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Cyclodextrins and drug permeability through semi-permeable cellophane membranes

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Abstract

Determinations of drug fluxes through semi-permeable cellophane membranes are used to evaluate cyclodextrin complexes and cyclodextrin containing drug formulations. In the present study we investigated how the cyclodextrin concentration, the membrane thickness and the molecular weight cut off (MWCO) of the membrane influence drug fluxes. The cyclodextrin used was 2-hydroxypropyl-β-cyclodextrin (HPβCD) and the sample drug was hydrocortisone. The MWCO of the membranes ranged from 500 to 14,000 and the HPβCD concentration ranged from 0 to 25% (w/v). The hydrocortisone flux from saturated solutions through the MWCO 500 membrane was unaffected by the cyclodextrin concentration. When MWCO of the membrane was greater than the molecular weight of the complex the flux from solutions saturated with hydrocortisone increased with increasing HPβCD concentration. This increase showed negative deviation from linearity. When the flux was corrected for the viscosity increase with increasing HPβCD concentration then the flux pattern could be described on the basis of Fick's first law and Stokes–Einstein equation. However, the flux did not correlate with the viscosity when it was increased by adding polymer to the saturated drug solutions. It was shown that the observed flux pattern was consistent with self-association of cyclodextrin complexes in the aqueous donor phase.

Keywords: Semi-permeable membranes; Cyclodextrin; Flux; Complexation; Self-association

1. Introduction

Cyclodextrins are cyclic oligosaccharides with hydrophilic outer surface and somewhat lipophilic central cavity. In aqueous solutions cyclodextrins are able to form inclusion complexes with many drugs by taking up some hydrophobic moieties of the drug molecules into the central cavity. No covalent bonds are formed or broken during the complex formation and in solution molecules bond within the complex are in dynamic equilibrium with free molecules (Loftsson and Brewster, 1996; Rajewski and Stella, 1996; Irie and Uekama, 1997). Cyclodextrin complexation of drugs will change many of the drugs physicochemical properties, such as their aqueous solubility and chemical stability, without affecting their intrinsic ability to permeate lipophilic biomembranes. Cyclodextrins have been used as penetra-

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tion enhancers in topical drug formulations (Uekama et al., 1998; Loftsson and Järvinen, 1999; Masson et al., 1999; Matsuda and Arima, 1999).

It is not well understood how cyclodextrins act as permeation enhancers and a number of possible mechanisms have been suggested (Loftsson and Ólafsson, 1998). However, it is generally believed that the hydrophilic cyclodextrins act as true carriers by keeping hydrophobic drug molecules in solution and delivering them to the lipophilic membrane surface where they partition from the cyclodextrin cavity into the lipophilic membrane (Uekama et al., 1998; Loftsson and Järvinen, 1999). The lipophilic membrane has low affinity for the large hydrophilic cyclodextrin molecules and their complexes, which thus remain in the aqueous exterior. For example, only 0.02% of topically applied 2-hydroxypropyl-β-cyclodextrin (HPβCD) was absorbed into hairless mouse skin under occlusive conditions (Tanaka et al., 1995). In aqueous cyclodextrin solutions saturated with drug, the drug flux through a biomembrane increases with increasing cyclodextrin concentration. On the other hand, if the drug concentration is kept constant and below saturation, the flux will decrease with increasing cyclodextrin concentration (Sigurdardottir and Loftsson, 1995; Jarho et al., 1996b,a; Masson et al., 1999). These observations have been explained by the existence of an aqueous diffusion barrier at the membrane surface (Masson et al., 1999; Loftsson and Masson, 2001). Such aqueous diffusion barriers could resemble hydrophilic semi-permeable membranes.

Determinations of drug fluxes through semi-permeable cellophane membranes have been used to investigate release of drugs from cyclodextrin containing vehicles as well as to obtain stability constants of drug/cyclodextrin complexes (Masson et al., 1999; Ono et al., 1999). Permeation of drug molecules from aqueous cyclodextrin containing vehicles through semi-permeable cellophane membranes follows the same pattern as permeation through lipophilic biomembranes. This flux pattern is obtained for membranes with molecular weight cut-off (MWCO) well above the molecular weight of cyclodextrin. Since both the cyclodextrin molecules and their complexes are able to permeate the membranes the suggestion of an aqueous diffusion barrier at the surface of a membrane, which is only permeable to the lipophilic drug but not to the hydrophilic cyclodextrin molecules, does not satisfactorily explain these observations.

