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The Effects of Cyclodextrins on Hydrocortisone Permeability Through Semi-Permeable Membranes

HAKON H. SIGURDSSON[1,2], AUDUR MAGNUSDOTTIR[2], MÁR MÁSSON[1] and THORSTEINN LOFTSSON[1]

[1]*Faculty of Pharmacy, University of Iceland, P.O. Box 7210, IS-127 Reykjavík, Iceland;* [2]*deCODE genetics, Sturlugata 8, IS-101 Reykjavík, Iceland*

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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 (HP-β-CD) and the sample drug was hydrocortisone. The MWCO of the membranes ranged from 500 to 14,000 and the HP-β-CD 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 HP-β-CD concentration. This increase showed negative deviation from linearity. When the flux was corrected for the viscosity increase with increasing HP-β-CD 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.

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 [1–3]. Cyclodextrins have been used as penetration enhancers in topical drug formulations but it is not well understood how cyclodextrins act as permeation enhancers and a number of possible mechanisms have been suggested [4]. 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 [5, 6]. The lipophilic membrane has low affinity for the large hydrophilic cyclodextrin molecules and their complexes, which thus remain in the aqueous exterior [7]. 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 [8–10]. These observations have been explained by the existence of an aqueous diffusion barrier at the membrane surface [10]. 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 [10, 11]. 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.

Materials and methods

Hydrocortisone was purchased from Norsk Medicinale Depot (Oslo, Norway), 2- hydroxypropyl-β-cyclodextrin with molar substitution of 0.64 (HP-β-CD; Encapsin^{TM} 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 12000–14000 (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 (Huston, Texas).

The permeability of hydrocortisone from aqueous HP-β-CD solutions through the semi-permeable cellophane membranes was studied using Franz diffusion cells (FDC 400 15FF, Vangard International, Neptune, New Jersey) 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) HP-β-CD, stirred with a magnetic bar. HP-β-CD 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 HP-β-CD solutions, which had been heated in an autoclave (121 °C for 20 min) to promote complex formation. After equilibration for at least three days at room temperature, 2 ml of the filtered donor phase (Spartan 0.45 μm membrane filters from Schleicher & Schuell, 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 hours 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-hour study period.

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

The solubilities of hydrocortisone in the various aqueous HP-β-CD 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 one 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.

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, 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.

Results and discussion

The phase-solubility diagram of hydrocortisone is of Higuchi's A_L-type and formation of a hydrocortisone/HP-β-CD 1:1 complex can be assumed.

Figure 1 shows the flux of hydrocortisone from aqueous HP-β-CD 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 12000–14000. Since the phase-solubility diagram is linear there should be a linear relationship between the flux and the HP-β-CD 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) HP-β-CD. 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} (mean ± SD, $n = 3$) when no HP-β-CD was present in the donor phase and 10.8 ± 4.8 μg h^{-1} cm^{-2} (mean ± SD, $n = 3$) when 20% (w/v) HP-β-CD was present. Similar observations were made when the double layer MWCO 500 membrane was used. Thus, increased HP-β-CD 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 HP-β-CD is 1404 and, thus, both the HP-β-CD molecule and the hydrocortisone/HP-β-CD complex are unable to permeate the MWCO 500 membrane. The difference between the hydrocortisone flux from pure aqueous solution and 20% (w/v) HP-β-CD solution gradually increased as the pores become larger.

It is possible that the negative deviation from linearity of the flux versus HP-β-CD 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%

