          | 6.58     | 310 ± 16             | 12.9            | 1.31                   | 8.5 ± 1.2                 | 45.0 ± 0.5             | 5.79 ± 0.46                     | 2.60 ± 0.13                   |
| F2          | 5.38     | 315 ± 38             | 11.6            | 1.18                   | 3.9 ± 0.9                 | 41.9 ± 0.9             | 2.50 ± 0.56                     | 2.53 ± 1.90                   |
| F3          | 5.55     | 344 ± 9              | 8.13            | 1.23                   | 5.7 ± 2.2                 | 41.5 ± 0.2             | 4.16 ± 0.53                     | 3.25 ± 0.16                   |
| F4          | 5.04     | 400 ± 6              | 7.69            | 1.09                   | 3.2 ± 1.6                 | 39.6 ± 0.5             | 3.44 ± 0.49                     | 2.62 ± 1.36                   |

1 Determined at 100 rpm, 25°C

Scanning Electron Microscopy (SEM)

SEM images of these formulations are shown in Figure 5. The particle sizes obtained by this method are in agreement with those obtained by laser diffraction and light microscopy. It was difficult to discriminate between dexamethasone and cyclodextrin particles. However, the particle shape was different when the formulation contained the stabilizer, i.e., 2.5% of poloxamer 407 gave cubic shape particles while the formulation containing 5% poloxamer 407 gave smaller and more irregular shaped particles.

Chemical stability

The dexamethasone eye drop formulations were chemically stable for at least 8 months under both storage conditions, i.e., at both 25°C and 40°C (Table 4). This indicates that CD and polymer combinations can provide aqueous ophthalmic dexamethasone preparations that are chemically stable.

Permeation studies

Table 5 displays both total amount and the amount of dissolved dexamethasone in the aqueous eye drop formulations as well as the permeation parameters for dexamethasone from the aqueous eye drop suspensions through semipermeable cellophane membrane with MWCO 12-14000 and porcine sclera. The polymer was unable to permeate the artificial and the biological membrane but the drug and drug/CD complexes were able to permeate through the membranes into the receptor medium. Other types of drug delivery systems have been shown to be able to deliver dexamethasone through sclera in in vitro models (37). In our previous study, the CE of dexamethasone/γCD/HPγCD 60/40 complex was almost 3 times higher than that of the dexamethasone/γCD/HPγCD 80/20 complex (32).
Table 4. Chemical stability of the dexamethasone eye drop formulations' storage at 25°C and 40°C

| Formulation | Time (months) | Temperature (°C) |
|-------------|---------------|------------------|
|             | 25            | 40               |
| F1          | 0             | 14.13 ±0.23      | 14.47 ±0.71 |
|             | 2             | 14.22 ±0.23      | 14.64 ±0.54 |
|             | 3             | 14.22 ±0.59      | 14.42 ±0.07 |
|             | 4             | 14.57 ±0.11      | 14.47 ±0.05 |
|             | 8             | 14.37 ±0.52      | 14.49 ±0.64 |
| F2          | 0             | 12.34 ±0.47      | 13.53 ±1.16 |
|             | 2             | 12.30 ±1.10      | 13.24 ±0.54 |
|             | 3             | 11.97 ±0.40      | 13.80 ±1.97 |
|             | 4             | 12.27 ±0.10      | 13.97 ±0.58 |
|             | 8             | 12.73 ±0.22      | 13.54 ±1.19 |
| F3          | 0             | 13.77 ±0.17      | 13.60 ±0.16 |
|             | 2             | 13.77 ±0.48      | 13.96 ±0.86 |
|             | 3             | 14.08 ±0.26      | 14.16 ±0.44 |
|             | 4             | 14.57 ±0.28      | 14.18 ±0.24 |
|             | 8             | 14.81 ±0.05      | 13.70 ±0.12 |
| F4          | 0             | 13.14 ±0.63      | 13.18 ±0.68 |
|             | 2             | 13.02 ±0.33      | 13.28 ±1.04 |
|             | 3             | 13.22 ±0.35      | 13.12 ±0.38 |
|             | 4             | 13.23 ±0.91      | 13.03 ±0.52 |
|             | 8             | 12.91 ±1.97      | 13.14 ±0.28 |