The purpose of the present study was to investigate the effects of cyclodextrins on drug permeability through semi-permeable membranes of different MWCO, in an effort to elucidate the mechanism of drug permeability from aqueous cyclodextrin solutions through semi-permeable cellophane membranes.

2. Materials and methods

2.1. Materials

Hydrocortisone was purchased from Norsk Medicinale Depot (Oslo, Norway), 2-hydroxypropyl-β-cyclodextrin with molar substitution of 0.64 (HPβCD; Encapsin™ HPB) from Janssen Biotech (Olen, Belgium), polyvinylpyrrolidone of average molecular weight 40,000 (PVP) from Sigma Chemical Co. (St. Louis, Missouri), and hydroxypropyl methylcellulose 4000 (HPMC) from Mecobenzon (Copenhagen, Denmark). All other chemicals were commercially available products of special reagent grade. Semi-permeable cellophane membranes (Spectra/Por® Dialysis Tubing from regenerated cellulose) of MWCO 3500 (No. 3), 6000–8000 (No. 1) and 12,000–14,000 (No. 2), as well as semi-permeable cellophane membrane (Spectra/Por® CE Dialysis membrane from cellulose esters) of MWCO 500 were purchased from Spectrum Laboratories (Houston, TX). The moisture content of HPβCD was periodically determined and corrected for (Scaltec SMO 01 Moisture Analyzer, Göttingen, Germany)

2.2. Permeation studies

The permeability of hydrocortisone from aqueous HPβCD solutions through the semi-permeable cellophane membranes was studied using Franz diffusion cells (FDC 400 15FF, Vangard International, Neptune, NJ) at room temperature

(22–23 °C). The receptor phase consisted of pH 7.4 aqueous 8.0×10^{-3} M phosphate buffer solution containing 0.7% (w/v) sodium chloride and 2.5% (w/v) HPβCD, stirred with a magnetic bar. HPβCD was added to the receptor phase to ensure sufficient drug solubility. The receptor phase was sonicated under vacuum to remove dissolved air before it was placed in the receptor chamber (12 ml). The donor phase consisted of solution of hydrocortisone in aqueous HPβCD solutions, which had been heated in an autoclave (121 °C for 20 min) to promote complex formation. After equilibration for at least 3 days at room temperature, 2 ml of the filtered donor phase (Spartan 0.45 μm membrane filters from Schleider&Schull, Dassel, Germany) were applied to the membrane surface (1.77 cm^2). When polymer was added to the donor phase to increase its viscosity then the polymer was added after the heating process and equilibration at room temperature. Samples (50 μl) were withdrawn from the receptor phase at various time points for up to 48 h and replaced with fresh receptor phase. The samples were kept frozen until analyzed by HPLC. The flux was calculated from the linear part of each permeability profile. No changes in the volumes of the donor and receptor phases were observed during the 48-h study period.

The viscosity of selected donor phases was determined at room temperature in a Brookfield digital viscometer Model DV-1 + (Brookfield, Middleboro, MA) equipped with a Brookfield UL adapter.

2.3. Solubility determinations

The solubilities of hydrocortisone in the various aqueous HPβCD solutions were determined by adding excess amounts of hydrocortisone to the solutions. The suspensions formed were heated in an autoclave (121 °C for 20 min) in sealed containers. After cooling to room temperature the containers were opened and small amounts of solid hydrocortisone added to each container to promote precipitation of hydrocortisone. Then the containers were closed and allowed to equilibrate for 1 week at room temperature. After equilibration was attained, an aliquot of the suspension was filtered through Spartan 0.45 μm membrane filter, diluted with 70% (v/v) methanol in water if necessary and analyzed by HPLC.

