REVIEW

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Influence of aqueous diffusion layer on passive drug diffusion from aqueous cyclodextrin solutions through biological membranes

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Most drugs permeate biological membranes via passive diffusion and it is generally assumed that the main barrier is the lipophilic structure of the membrane. However, we have observed that the unstirred water layer adjacent to the membrane surface can in some cases be a barrier just as effective as the lipophilic membrane itself. Hydrophilic cyclodextrins can enhance drug delivery through biological membranes by increasing the availability of dissolved drug molecules immediate to the membrane surface, i.e. by increasing drug delivery through the unstirred water layer. Cyclodextrins and drug/cyclodextrin complexes are, in most cases, unable to permeate lipophilic membranes. Thus, excess cyclodextrin, more than is needed to solubilize the drug in the aqueous exterior, will hamper drug delivery through biological membranes.

1. Cyclodextrins and their pharmaceutical applications

Cyclodextrins are cone-shaped cyclic oligosaccharides with a hydrophilic outer surface and a somewhat lipophilic central cavity. The most common natural cyclodextrins consist of six (α-cyclodextrin), seven (β-cyclodextrin) and eight (γ-cyclodextrin) α-1,4-linked α-D-glucopyranose units. The secondary hydroxy groups are extended from the wider edge of the cyclodextrin cone and the primary hydroxy groups from the narrow edge. Although the cyclodextrin molecules are able to form numerous hydrogen bonds with the surrounding water molecules their solubility in water is limited, especially that of β-cyclodextrin. This is thought to be due to relatively strong binding of the cyclodextrin molecules in the crystal state (i.e. relatively high crystal lattice energy) (Frömming and Szejtli 1994; Loftsson and Brewster 1996). Random substitution of the hydroxy groups, and consequent formation of amorphous mixtures of isomeric derivatives, will result in dramatic improvements in their solubility. Cyclodextrin derivatives of pharmaceutical interest include hydroxypropyl derivatives of β- and γ-cyclodextrin, sulfobutylether β-cyclodextrin and randomly methylated β-cyclodextrin. More than 30 different pharmaceutical products containing cyclodextrins are now on the market worldwide. In the pharmaceutical industry, cyclodextrins have mainly been used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, and to increase their bioavailability and stability (Loftsson and Brewster 1996; Uekama 2002; Loftsson et al. 2004a; Loftsson et al. 2005a).

Cyclodextrins are able to form inclusion complexes with many drugs by incorporating a drug molecule, or more commonly a lipophilic moiety of the molecule, into the central cavity. No covalent bonds are formed or broken during the drug/cyclodextrin complex formation. The driving forces leading to the inclusion complex formation include release of enthalpy-rich water molecules from the cyclodextrin cavity, electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding, release of conformational strain and charge-transfer interaction (Bergeron 1984; Loftsson and Brewster 1996; Liu and Guo 2002). All these forces are relatively weak allowing free drug molecules in solution to be in rapid equilibrium with drug molecules bound within the cavity (Stella and Rajewski 1997). Most drug molecules form 1 : 1 complexes with cyclodextrin molecules and the value of the stability constant is most often between 50 and 2000 M^{-1} with a mean value of 129, 490 and 355 M^{-1} for α-, β- and γ-cyclodextrin, respectively (Connors 1995; Connors 1997; Stella and Rajewski 1997; Rao and Stella 2003). Cyclodextrins and cyclodextrin complexes self-associate to form aggregates and that those aggregates can also act as solubilizers themselves (Mele et al. 1998; González-Gaitano et al. 2002; Magnusdottir et al. 2002; Loftsson et al. 2004b). There are some indications that the water-soluble polymers and certain organic and inorganic salts enhance the complexation efficiency by stabilizing these aggregates through formation of non-inclusion complexes with the cyclodextrin complexes and aggregates (Loftsson et al. 2003a; Loftsson and Másson 2004; Loftsson et al. 2004b; Duan et al. 2005; Loftsson et al. 2005b). For example, it has been shown that both 2-hydroxypropyl-β-cyclodextrin and randomly methylated β-cyclodextrin form aggregates consisting of, on the average, two to three cyclodextrin mole-

cules and other excipients, such as lysine and polyvinylpyrrolidone, forming non-inclusion complexes with the cyclodextrin aggregates. The critical cyclodextrin concentration of the aggregate formation is about 5.4% (w/v) (Duan et al. 2005; Loftsson et al. 2005b). Thus, aqueous cyclodextrin solutions can consist of multiple structures of ternary, quaternary and higher order complexes and aggregates.