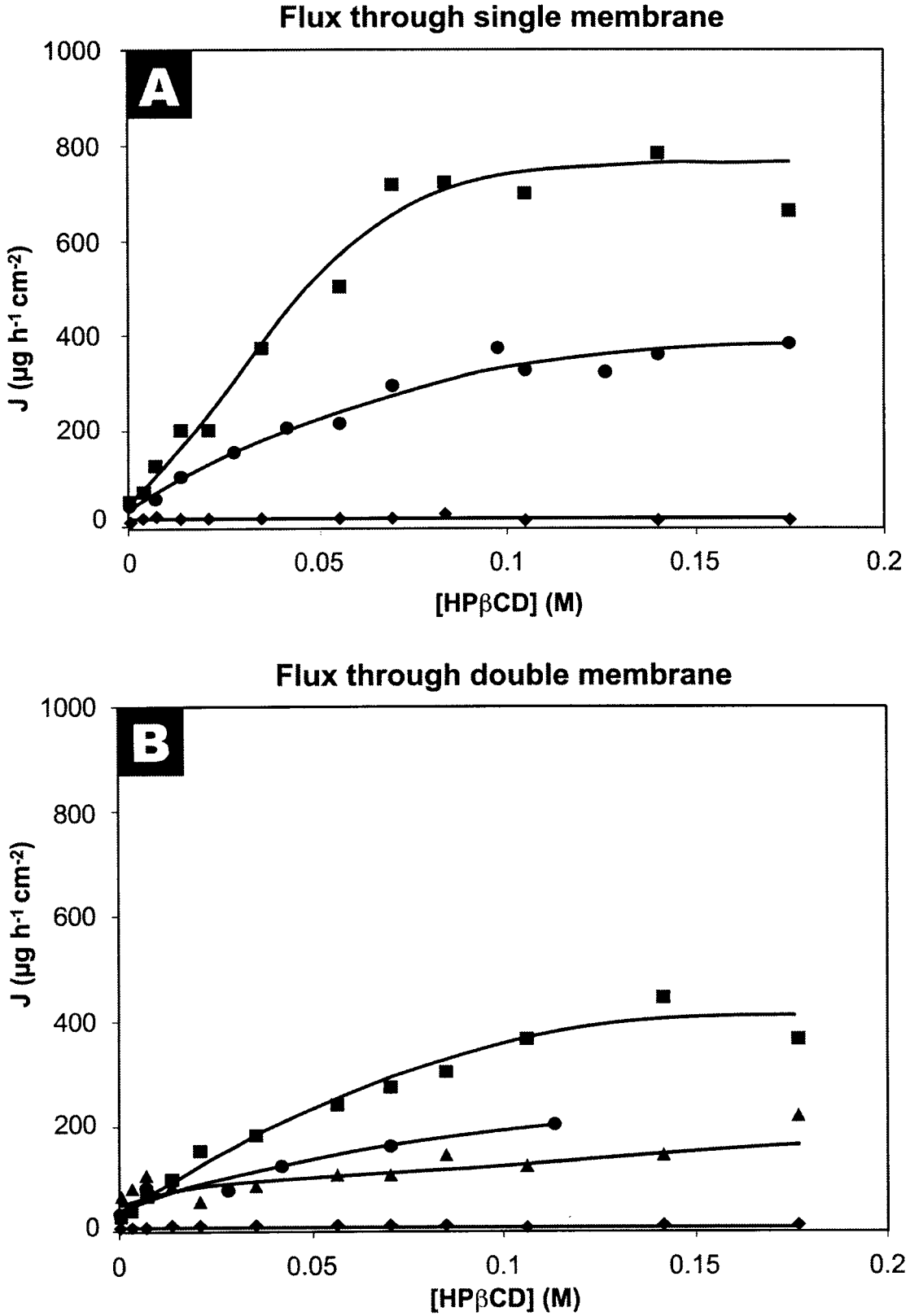
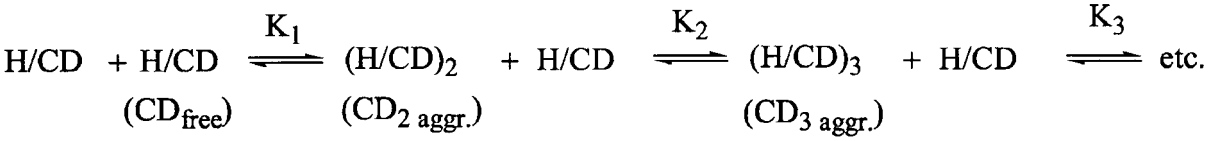


Figure 1. The effect of HP-β-CD concentration on the flux (J) of hydrocortisone from aqueous solution, saturated with hydrocortisone, through a single (A) and double (B) layer semi-permeable cellophane membrane. MWCO 500 (♦), MWCO 3500 (▲), MWCO 6000–8000 (●), and MWCO 12000–14000 (■), mean values of 3 determinations.

Simple complexation:

Self-association:



Scheme 1. Examples of self-association of hydrocortisone/HP-β-CD complexes.