2.4. HPLC analysis

The quantitative determination of hydrocortisone was performed on a high performance liquid chromatographic (HPLC) component system consisting of ConstaMetric 3200 isocratic solvent delivery system operated at 1.50 ml min^{-1}, a Merck-Hitachi AS4000 autosampler, a Luna C_{18} 5 μm (4.6×150 mm) column, a SpectroMonitor 3200 UV/VIS variable-wavelength detector operated at 254 nm and a Merck-Hitachi D-2500 Chromato-Integrator. The mobile phase consisted of acetonitrile/tetrahydrofuran/water (25:1:64, v/v) and the retention time was 2.4 min.

3. Results and discussion

The phase-solubility diagram of hydrocortisone in aqueous HPβCD solutions is shown in Fig. 1. The solubility of hydrocortisone increases linearly with increasing HPβCD concentration. Thus, the phase-solubility diagram is of Higuchi's A_L-type and formation of a hydrocortisone/HPβCD 1:1 complex can be assumed. At room temperature the aqueous solubility of hydrocortisone was determined to be about 0.4 mg ml^{-1} or 1×10^{-3} M^{-1} and apparent stability constant of the hydrocortisone/HPβCD 1:1 complex to be 1400 M^{-1}, as determined by the phase-solubility method (Higuchi and Connors, 1965).

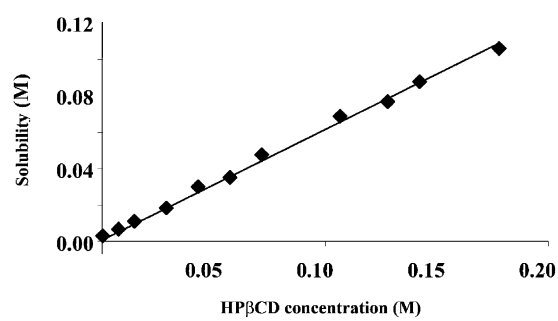


Fig. 1. The phase-solubility diagram of hydrocortisone in aqueous HPβCD solution at room temperature (22–23 °C).

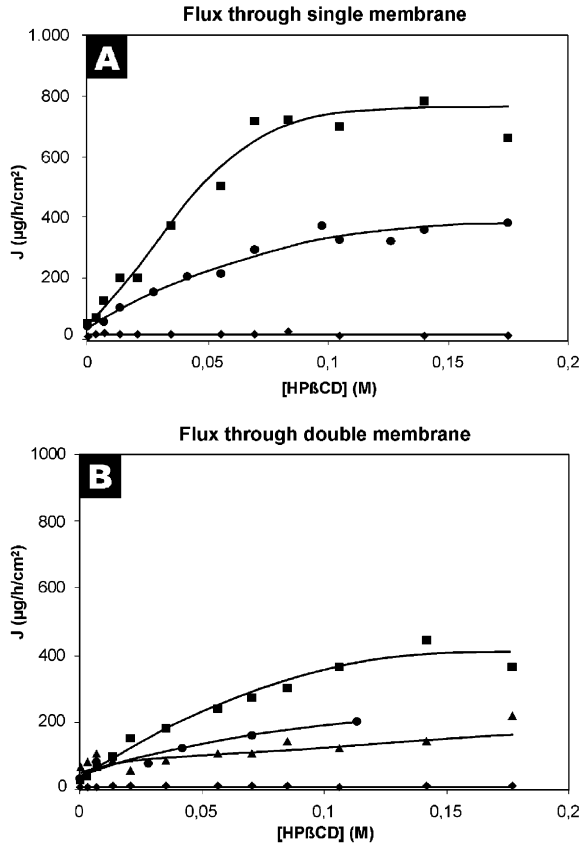


Fig. 2. The effect of HPβCD concentration on the flux (J) of hydrocortisone from aqueous solution, saturated with hydrocortisone, through a single (A) and double (B) layer semi-permeable cellophane membrane. MWCO 500 (◆), MWCO 3500 (▲), MWCO 6000–8000 (●), and MWCO 12,000–14,000 (■).