1Data represent to dexamethasone concentration (mg/ml), (mean±S.D.); n=3

Therefore, the γCD/HPγCD 80/20 and 60/40 complexes were selected to investigate the complexation effects on dexamethasone membrane permeability. Although the concentration of dissolved dexamethasone and the dexamethasone flux (J) from the γCD formulations (Table 5) were smaller than those of formulations containing mixtures of γCD/HPγCD (i.e., F1 to F4), the permeation coefficient (P_{app}) was 1.5 times higher most probably due to the smaller MW (i.e., smaller hydrodynamic radius) of the dexamethasone/γCD complex. The concentration of dissolved dexamethasone in the eye drop formulations consisting of γCD/HPγCD 60/40 (i.e., F3 and F4) was 2-fold higher than those containing γCD/HPγCD 80/20 (i.e., F1 and F2) leading to significant higher permeation flux through semipermeable membrane (p < 0.05). Increasing the poloxamer 407 concentration from 2.5% (i.e., F1 and F3) to 5% w/v (i.e., F2 and F4) resulted in small increase of dissolved dexamethasone. However, poloxamer 407 affected values of both J and P_{app}, i.e., increasing the poloxamer 407 concentration decreased the J and P_{app} values. The dexamethasone fluxes from eye drop suspensions through semipermeable cellophane membrane MWCO 12-14,000 or sclera increased in the following order: F3 > F4 > F1 > F2. Sclera was about 20 to 50 times less permeable than the semipermeable cellophane membrane.

DISCUSSION

The FT-IR spectroscopic studies indicate that some fraction of dexamethasone is in the free uncomplexed form in the HPγCD and γCD/HPγCD (20:80) samples while the drug is mainly found as CD complexes in the γCD and γCD/HPγCD (80:20) samples. The observed slight change in the DSC thermographs in the PM samples relative to the peak of pure dexamethasone suggests a weak interaction between the pure dexamethasone and CD attributed to the mixing or sample heating during DSC scanning. For all complexed systems, the dehydration temperature of CDs and mixtures thereof are lower than the dehydration temperature of pure CDs, indicating that the inclusion of dexamethasone into cavities results in weakening of bonds between the remaining water molecules. Similar observations have been reported in the literature (38, 39).
Table 5. Total and dissolved dexamethasone content, flux and apparent permeation coefficient ($P_{app}$) of the four dexamethasone eye drop formulations, and two reference formulations, through semipermeable membrane MWCO 12-14000 or porcine sclera.

| Formulation | Total dexamethasone content (mg/ml) | Dissolved dexamethasone content (mg/ml) | % dissolved dexamethasone | Permeation studies (MWCO 12-14000)$^3$ | Permeation studies (sclera)$^4$ |
|-------------|-----------------------------------|----------------------------------------|---------------------------|-----------------------------------------|---------------------------------|
|             | Flux ± S.D. ($\mu$g h$^{-1}$ cm$^{-2}$) | $P_{app}$ ± S.D. ($x$ 10$^{-6}$ cm s$^{-1}$) | Flux ± S.D. ($\mu$g h$^{-1}$ cm$^{-2}$) | $P_{app}$ ± S.D. ($x$ 10$^{-6}$ cm s$^{-1}$) |
| Ref.1$^1$  | 12.50 ± 0.67                          | 1.71 ± 0.01                            | 13.66                      | 60.29 ± 0.47                           | 9.79 ± 0.08 n.d. n.d.          |
| Ref.2$^2$  | 9.47 ± 0.29                           | 1.45 ± 0.04                            | 15.31                      | 50.84 ± 2.01                           | 9.74 ± 0.39 n.d. n.d.          |
| F1         | 13.69 ± 0.09                           | 4.86 ± 0.05                            | 35.49                      | 114.47 ± 16.34                         | 6.54 ± 0.93 4.81 ± 0.78 0.27 ± 0.04 |
| F2         | 12.07 ± 0.91                           | 4.87 ± 0.02                            | 40.60                      | 107.49 ± 7.01                          | 6.13 ± 0.40 2.10 ± 1.07 0.12 ± 0.05 |
| F3         | 13.94 ± 0.16                           | 8.52 ± 0.06                            | 61.13                      | 198.07 ± 15.58                         | 6.46 ± 0.51 6.55 ± 1.67 0.21 ± 0.04 |
| F4         | 12.60 ± 0.04                           | 8.75 ± 0.01                            | 69.48                      | 176.42 ± 9.36                          | 5.60 ± 0.30 5.22 ± 3.13 0.17 ± 0.07 |

$^1$Reference dexamethasone eye drop formulation containing (18% w/v) γCD and (2.5% w/v) poloxamer 407.