2. Drug permeation through biological membrane barriers

In general, biological membranes have a hydrophilic exterior and a lipophilic interior. Although many of them contain specialized transport systems that assist passage of some selected compounds most drugs permeate these membranes, transcellular or paracellular, via passive diffusion. The fundamental equation describing passive drug transport through the membranes is based on Fick's first law:

$$J = P \cdot C_{Aq} \quad (1)$$

where J is the drug flux through a membrane (mass/area/time), P is the permeability coefficient of the drug through the lipophilic membrane and C_{Aq} is the drug concentration at the aqueous exterior. The permeability coefficient is defined as:

$$P = \frac{D \cdot K}{h} \quad (2)$$

where D is the diffusion coefficient of the drug within the membrane, K is the partition coefficient of the drug from the aqueous exterior into membrane and h is the effective thickness of the membrane. The equations show that for a drug molecule to be successfully delivered through a membrane, the drug must possess sufficient aqueous solubility (or high C_{Aq} value) but at the same time the drug must possess sufficient lipophilicity to be able to partition from the aqueous exterior into the lipophilic membrane (or high K value). Adjacent to the membrane surface is an unstirred or stagnant water layer that acts as a diffusion barrier for rapidly permeating drugs. The thickness of this diffusion barrier and its significance in the overall barrier function of the membrane depends on the physicochemical properties of both the membrane and the penetrating drug. For example, at the skin surface the aqueous diffusion barrier can, under certain conditions, be relatively thin and insignificant for the overall barrier function of the skin while the aqueous mucin layer on the eye surface and in the gastrointestinal tract can have significant contribution to the overall barrier function. The total drug permeation resistance is the sum of resistance within the unstirred water layer (R_{Aq}) and the lipophilic membrane (R_M), and their relative importance depends on the physicochemical properties of both the drug and the membrane (Flynn et al. 1972; Florence and Attwood 1998; Masson et al. 1999; Loftsson and Masson 2001). Since the permeability constants are the reciprocals of the resistance the following equations are obtained:

$$J = P \cdot C_{Aq} = (R_{Aq} + R_M)^{-1} \cdot C_{Aq} = \left(\frac{1}{P_{Aq}} + \frac{1}{P_M}\right)^{-1} \cdot C_{Aq} \quad (3)$$

$$J = \left(\frac{P_{Aq} \cdot P_M}{P_{Aq} + P_M}\right) \cdot C_{Aq} \quad (4)$$

If the value of the permeability constant through the lipophilic membrane (P_M) is much greater than the value of the permeability constant through the diffusion layer (P_{Aq}) then Eq. (4) becomes:

$$J = \left(\frac{P_{Aq} \cdot P_M}{P_{Aq} + P_M}\right) \cdot C_{Aq} \approx \left(\frac{P_{Aq} \cdot P_M}{P_M}\right) \cdot C_{Aq} = P_{Aq} \cdot C_{Aq} \quad (5)$$

and the unstirred water layer becomes the main barrier, i.e. the permeation is diffusion controlled. On the other hand, if P_{Aq} is much greater than P_M then Eq. (6) is obtained:

$$J = \left(\frac{P_{Aq} \cdot P_M}{P_{Aq} + P_M}\right) \cdot C_{Aq} \approx \left(\frac{P_{Aq} \cdot P_M}{P_{Aq}}\right) \cdot C_{Aq} = P_M \cdot C_{Aq} \quad (6)$$

and the permeation will be membrane controlled. Finally the diffusion coefficient can be estimated from the Stokes-Einstein equation:

$$D = \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N} \quad (7)$$

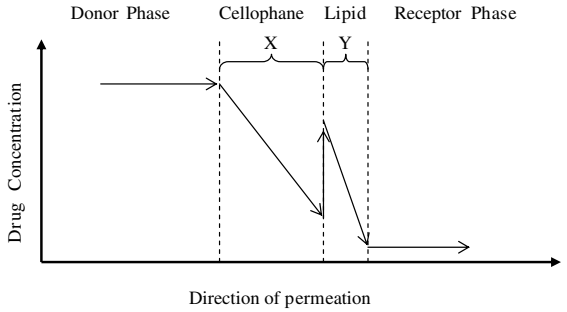
where R is the molar gas constant, T is the absolute temperature, g is the apparent viscosity within the unstirred water layer or the lipophilic membrane, r is the radius of the permeating drug molecule, and N is Avogadro's number. Thus, the diffusion constant within the unstirred water layer (D_{Aq}) will decrease with increasing viscosity of the layer as well as with increasing molecular weight of the drug. For example, small lipophilic drug molecules frequently possess a large permeability coefficient through the lipophilic membrane (i.e. large P_M value) and, thus, may be able to permeate across the lipophilic membrane much faster that they can be transported through the unstirred water layer. Under such conditions diffusion through the water layer becomes the rate-limiting step in the absorption process. Such permeation conditions are shown in Table 1. The semi-permeable cellophane with a molecular weight cutoff 12–14,000 acts as unstirred water layer through which the drug (hydrocortisone) permeates relatively rapid ($P_{Aq} = 4.22\ 10^{-6}\ cm \cdot s^{-1}$). Two different lipophilic membranes were fused to this cellophane membrane, an octanol collodium membrane and a dodecanol collodium membrane, prepared by methods described by Neubert (Neubert and Fürst 1989; Mrestani et al. 2004). The drug permeates relatively rapidly through the octanol membrane ($P_M > P_{Aq}$) but significantly slower through the more viscose dodecanol membrane ($P_M < P_{Aq}$). Thus, the permeation through the cellophane/octanol membrane is diffusion controlled but membrane controlled through the cellophane/dodecanol membrane (Table 1).

Conventional penetration enhancers, such as fatty acids, fatty amines and fatty alcohols, enhance drug delivery through biological membranes by permeating into the membrane and disrupting its barrier properties (Williams and Barry 2004) without affecting the unstirred aqueous layer.

3. Effect of cyclodextrins on drug permeability through membranes

There is very strong evidence that hydrophilic cyclodextrins enhance drug delivery through biological membranes without affecting their barrier function (Loftsson and Masson 2001; Loftsson et al. 2003b; Sinha et al. 2003). Some general observations regarding cyclodextrins and drug permeation through biological membranes are summarized in Table 2. These observations indicate that under normal

Table 1: Permeation of hydrocortisone ($logK_{octanol/water}$ 1.6, MW 362 Dalton) through an artificial membrane consisting of semi-permeable cellophane membrane, semi-permeable cellophane membrane (diffusion layer) with a fused octanol collodium membrane (lipid membrane) at the receptor side, and a semi-permeable cellophane membrane with a fused dodecanol collodium membrane (lipid membrane)



Property	Cellophane	Cellophane/ Octanol*	Cellophane/ Dodecanol*
Thickness (μm):			
Diffusion layer (X)	230	213	~230
Lipid membrane (Y)	0	140	140
Permeability (cm s^{-1}) Mean ± standard deviation	$4.19 \pm 0.46 \times 10^{-6}$	$4.05 \pm 0.47 \times 10^{-6}$	$1.19 \pm 0.35 \times 10^{-6}$

* 1-Octanol: melting point −17 to −16 °C, viscosity (30 °C) 3.45 cP; 1-dodecanol: melting point 24 °C, viscosity (30 °C) 6.51 cP (Saleh et al. 2004)
The donor phase consisted of an aqueous 5% (w/v) 2-hydroxypropyl-β-cyclodextrin solution saturated with hydrocortisone (hydrocortisone conc. 10 mg/ml) and the receptor phase consisted of 10% (w/v)) 2-hydroxypropyl-β-cyclodextrin solution (23 °C)