Fig. 2 shows the flux of hydrocortisone from aqueous HPβCD solution, saturated with hydrocortisone, through a single layer or a double layer of semi-permeable cellophane membranes with MWCO of 500, 3500, 6000–8000 and 12,000–14,000. The semi-permeable cellophane membranes are size exclusion membranes, where molecules permeate through pores in the membrane. According to Fick's first law

$$J = D\frac{(C_1 - C_2)}{h} \approx \frac{D \times C_1}{h}, \quad (1)$$

the flux (J) through a porous membrane is proportional to the diffusion coefficient of the penetrating drug molecules (D) and the drug concentration difference between the donor phase (C_1) and the receptor phase (C_2), divided by the effective thickness of the membrane (h). Under sink conditions, C_2 can be omitted since $C_1 - C_2 \approx C_1$. The flux values in Fig. 2 were determined from saturated solutions, and under such conditions that C_1 is equal to the hydrocortisone solubility at the given HPβCD concentration. Since the phase-solubility diagram is linear (Fig. 1) there should be a linear relationship between the flux and the HPβCD concentration. However, all the diagrams show negative deviation from linearity. The passive diffusion of hydrocortisone through the semi-permeable cellophane membranes appears to be a capacity limited process. The capacity appears to depend on the pore size of the membrane but maximum capacity is usually attained at or below 20% (w/v) HPβCD. In the case of the MWCO 500 membrane the hydrocortisone flux through a single layer membrane was determined to be 8.9 ± 1.1 µg h^{-1} cm^{-2} when no HPβCD was present in the donor phase and 10.8 ± 4.8 µg h^{-1} cm^{-2} when 20% (w/v) HPβCD was present. Similar observations were made when the double layer MWCO 500 membrane was used. Thus, increased HPβCD concentration, and consequent increase in total amount of dissolved hydrocortisone, did not have any noticeable effect on the flux of hydrocortisone through the membrane. This is what should be expected since in saturated solutions the concentration of free hydrocortisone is constant and equal to its intrinsic solubility. The molecular weight of HPβCD is 1404 and, thus, both the HPβCD molecule and the hydrocortisone/HPβCD complex are unable to permeate the MWCO 500 membrane. The difference between the hydrocortisone flux from pure aqueous solution and 20% (w/v) HPβCD solution gradually increased as the pores become larger. The hydrocortisone flux through a single layer of the MWCO 6000–8000 membrane was determined to be 41 ± 6 µg h^{-1} cm^{-2} when no HPβCD was present in the donor phase and 350 ± 20 µg h^{-1} cm^{-2} when 20% (w/v) HPβCD was present. The values for a single layer of the MWCO 12,000–14,000 membrane determined to be 48 ± 1 µg h^{-1}

cm^{-2} and 770 ± 70 μg h^{-1} cm^{-2}, respectively. In the case of the double layer MWCO 12,000–14,000 membrane the values were 26 ± 2 and 440 ± 10 μg h^{-1} cm^{-2}, respectively. These are all average values of three experiments ± the standard deviation.

It is possible that the negative deviation from linearity of the flux versus HPβCD concentrations profile is not a capacity limited process but rather a result of increased viscosity of the donor phase. The viscosity of pure aqueous hydrocortisone solution is 1.00 cPoise but that of aqueous 20% (w/v) HPβCD solution 2.10 cPoise. Fig. 3 shows the effect of HPβCD concentration on the flux of hydrocortisone from aqueous HPβCD solutions through a single layer of the MWCO 6000–8000 membrane. The relationship between the viscosity (η) of the diffusion medium and the diffusion coefficient is given by Stokes-Einstein equation

$$D = \frac{RT}{6\pi\eta r N}, \tag{2}$$

where R is the molar gas constant, T is the absolute temperature, r is the radius of the diffusing molecule (assuming it can be represented by a spherical particle) and N is Avogadro's number. Combining Eqs. (1) and (2) and rearrangement gives

$$J\eta = \frac{RT}{6\pi r N}\frac{C_1}{h}. \tag{3}$$

Since the viscosity of pure aqueous hydrocortisone solution is equal to unity we can define the flux corrected for the apparent viscosity effect (J') as

$$J' = J\frac{\eta}{\eta_{Aq}}, \tag{4}$$

where η is the viscosity of the donor phase, i.e. the aqueous HPβCD solution, and η_{Aq} is the viscosity of water. Fig. 3B shows the relationship between the corrected flux, i.e. J', and the HPβCD concentration. The linear profile obtained indicates that increased viscosity could be the main reason for the negative deviation observed in Figs. 2 and 3A. Thus, the observed increase in viscosity with increasing HPβCD concentration was used to correct for the non-ideality of the donor phase.