$^2$Reference dexamethasone eye drop formulation containing (18% w/v) γCD and (5% w/v) poloxamer 407.

$^3$Single membrane; n = 3-5

$^4$n.d. = not determined

The complete disappearance of the dexamethasone peak in the thermograms indicates loss of crystallinity and formation of an amorphous solid dispersion, possibly due to complex formation. Also the XPD patterns of the dexamethasone/CD complexes show that they are completely amorphous. Once the data is not a strong evidence of dexamethasone/CD complex formation. Loss of drug crystallinity can be a consequence of the lyophilization process, i.e., the X-ray data can not distinguish between true inclusion complexes and homogeneous molecular mixtures of drug and CD (40). The conclusion of these solid-state characterization studies is that dexamethasone forms partial solid inclusion complexes with γCD while it most likely formed both inclusion and non-inclusion complexes with HPγCD and γCD/HPγCD mixtures.

The dissolution studies show that HPγCD complexation of dexamethasone results in faster dissolution and higher dissolution efficiency than the parent γCD, most probably due to higher solubility of the dexamethasone/HPγCD complex. The dissolution efficiency depended also on the amount of CD, i.e., the dissolution efficiency decreased with increasing drug/CD ratio. Thus, the rapid release or retard release characteristics could be predesigned by adjusting the ratio of γCD/HPγCD and/or the total amount of CD included in the formulation.

The aqueous solubility of the dexamethasone/CD complexes can be controlled by adjusting the γCD/HPγCD ratio in the complex. Thus mixtures of γCD/HPγCD were selected for further development of dexamethasone eye drop formulations (Table 1). Although the ideal pH for comfortable instillation the eye drops to the eye is 7.2 ± 0.2 (41) the buffer capacity of the tears is able to adjust the pH to physiological levels upon administration of eye drops with no or very low buffer capacity. The osmolality, viscosity, degree of flocculation and re-dispersion time were acceptable for an eye drop suspension (Table 3). All tested formulations were physically stable, i.e., the particles did not aggregate and were easily resuspended by a moderate amount of agitation (33, 42). As expected, the surface tension decreased with increasing poloxamer (P407) concentration. When the formulations are compared it can be seen that the particle size decreases with increasing HPγCD and poloxamer concentration.
The particulate samples frequently contain agglomerates or aggregates and laser diffraction (LD) techniques cannot distinguish between scattering by single particles and scattering by clusters of primary particles. Therefore, in most cases, the particle size obtained by the laser diffraction technique is larger than those obtained by the optical microscopic method. The particle sizes in all four formulations were less than 10 μm which minimizes the likelihood of particle eye irritation and consequent increase in tear flow that can lead to decreased drug residence time on the eye surface (43). SEM images of these formulations confirmed that the particle size and shape were influenced by poloxamer concentration (Figure 5). All investigated dexamethasone eye drop formulations are chemically stable for at least 8 months under ambient and accelerated storage conditions. These formulations were investigated further for the effect of CDs and polymer on *in vitro* permeation through semipermeable membrane and *ex vivo* permeation through porcine sclera tissue.

