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Note

Effect of the unstirred water layer on permeability enhancement by hydrophilic cyclodextrins

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Abstract

Saturated solutions of three test compounds, carbamazepine, griseofulvin and hydrocortisone, were prepared in aqueous buffer (pH 7.4) containing 0, 1, 5 and 10% HPβCD. The permeability and flux of the drugs though a PAMPA membrane at different unstirred water layer (UWL) thicknesses was determined. In absence of HPβCD, permeability coefficients increased two- to three-fold with decreasing UWL thickness to a certain minimum values of about 40 μm. Addition of HPβCD to systems exhibiting larger UWL thicknesses significantly increased compound flux. The effect of HPβCD was linked to its association constant ($K_{1:1}$) with the model drugs and decreased with decreasing UWL thickness to a certain minimum value. This suggests that hydrophilic cyclodextrins enhance flux when the UWL resistance significantly contributes to the overall barrier resistance.
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Biological membranes normally have a hydrophilic exterior and a lipophilic interior and, although many biological membranes contain specialized transport systems, most drugs permeate the membranes via passive diffusion. Adjacent to the membrane surfaces is an unstirred or stagnant water layer (UWL) that can act as diffusion barrier for rapidly penetrating drugs. The flux (J) of a drug through the membrane can be described by the following equation (Loftsson et al., 2006a):

$$J = PC_V = (R_D + R_M + R_R)^{-1} C_V = \left(\frac{1}{P_D} + \frac{1}{P_M} + \frac{1}{P_R}\right)^{-1} C_V \quad (1)$$

where J is the flux of the compound through the membrane, P the overall permeability coefficient, C_V the concentration of the compound in the vehicle (i.e. donor phase), R_D, R_M and R_R the resistances in the UWL at the donor side, within the membrane and in the UWL at the receptor side, respectively, and P_D, P_M and P_R are the corresponding permeability coefficients. In the present study, R_R was assumed to be negligible due to stirring of the receptor phase and thus Eq. (2) is obtained from Eq. (1):

$$J = \left(\frac{P_D P_M}{P_D + P_M}\right) C_V \quad (2)$$

The permeability constant is composed of the diffusion constant (D), the partition constant between the aqueous exterior and the lipophilic membrane (K) and the thickness (h) of the membrane:

$$P = \frac{DK}{h} \quad (3)$$

For P_D the value of K is unity and h is the thickness of the UWL. The thickness of the UWL ranges from negligible to greater than 100 μm such that its influence on the overall drug permeability through the membrane can range from being insignificant to being the rate determining factor (Loftsson et al., 2006b).

It is well documented that cyclodextrins can, under certain conditions, enhance drug delivery through biological membranes (Cho et al., 1995; Uekama et al., 1998; Matsuda and Arima, 1999; Loftsson et al., 2004, 2005). However, cyclodextrins and cyclodextrin complexes are unable to permeate lipophilic biological membranes. The parallel artificial membrane permeation assay (PAMPA) is widely used for drug screening and it has been shown that PAMPA is as good an

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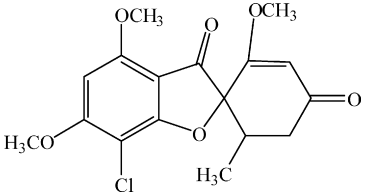
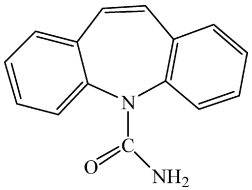
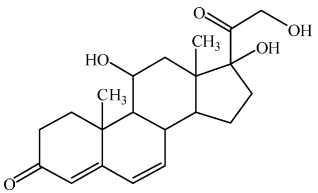
Table 1
The effective permeability constant and the flux in the PAMPA system for griseofulvin, carbamazepine and hydrocortisone