(w/v) HP-β-CD solution 2.10 cPoise. However, increased HP-β-CD 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) HP-β-CD solutions, which had previously been saturated with hydrocortisone and the hydrocortisone flux through the membrane determined. This experiment was repeated with 10% (w/v) HP-β-CD solution and PVP. Although the polymers increased the viscosity of the donor phase no decrease in the hydrocortisone flux was observed. 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 (Figure 1) must lay within the membrane itself (i.e., be a capacity limited process) or be a consequence of some structures formed by the hydrocortisone/HP-β-CD complex in the aqueous donor phase. However, the viscosity increase observed with increasing HP-β-CD concentration is due to increased overall HP-β-CD-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 [12, 13]. Depending on the MWCO of the membranes aggregates consisting of more that 2 to 8 hydrocortisone/HP-β-CD 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 HP-β-CD, can then be expressed by Taylor series:

$$\begin{aligned}[CD]_{total} &= \lfloor CD_{free}\rfloor + 2\lfloor CD_{2\,aggr}\rfloor + 3\lfloor CD_{3\,aggr}\rfloor \\ &\quad +4\lfloor CD_{4\,aggr}\rfloor + \cdots \\ &= [CD_{free}] + 2K_1[CD_{free}] + 3K_1K_2[CD_{free}]^2 \\ &\quad +4K_1K_2K_3[CD_{free}]^3 \cdots \end{aligned} \quad (1)$$

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 Equation (1) can be simplified further by assuming that $K_1 = K_2 = K_3 = \cdots = K$. Thus Equation (2) is obtained, which gives $[CD_{free}]$ as a function of $[CD]_{total}$ and K.

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

The value of K (9 M^{-1}) was estimated by fitting the observed flux values to the molar concentration of free hydrocortisone/HP-β-CD complex. Equation (2) was then used to obtain $[CD]_{free}$ at the various HP-β-CD concentrations and the values obtained used to correct the flux values (J) with regard to aggregation:

$$\ddot{J} = J \cdot \frac{[CD]_{total}}{[CD_{free}]} \quad (3)$$

A linear profile is obtained which indicates that the negative deviation from linearity (Figure 1) could be explained by aggregation, or self-association, of the hydrocortisone/HP-β-CD complexes.

The diameter of the HP-β-CD molecule is only about 2 nm and, thus, it can be difficult to characterize the weakly associated aggregates consisting of small number of hydrocortisone/HP-β-CD complexes. However, changes in physicochemical properties due to formation of such aggregates, for example changes in trans-membrane fluxes, can easily be observed. Any kind of self-association between the hydrocortisone/HP-β-CD complexes, as well as between unoccupied HP-β-CD molecules and the complexes, can explain the observed negative deviation from linearity of the hydrocortisone flux versus total HP-β-CD concentration (Figure 1).

Conclusion

The flux of hydrocortisone from aqueous HP-β-CD solutions saturated with the drug through semi-permeable cellophane membranes show negative deviation from linearity upon increasing HP-β-CD concentration. The viscosity of the aqueous HP-β-CD 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 HP-β-CD 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/HP-β-CD complex.

The deviation from linearity can also be corrected for by assuming that hydrocortisone/HP-β-CD complexes, as well as HP-β-CD 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

1. T. Loftsson and M. E. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).
2. R.A. Rajewski and V.J. Stella: *J. Pharm. Sci.* **85**, 1142 (1996).
3. T. Irie and K. Uekama: *J. Pharm. Sci.* **86**, 147 (1997).
4. T. Loftsson and J.H. Ólafsson: *Int. J. Dermatol.* **37**, 241 (1998).
5. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
6. T. Loftsson and T. Järvinen: *Adv. Drug Deliv. Rev.* **36**, 59 (1999).
7. M. Tanaka, Y. Iwata, Y. Kouzuki, K. Taniguchi, H. Matsuda, H. Arima, and S. Tsuchiya: *J. Pharm. Pharmacol.* **47**, 897 (1995).
8. A.M. Sigurdardottir and T. Loftsson: *Int. J. Pharm.* **126**, 73 (1995).
9. P. Jarho, A. Urtti, D.W. Pate, P. Suhonen, and T. Järvinen: *Int. J. Pharm.* **137**, 209 (1996).
10. M. Masson, T. Loftsson, G. Masson, and E. Stefansson: *J. Contr. Rel.* **59**, 107 (1999).
11. N. Ono, F. Hirayama, H. Arima, and K. Uekama: *Eur. J. Pharm. Sci.* **8**, 133 (1999).
12. L. Szente, J. Szejtli, and G.L. Kis: *J. Pharm. Sci.* **87**, 778 (1998).
13. A. Angelova, C. Ringard-Lefebvre, and A. Baszkin: *J. Coll. Interf. Sci.* **212**, 280 (1999).