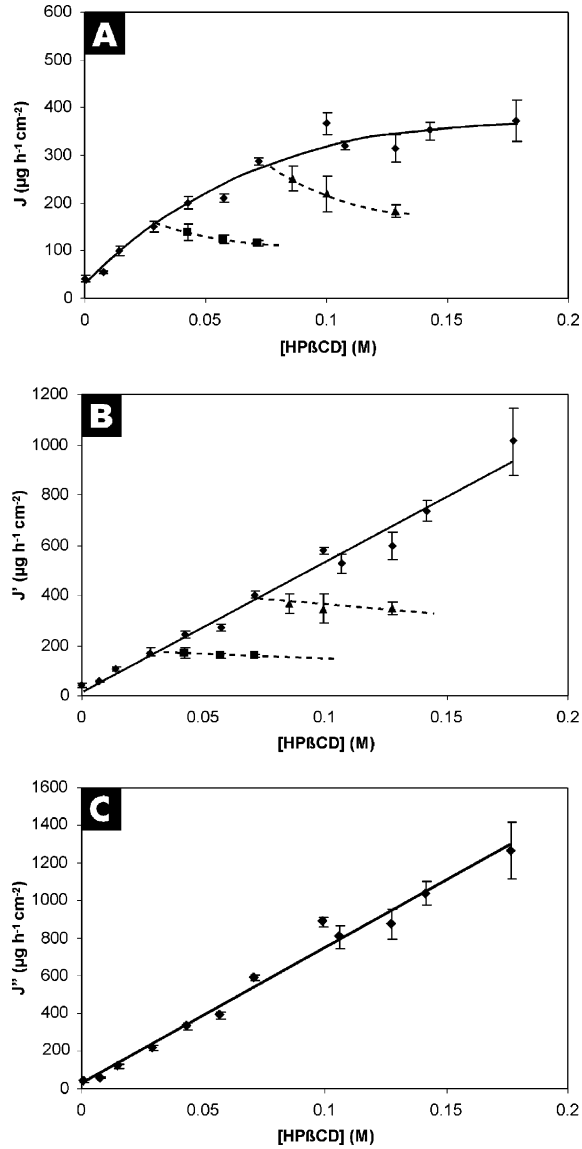


Fig. 3. The effect of HPβCD concentration on the flux of hydrocortisone from aqueous solution, saturated or unsaturated with hydrocortisone, through a single layer of a MWCO 6000–8000 semi-permeable cellophane membrane; as determined (J) (A), corrected for the effect of increased viscosity (J') (B) and corrected for the effect of self-association of the hydrocortisone/HPβCD complexes (J'') (C). Saturated solutions (◆); unsaturated solutions at HPβCD conc. ≥4%, where the hydrocortisone concentration is kept constant and at the same level as in saturated 4% HPβCD solution (■); unsaturated solutions at HPβCD conc. ≥10%, where the hydrocortisone concentration is kept constant and at the same level as in saturated 10% HPβCD solution (▲). The average of three experiments ± standard deviation.

Table 1
The permeability coefficient of free hydrocortisone molecules (P_H) and the viscosity corrected permeability coefficient for the hydrocortisone/HPβCD complex ($P'_{H/CD}$) through single and double layers of the semi-permeable cellophane membranes

MWCO of the cellophane membrane	Single layer membrane		Double layer membrane	
	P_H (cm h^{-1})	($P'_{H/CD}$) (cm h^{-1})	P_H (cm h^{-1})	($P'_{H/CD}$) (cm h^{-1})
500	$2.1 \pm 0.3 \times 10^{-2}$	–	$0.5 \pm 0.1 \times 10^{-2}$	–
6000–8000	$3.9 \pm 0.6 \times 10^{-2}$	2.3×10^{-2}	$2.8 \pm 0.2 \times 10^{-2}$	1.2×10^{-2}
12,000–14,000	$10.1 \pm 0.1 \times 10^{-2}$	4.8×10^{-2}	$5.3 \pm 0.4 \times 10^{-2}$	2.6×10^{-2}