The structure and physicochemical characteristics[a]	CD concentration % (w/v)	C_d (mg/ml)	Effective permeability (P_{eff}) ± S.D. × 10^6 (cm s^{-1})					Flux (J) × 10^6 (mg cm^{-2} s^{-1})		J_{40}/J_{100}
			No cyclodextrin[b]			10% (w/v) HPβCD[b]		10% (w/v) HPβCD[b]		
			UWL > 100 μm	UWL 40 μm	UWL 25 μm	UWL > 100 μm	UWL 40 μm	UWL > 100 μm	UWL 40 μm	
										
	Apparent $K_{1:1}$ = 23 M^{-1}									
MW: 352.8 Da										
MP: 217–224 °C	0	0.027	3.52 ± 0.52	9.40 ± 1.36	9.68 ± 2.60	–	–	0.103	0.254	2.5
Solubility in water: 0.03 mg/ml	1	0.031				14.3 ± 1.10	50.0 ± 10.0	0.443	1.55	3.5
$C\log P_{octanol/water}$: 2.2	5	0.048				14.8 ± 5.43	49.6 ± 8.0	0.710	2.38	3.4
										
	Apparent $K_{1:1}$ = 650 M^{-1}									
MW: 236.3 Da	0	0.15	23.7 ± 1.8	83.0 ± 6.2	81.5 ± 7.6			4.1	12	2.9
MP: 189–193 °C	1	0.64				13.8 ± 1.4	19.3 ± 0.9	8.8	12	1.4
Solubility in water: 0.15 mg/ml	5	2.62				5.4 ± 2.4	5.8 ± 1.6	14	15	1.1
$C\log P_{octanol/water}$: 2.5	10	5.09				3.4 ± 0.9	3.4 ± 0.9	17	17	1.0
										
	Apparent $K_{1:1}$ = 1340 M^{-1}									
MW: 362.53 Da	0	0.33	14.6 ± 3.3	35.6 ± 2.2	32.5 ± 3.8			5.3	11.7	2.2
MP: 214 °C	1	1.73				9.1 ± 3.5	7.4 ± 0.1	15.7	12.8	0.8
Solubility in water: 0.33 mg/ml	5	7.52				3.3 ± 1.3	2.0 ± 0.3	24.8	15.0	0.6
$C\log P_{octanol/water}$: 1.6	10	14.6				1.3 ± 0.3	1.0 ± 0.3	19.0	14.6	0.8

The donor phase contained saturated drug solution prepared in pH 7.4 pIon buffer and in the same buffer containing 1, 5 and 10% (w/v) HPβCD. Receptor phase buffers were either the pIon buffer or the buffer containing 10% (w/v) HPβCD.

[a] Data from (Moffat et al., 2004).

[b] Receptor phase.

indicator for oral drug absorption as Caco-2 (Zhu et al., 2002; Avdeef, 2003; Bermejo et al., 2004). In PAMPA the UWL can be a rate-determining barrier to transport of lipophilic molecules (Bermejo et al., 2004; Nielsen and Avdeef, 2004). Furthermore, it has been shown that hydrophilic complexing agents, such as cyclodextrins, can enhance permeation of lipophilic compounds through the UWL (Mayer et al., 2005). The purpose of the present study was to assess the importance of the UWL in mechanisms associated with permeability enhancement afforded by hydrophilic cyclodextrins such as 2-hydroxypropyl-β-cycluextrin (HPβCD) using the PAMPA system.