Increasing the HPγCD concentration increased the fraction of dissolved dexamethasone that again lead to significant increase in the dexamethasone flux through the semipermeable cellophane membrane. On the other hand increasing the poloxamer concentration resulted in decreased flux even though this lead to increased dexamethasone solubility (Table 5). Frequently, combination of different solubility techniques, such as combinations of CD complexation and polymer addition, results in synergistic effect. For example,

![SEM images of the dexamethasone eye drop formulations; F1 (A); F2 (B); F3 (C); F4 (D)](image)

*Figure 5. SEM images of the dexamethasone eye drop formulations; F1 (A); F2 (B); F3 (C); F4 (D)*
combinations of γCD and HPγCD and combinations of αCD and HPβCD have been shown to have synergistic effect although the mechanism of the effect is not known (32, 44). Addition of polymer leads to formation of co-complex consisting of the drug, CD and polymer that enhances the intraocular availability of drug (45). Also here poloxamers increases the solubilization but, perhaps due to micellar formation, the drug permeation was decreased (see $P_{\text{app}}$ values obtained from formulation 4) (46). The surface area of sclera has been estimated to be about 17-fold larger than that of cornea (47). Transscleral drug delivery is being investigated as an approach for the delivery of therapeutic agents such as steroids, carboplatin, and methotrexate to the back of the eye for the treatment of posterior segment diseases (48). Also the in vitro transscleral permeation studies indicate that although poloxamers increases the fraction of dissolved drug in the aqueous eye drop formulation increasing the poloxamer concentration will most likely result in decreased topical drug availability.

CONCLUSIONS

Formation of dissolved and solid dexamethasone/CD complexes affected the drug permeation through both cellophane and sclera. The composition of formulation has to be carefully designed to the desired drug delivery profile. Type and amount of both CD and stabilizer lead to important modifications in the physicochemical and biological properties of the eye drop formulation. Furthermore, dissolution and permeation studies have shown that drug availability from formulation containing CD mixtures can be much greater than from comparable formulations of individual CDs. In addition, this study shows that it is possible to reduce amount of γCD in the preparation by adding HPγCD to the medium without decreasing bioavailability of drug. The sustained or rapid release of drug can be designed by changing the γCD/HPγCD ratio in the aqueous eye drop formulation and by changing the amount of polymer stabilizer. The topical dexamethasone eye drop suspension does possess adequate physical and chemical stability and appears to be able to deliver drug through sclera.

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NOVELTY OF THE WORK

This study describes the benefits of γCD/HPγCD mixtures on drug solubilization, dissolution and permeation all of which are important features of an effective aqueous eye drop formulation. One of the most important findings was that the drug release could be controlled by adjusting the γCD:HPγCD ratio in the solid drug complexes which enabled anything from rapid to sustained drug release. Furthermore, the diameter of the drug/γCD/HPγCD complex particles could be controlled by the γCD:HPγCD ratio and by the amount of stabilizer added to the aqueous eye drop suspension.