P_H was obtained by determining the flux from water saturated with hydrocortisone through the membrane. The values shown are the mean values ± standard deviation. ($P'_{H/CD}$) was obtained by dividing the slope of the phase solubility diagram (Fig. 1) into the slope of the J' vs. [HPβCD] diagram, according to Eq. (6).

The permeability coefficient (P) is defined as the diffusion coefficient (D) divided by the thickness of the membrane (h):

$$P = \frac{D}{h}. \tag{5}$$

The corrected permeability coefficient of the hydrocortisone/HPβCD complex $P'_{H/CD}$, i.e. when effects of the HPβCD concentration on the viscosity has been accounted for, was calculated from the slope of the J' vs. [HPβCD] diagram (Slope 1) and the slope of the phase-solubility diagram (Slope 2):

$$P' = \frac{J'}{C_1} = \frac{\text{Slope 1}}{\text{Slope 2}}. \tag{6}$$

The permeability coefficient of the free hydrocortisone molecules (P_H) was calculated by determining the hydrocortisone flux from water, saturated with hydrocortisone, through the membranes. The permeability coefficients increase with increasing pore size and decrease with increasing thickness of the membrane (Table 1). The increase in pore size will result in decrease in permeation resistance, which can explain the observed increase in the permeability coefficient. However, the resistance will also depend on the number of pores per unit surface area, which in our case is unknown. Within experimental error the effect of increased membrane thickness is in an agreement with Eq. (5), which states that, the permeability coefficient is in reverse proportion to the effective thickness of the membrane ($P \propto h^{-1}$). Furthermore, the free versus complex permeability coefficient ratio (i.e. $P_H/P'_{H/CD}$-ratio) is approximately two, which is not far from the calculated value of 1.7. The calculated value is based on the assumption that the molecules are of equal density and spherical shaped (i.e. that both the hydrocortisone molecule and the hydrocortisone/HPβCD have a spherical shape), and that the radius of the molecules are proportional to the third square of the molecular weights ($r \propto \sqrt[3]{MW}$) of hydrocortisone and the unhydrated hydrocortisone/HPβCD 1:1 complex, and Eq. (2). Thus, when the non-ideality of the system has been accounted for (using Eq. (4)) the permeability appears to follow Fick's first law (Eq. (1)) and the Stokes–Einstein equation (Eq. (2)). However, increased HPβCD concentration, and the consequent increased viscosity, did not have any detectable effect on the hydrocortisone flux through the MWCO 500 membrane. Thus, the viscosity effect was investigated further. Small amounts of HPMC were added to 5% (w/v) HPβCD solutions, which had previously been saturated with hydrocortisone and the hydrocortisone flux through the membrane determined. This experiment was repeated with 10% (w/v) HPβCD solution and PVP. Although the polymers increased the viscosity of the donor phase no decrease in the hydrocortisone flux was observed (Table 2). Thus, under these conditions the viscosity of the donor phase does not affect the hydrocortisone permeability through the membrane and the source for the observed negative deviation from linearity (Fig. 2) must lay within the membrane itself (i.e. be a capacity limited process) or be a consequence of

some structures formed by the hydrocortisone/HPβCD complex in the aqueous donor phase. However, the viscosity increase observed with increasing HPβCD concentration is due to increased overall HPβCD–water interactions in the donor phase and subsequent decreased fluidity of the system. Viscosity is a macroscopic property that does not reflect conditions in the microscopic environment found within the pores of the cellophane membranes.

Cyclodextrins and cyclodextrin complexes are known to self-associate to form some kind of aggregates or micelles (Szente et al., 1998; Angelova et al., 1999). Depending on the MWCO of the membranes aggregates consisting of more that 2 to 8 hydrocortisone/HPβCD complexes will be unable to permeate membranes.