Carbamazepine, griseofulvin and hydrocortisone were obtained from Sigma–Aldrich (Bornem, Belgium). HPβCD (hydroxypropylbetadex, Ph. Eur. 5.0) with degree of substitution 4.2 was obtained from Roquette (France). Materials for the PAMPA method including buffers, disposables and lipid mixtures for preparing the membranes were obtained from pIon (Woburn, MA, USA). PAMPA is based on permeation through a membrane "sandwich" formed in 96-well microtiter plates (PAMPA Evolution version 2.2, Woburn, MA, USA). The plate is constructed so that each well is divided into two chambers (a donor on the bottom and an acceptor on the top) separated by a 125 μm microfilter disc. The disc is coated with a 2% (w/v) dodecane solution of dioleylphosphadityl choline under conditions that favor the formation of a multilamellar structure. The donor phase contained saturated drug solution prepared in pH 7.4 pIon buffer and in the same buffer containing 1, 5 and 10% HPβCD, w/v. Receptor phase buffers were either the pIon buffer or the same buffer containing 10% HPβCD. The PAMPA Evolution permeability analyzer hardware was dedicated to the Gut-Box system so that the diffusion cells could be agitated and the thickness of the UWL could be controlled. The UWL thickness can be adjusted from approximately 25 to >100 μm. The permeability coefficients and the flux for carbamazepine, griseofulvin and hydrocortisone were determined in buffer or in buffer containing various cyclodextrin concentrations at three distinct UWL thicknesses (>100, 40 and 25 μm). All donor solutions were saturated with respect to the drug. Phase-solubility analysis and calculated 1:1 apparent drug/cyclodextrin complexation constants ($K_{1:1}$) were completed as previously described (Brewster and Loftsson, 1999).

Structural and physicochemical characterization data, as well as permeability and flux data for griseofulvin, carbamazepine and hydrocortisone are shown in Table 1. The data indicated compounds with varying degrees of interaction with HPβCD, lipophilicities and water solubilities. Permeability coefficients determined without cyclodextrins resulted in lower permeability coefficients at UWL > 100 μm compared with those of 40 μm. Values at 25 μm were essentially the same as those at 40 μm. In the case of carbamazepine, the difference was three-fold, for griseofulvin 2.5-fold and for hydrocortisone 2.2-fold (Table 1). The flux differences between UWL of >100 μm and 40 μm tended to decrease as a function of increasing HPβCD concentrations. For carbamazepine, the flux in the absence of cyclodextrin was approximately three-fold different when assessing the two stirring rates. At 1%, w/v, HPβCD concentration the difference was less than 40%, less than 10% at 5%, w/v, HPβCD and identical at 10% HPβCD. For hydrocortisone, the greater than two-fold difference in flux seen in the absence of HPβCD was abolished with addition of as little as 1% HPβCD to the donor phase. A slightly different profile was observed for griseofulvin where the 2.5-fold flux difference observed in the absence of HPβCD was maintained in presence of HPβCD. In these experiments, the donor phase was saturated with the drug and the total amount of dissolved drug increases with increasing HPβCD. In HPβCD solutions saturated with drug the concentration of free drug is constant and equal to the aqueous solubility of the drug. Thus, the flux should be constant and independent of the HPβCD concentration. In all cases, the flux increased with increasing amount of HPβCD, i.e. increasing total amount of dissolved drug which includes both free and material bound to HPβCD. The effect of HPβCD appears to be related to $K_{1:1}$ with flux increasing with increasing $K_{1:1}$. Also, the effect of HPβCD decreases with a decreasing UWL thickness. This suggests that the diffusion resistance of the UWL (R_{Aq}) has to be greater than the resistance of the membrane (R_M). In other words, for HPβCD to have enhancing effect—R_{Aq} must have a significant contribution to the overall barrier resistance. Finally, the results show that the resistance of the 40 and 25 μm thick UWL are much smaller than the resistance of the membrane ($R_{Aq} \ll R_M$). These results agree with previous observations that medium constituents, which normally are believed to bind hydrophobic compounds, actually can enhance permeation through UWL adjacent to a lipophilic membrane surface (Cho et al., 1995; Mayer et al., 2005).

Data based on PAMPA with stirring of the bulk media suggest that the observed increases in flux are associated with the ability of HPβCD, and by extension other hydrophilic cyclodextrins, to transport a drug molecule though the UWL so that it is brought in close proximity to the lipophilic membrane. That suggests that hydrophilic cyclodextrins can exert an improvement in drug permeation only if UWL significantly contributes to the barrier function of the membrane for the compounds of interest. It also appears that a minimum complexation affinity is required for this enhancement to occur. Conversely, an extremely high complexation affinity is expected to reduce drug uptake though membranes by reducing the availability of the drug.

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