REFERENCES

1. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Delivery Rev, 2007; 59:645-666.
2. Loftsson T, Duchene D. Cyclodextrins and their pharmaceutical applications. Int J Pharm, 2007; 329:1-11.
3. Loftsson T, Vogensen SB, Brewster ME, Konradsdottir F. Effects of cyclodextrins on drug delivery through biological membranes. J Pharm Sci, 2007; 96:2532-2546.
4. Luo LB, Chen HL, Wang F, Dai QP, Tang WX. Thermolysis of β-cyclodextrin/alkylcobaloxime inclusion complexes in the solid state. Thermochim Acta, 1997; 298:129-134.
5. Palmieri GF, Giovannucci G, Antonini I, Martelli S. Inclusion complexation of fenofibrate with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. Evaluation of interactions in solution and solid complex characterization. STP Pharma Sci, 1997; 7:174-181.
6. Mielcarek J. Photochemical stability of the inclusion complexes of nicardipine with α-, γ-cyclodextrin, methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin in the solid state and in solution. Pharmazie, 1996; 51:477-479.
7. Spulber M, Pinteala M, Fifere A, Harabagiu V, Simionescu BC. Inclusion complexes of 5-flucytosine with β-cyclodextrin and hydroxypropyl-β-cyclodextrin: characterization in aqueous solution and in solid state. J Inclusion Phenom Macro cyclic Chem, 2008; 62:117-125.
8. Amato ME, Lipkowitz KB, Lombardo GM, Pappalardo GC. NMR spectroscopic evidence and molecular dynamics studies on inclusion and non-inclusion phenomena between β-cyclodextrin and new anti-Alzheimer’s drugs tacrine (CI-970), velnacrine (HP-029) and suronacrine (HP-128). J Chem Soc., Perkin Trans, 1996; 2:321-325.
9. Duan MS, Zhao N, Ossurardottir IB, Thorsteinsson T, Loftsson T. Cyclodextrin solubilization of the antibacterial agents triclosan and triclocarban:
Formation of aggregates and higher-order complexes. Int J Pharm, 2005; 297:213-222.
10. Loftsson T, Masson M, Brewster ME. Self-association of cyclodextrins and cyclodextrin complexes. J Pharm Sci, 2004; 93:1091-1099.
11. He YF, Fu P, Shen XM, Gao HC. Cyclodextrin-based aggregates and characterization by microscopy. Micron, 2008; 39:495-516.
12. Jansook P, Kurkov SV, Loftsson T. Cyclodextrin as solubilizers: Formation of complex aggregates. J Pharm Sci, 2010; 99:719-729.
13. Loftsson T, Jarvinen T. Cyclodextrins in ophthalmic drug delivery. Adv Drug Delivery Rev, 1999; 36:59-79.
14. Loftsson T, Frioriksdottir H, Sigurardottir AM, Ueda H. The effect of water-soluble polymers on drug-cyclodextrin complexation. Int J Pharm, 1994; 110:169-177.
15. Jarho P, Pate DW, Brenneisen R, Jarvinen T. Hydroxypropyl-β-cyclodextrin and its combination with hydroxypropyl-methylcellulose increases aqueous solubility of Δ(9)-tetrahydrocannabinol. Life Sci, 1998; 63:PI381-PI384.
16. Loftsson T, Frioriksdottir H. The effect of water-soluble polymers on the aqueous solubility and complexing abilities of β-cyclodextrin. Int J Pharm, 1998; 163:113-121.
17. Ribeiro LSS, Ferreira DC, Veiga FJB. Physicochemical investigation of the effects of water-soluble polymers on vinpocetine complexation with β-cyclodextrin and its sulfobutyl ether derivative in solution and solid state. Eur J Pharm Sci, 2003; 20:253-266.
18. Kaur IP, Smitha R, Aggarwal D, Kapil M. Acetazolamide: future perspective in topical glaucoma therapeutics. Int J Pharm, 2002; 248:1-14.
19. Cappello B, Carmignani C, Iervolino M, Immacolata La Rotonda M, Fabrizio Saettone M. Solubilization of tropicamide by hydroxypropyl-β-cyclodextrin and water-soluble polymers: in vitro/in vivo studies. Int J Pharm, 2001; 213:75-81.
20. Ludwig A. The use of mucoadhesive polymers in oculcar drug delivery. Adv Drug Delivery Rev, 2005; 57:1595-1639.
21. Ma WD, Xu H, Wang C, Nie SF, Pan WS. Pluronic F127-g-poly(acrylic acid) copolymers as in situ gelling vehicle for ophthalmic drug delivery system. Int J Pharm, 2008; 350:247-256.
22. El-Kamel AH. In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate. Int J Pharm, 2002; 241:47-55.
23. Qi H, Li L, Huang C, Li W, Wu C. Optimization and physicochemical characterization of thermosensitive polymers gel containing pantar in for ophthalmic use. Chem Pharm Bull (Tokyo), 2006; 54:1500-1507.