Self-association (Scheme 1) should increase with increasing concentration of cyclodextrin and the cyclodextrin complex. The total concentration of cyclodextrin ($[CD]_{total}$), i.e. the concentration of dissolved HPβCD, can then be expressed by Taylor series:

$$[CD]_{total} = [CD_{free}] + 2[CD_{2\ aggr.}] + 3[CD_{3\ aggr.}] + 4[CD_{4\ aggr.}] + \cdots \quad (7)$$
$$= [CD_{free}] + 2K_1[CD_{free}] + 3K_1K_2[CD_{free}]^2 + 4K_1K_2K_3[CD_{free}]^3 + \cdots$$

If $CD_{2\ aggr.}$ and larger aggregates are unable to permeate the membrane then, according to Fick's law, the flux should correlate with $[CD_{free}]$. The expression in Eq. (7) can be simplified further by assuming that $K_1 = K_2 = K_3 = \cdots = K$. Thus Eq. (8) is obtained, which gives $[CD_{free}]$ as a function of $[CD]_{total}$ and K.

$$[CD_{free}] = \frac{2[CD]_{total}}{1 + 2K[CD]_{total} + \sqrt{1 + 4K[CD]_{total}}}. \quad (8)$$

The value of K (9 M^{-1}) was estimated by fitting the observed flux values (Fig. 3A) to the molar concentration of free hydrocortisone/HPβCD complex. Eq. (8) was then used to obtain $[CD]_{free}$ at the various HPβCD concentrations and the values obtained used to correct the flux values (J) with regard to aggregation:

$$J'' = J\frac{[CD]_{total}}{[CD]_{free}}. \quad (9)$$

The linear profile obtained (Fig. 3C) indicates that the negative deviation from linearity (Fig. 3A) could be explained by aggregation, or self-association, of the hydrocortisone/HPβCD complexes.

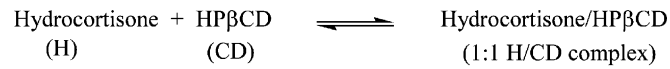
The diameter of the HPβCD molecule is only about 2 nm and, thus, it can be difficult to characterize the weakly associated aggregates consisting of small number of hydrocortisone/HPβCD complexes. However, changes in physicochemical properties due to formation of such aggregates, for example changes in transmembrane fluxes, can easily be observed. Any kind of self-association between the hydrocortisone/HPβCD complexes (the solid lines in Fig. 3),

Table 2
The effect of viscosity on the hydrocortisone flux from aqueous HPβCD solutions saturated with hydrocortisone through single layer of MWCO 6000–8000 semi-permeable cellophane membrane

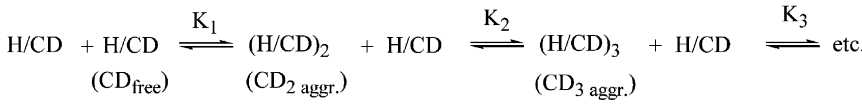
HPβCD concentration (% w/v)	Polymer concentration (% w/v)	Viscosity (cPoise)	Flux ± SD (μg h^{-1} cm^{-2})
5	0.00% HPMC	1.36	190 ± 20
5	0.05% HPMC	1.81	180 ± 3
5	0.11% HPMC	2.53	190 ± 10
5	0.22% HPMC	5.45	190 ± 9
10	0.00% PVP	1.42	310 ± 40
10	0.30% PVP	1.53	290 ± 10
10	0.60% PVP	1.62	290 ± 40
10	0.90% PVP	1.72	270 ± 20

The viscosity was increased through addition of either HPMC or PVP. The values shown are the mean values ± standard deviation (SD).

Simple complexation:



Self-association:



Scheme 1. Examples of self-association of hydrocortisone/HPβCD complexes.

as well as between unoccupied HPβCD molecules and the complexes (the dotted lines in Fig. 3), can explain the observed negative deviation from linearity of the hydrocortisone flux versus total HPβCD concentration plots shown in Figs. 2 and 3.