24. Kim EY, Gao ZG, Park JS, Li H, Han K. rhEGF/HP-β-CD complex in polymer gel for ophthalmic delivery. Int J Pharm, 2002; 233:159-167.
25. Edsman K, Carlforss J, Petersson R. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur J Pharm Sci, 1998; 6:105-112.
26. Bejjani RA, Behar-Cohen F, Benezra D, Gurny R, Delie F. Polymeric nanoparticles for drug delivery to the posterior segment of the eye. Chimia, 2005; 59:344-347.
27. Kim SH, Lutz RJ, Wang NS, Robinson MR. Transport barriers in transscleral drug delivery for retinal diseases. Ophthalmic Res, 2007; 39:244-254.
28. Loftsson T, Heinsdottir D, Stefansson E. Cyclodextrin microparticles for drug delivery to the posterior segment of the eye: aqueous dexamethasone eye drops. J Pharm Pharmacol, 2007; 59:629-635.
29. Sigurdsson HH, Konraethsdottir F, Loftsson T, Stefansson E. Topical and systemic absorption in delivery of dexamethasone to the anterior and posterior segments of the eye. Acta Ophthalmol Scand, 2007; 85:598-602.
30. Sigurdsson HH, Stefansson E, Gudmundsdottir E, Eysteinsson T, Thorsteinsdottir M, Loftsson T. Cyclodextrin formulation of dorzolamide and its distribution in the eye after topical administration. J Control Release, 2005; 102:255-262.
31. Jansook P, Loftsson T. CDs as solubilizers: Effects of excipients and competing drugs. Int J Pharm, 2009; 379:32-40.
32. Jansook P, Loftsson T. γCD/HP]-CD: Synergistic solubilization. Int J Pharm, 2008; 363:217-219.
33. Martin, A. Physical Pharmacy. 4th ed., Lippincott William an Wilkins, Baltimore, USA, pp 478-481, 2001.
34. Council of Europe. European Pharmacopoeia. 6th ed., Strasbourg, France, 2006.
35. Khan KA. Concept of dissolution efficiency. J Pharm Pharmac, 1975; 27:48-49.
36. Ciba-Geigly., Wissenschaftliche Tabellen Geigy. 8th ed. Basel: Ciba-Geigy, 1977.
37. Cruysberg LP, Nuijts RM, Geroski DH, Koole LH, Hendrikse F, Edelhauser HF. In vitro human scleral permeability of fluorescein, dexamethasone-fluorescein, methotrexate-fluorescein and rhodamine 6G and the use of a coated coil as a new drug delivery system. J Ocul Pharmacol Ther, 2002; 18:559-569.
38. Meier MM, Luiz MTB, Szpoganicz B, Soldi V. Thermal analysis behavior of β- and γ-cyclodextrin inclusion complexes with capric and caprilic acid. Thermochim Acta, 2001; 375:153-160.
39. Yilmaz VT, Karadag A, Icbudak H. Thermal-decomposition of β-cyclodextrin inclusion complexes of ferrocene and their derivatives. Thermochim Acta, 1995; 261:107-118.
40. Ribeiro L, Loftsson T, Ferreira D, Veiga F. Investigation and physicochemical characterization of vinpocetine-sulfobutyl ether β-cyclodextrin binary and ternary complexes. Chem Pharm Bull (Tokyo), 2003; 51:914-922.
41. Mathis GA. Clinical ophthalmic pharmacology and therapeutics: ocular drug delivery. In Gelatt KN (ed), Veterinary Ophthalmology. Lippincott Williams & Wilkins, Florida pp 291-297, 1999.
42. Arias JL, Lopez-Viota M, Clares B, Ruiz MA. Stability of fenbendazole suspensions for veterinary use Correlation between zeta potential and sedimentation. Eur J Pharm Sci, 2008; 34:257-262.
43. Kassem MA, Rahman AAA, Ghorab MM, Ahmed MB, Khalil RM. Nanosuspension as an ophthalmic delivery system for certain glucocorticoid drugs. Int J Pharm, 2007; 340:126-133.
44. Malaekeh-Nikouei B, Nassirli H, Davies N. Enhancement of cyclosporine aqueous solubility using α- and hydroxypropyl β-cyclodextrin mixtures. J Inclusion Phenom Macrocyclic Chem, 2007; 59: 245-250.
45. Kristinsson JK, Fridriksdottir H, Thorisdottir S, Sigurdardottir AM, Stefansson E, Loftsson T. Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops - Aqueous humor pharmacokinetics in humans. Invest Ophthalmol Vis Sci, 1996; 37: 1199-1203.
46. Amrite AC, Edelhauser HF, Singh SR, Kompella UB. Effect of circulation on the disposition and ocular tissue distribution of 20 nm nanoparticles after periocular administration. Mol Vis, 2008; 14:150-160.
47. Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. Adv Drug Delivery Rev, 2001; 52:37-48.
48. Koevary SB. Pharmacokinetics of topical ocular drug delivery: Potential uses for the treatment of diseases of the posterior segment and beyond. Curr Drug Metab, 2003; 4:213-222.