4. Conclusion

The flux of hydrocortisone from aqueous HPβCD solutions saturated with the drug through semi-permeable cellophane membranes show negative deviation from linearity upon increasing HPβCD concentration. The viscosity of the aqueous HPβCD solution could be used to correct for this deviation from linearity. However, the flux was not affected when the viscosity was increased by addition of polymers to the aqueous HPβCD solutions. The definition of viscosity is based on the bulk properties of solutions and does not apply to individual molecules. Furthermore, the definition of viscosity does not apply in the microscopic environment in the membrane pores which have diameters only slightly larger than the diameter of the hydrocortisone/HPβCD complex.

The deviation from linearity can also be corrected for by assuming that hydrocortisone/HPβCD complexes, as well as HPβCD molecules, self-associate to form aggregates in aqueous solutions. Aggregation is a microscopic property on the scale of the microscopic membrane pores. This explanation is supported by the experimental results and it is in an agreement with previous observations by other researchers.

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References

Angelova, A., Ringard-Lefebvre, C., Baszkin, A., 1999. Drug-cyclodextrin association constants determined by surface tension and surface pressure measurements. II. Sequestration of water insoluble drugs from air–water interface: retinol-βcyclodextrin system. J. Coll. Interf. Sci. 212, 280–285.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instrum. 4, 117–212.

Irie, T., Uekama, K., 1997. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. J. Pharm. Sci. 86, 147–162.

Jarho, P., Urtti, A., Pate, D.W., Suhonen, P., Järvinen, T., 1996a. The effects of HP-β-CD on aqueous solubility, stability and in vitro permeation of anandamide. In: Szejtli, J., Szente, L. (Eds.), Proceedings of the Eighth International Symposium of Cyclodextrins. Kluwer Academic Publishers, Dordrecht, pp. 395–398.

Jarho, P., Urtti, A., Pate, D.W., Suhonen, P., Järvinen, T., 1996b. Increase in aqueous solubility, stability and in vitro corneal permeability of anandamide by hydroxypropyl-β-cyclodextrin. Int. J. Pharm. 137, 209–217.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Järvinen, T., 1999. Cyclodextrins in ophthalmic drug delivery. Adv. Drug Deliv. Rev. 36, 59–79.

Loftsson, T., Masson, M., 2001. Cyclodextrins in topical drug formulations: theory and practice. Int. J. Pharm., 225, 15–30.

Loftsson, T., Ólafsson, J.H., 1998. Cyclodextrins: new drug delivery systems in dermatology. Int. J. Dermatol. 37, 241–246.

Masson, M., Loftsson, T., Masson, G., Stefansson, E., 1999. Cyclodextrins as permeation enhancers: some theoretical evaluations and *in vitro* testing. J. Contr. Rel. 59, 107–118.

Matsuda, H., Arima, H., 1999. Cyclodextrins in transdermal and rectal delivery. Adv. Drug Deliv. Rev. 36, 81–99.

Ono, N., Hirayama, F., Arima, H., Uekama, K., 1999. Determination of stability constant of β-cyclodextrin complexes using the membrane permeation technique and the permeation behavior of drug-competing agent-β-cyclodextrin ternary systems. Eur. J. Pharm. Sci. 8, 133–139.

Rajewski, R.A., Stella, V.J., 1996. Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J. Pharm. Sci. 85, 1142–1168.

Sigurdardottir, A.M., Loftsson, T., 1995. The effect of polyvinylpyrrolidone on cyclodextrin complexation of hydrocortisone and its diffusion through hairless mouse skin. Int. J. Pharm. 126, 73–78.

Szente, L., Szejtli, J., Kis, G.L., 1998. Spontaneous opalescence of aqueous γ-cyclodextrin solutions: complex formation or self-aggregation. J. Pharm Sci. 87, 778–781.

Tanaka, M., Iwata, Y., Kouzuki, Y., Taniguchi, K., Matsuda, H., Arima, H., Tsuchiya, S., 1995. Effect of 2-hydroxypropyl-β-cyclodextrin on percutaneous absorption of methyl paraben. J. Pharm. Pharmacol. 47, 897–900.

Uekama, K., Hirayama, F., Irie, T., 1998. Cyclodextrin drug carrier systems. Chem. Rev. 98, 2045–2076.