conditions neither the free hydrophilic cyclodextrin molecules nor their complexes are able to permeate lipophilic biological membranes such as skin, the gastrointestinal epithelium, the eye cornea and nasal mucosa. Also, the chemical structure of cyclodextrins (i.e. the large number of hydrogen donors and acceptors), their molecular weight (i.e. greater than 970 Dalton) and their very low octanol/water partition coefficient ($logK_{o/w}$ less than −3 (Moldenhauer 2004)) are all characteristics of compounds that do not readily permeate biological membranes (Amidon et al. 1995; Lipinski et al. 2001). Based on these observations it has been suggested that cyclodextrins enhance drug delivery through biological membranes by increasing the availability of dissolved drug molecules in an aqueous layer immediate to the lipophilic membrane surface (Loftsson and Masson 2001; Sinha et al. 2003). According to this model cyclodextrins solubilize the lipophilic water-insoluble drug molecules in an aqueous vehicle and enhance their permeation through an aqueous diffusion layer at the membrane surface. Cyclodextrins can only act as penetration enhancers if $P_{Aq} \leq P_M$, or in other words when permeation through the unstirred water layer contributes to the overall barrier function of the biological membrane. Furthermore, the physicochemical properties of the drug (e.g. its solubility in water), the drug : cyclodextrin concentration ratio and the composition of the drug formulation (e.g. aqueous or non-aqueous) will also determine whether cyclodextrins will enhance or hamper drug delivery through a biological membrane. Cyclodextrins are in most cases unable to enhance drug permeation through a lipophilic membrane barrier and excess cyclodextrin (more than is needed to dissolve the drug) will hamper drug permeation through the membrane (Loftsson et al. 2003b).
However, there is one exception. The somewhat lipophilic and surface active methylated cyclodextrins ($logK_{o/w}$ about −1) are known, under certain conditions, to act as conventional penetration enhancers increasing drug permeability through biological membranes by decreasing their barrier function (Merkus et al. 1999; Yang et al. 2004). Since cyclodextrins can both enhance and hamper drug delivery through biological membranes it is of uttermost importance to optimize cyclodextrin containing drug formulations with regard to drug delivery from the formulations (Loftsson and Masson 2001). Too much or too little cyclodextrin can result in less than optimum drug bioavailability.
Many experimental methods used for *in vitro* evaluation of drug delivery through membranes are equipped with stirrable donor chambers. In such devices the thickness of the aqueous diffusion layer (i.e. the unstirred water-layer closest to the membrane) will depend on the stirring rate, disappearing completely under vigorous stirring. Thus, in such systems cyclodextrins can act as enhancers at low stirring but then have no effect when the aqueous donor phase is rapidly stirred. An example of such an experimental model is hairless mouse skin (membrane) in a horizontal side-bi-side diffusion cell with an aqueous donor phase (Table 3).

4. Cyclodextrins and biological membranes

The effects of cyclodextrins on drug permeation through various membranes are shown in Table 3. The two semi-permeable cellophane membranes are hydrophilic membranes with no lipophilic membrane barrier, and can be regarded as unstirred aqueous layer (i.e. an aqueous diffusion barrier). Both the drug (MW < 500) and the drug/cyclodextrin complex are able to permeate the membrane with molecular weight cutoff (MWCO) 12–14,000 but only the free drug is able to permeate the membrane with MWCO 500. Thus, cyclodextrins are able to enhance drug delivery through the MWCO 12–14,000 membrane but not through the MWCO 500 membrane. Since the complex is able to permeate the MWCO 12–14,000 membrane adding excess cyclodextrin, more than is needed to solubilize the drug, to the aqueous donor phase does not reduce drug permeation. On the other hand, addition of excess cyclodextrin to the donor phase reduces the amount of free drug and, thus, reduces the drug permeation through the MWCO 500 membrane. The properties of the fish skin ap-

Table 2: Some common observations regarding cyclodextrins and drug permeation through biological membranes

Observation	Reference
Only negligible amounts of hydrophilic cyclodextrins and cyclodextrin complexes are able to penetrate biological membranes such as skin and gastrointestinal mucosa.	(Uekama et al. 1987; Gerlóczy et al. 1988; Tanaka et al. 1995; Uekama et al. 1998; Matsuda and Arima 1999; Loftsson and Masson 2001)
Cyclodextrins do not, in general, enhance permeability of hydrophilic water-soluble drugs through lipophilic biological membranes.	(Loftsson and Stefánsson 1997; Siefert and Keipert 1997; Loftsson and Masson 2001; Loftsson et al. 2003b)
Cyclodextrins are able to alleviate local drug irritation after topical and oral application.	(Amdidouche et al. 1994; Loftsson et al. 2004a)
Cyclodextrins are able to extract lipophilic components from biomembranes such as stratum corneum but pretreatment with hydrophilic cyclodextrins does not usually enhance permeability and reduced permeability is commonly observed at relatively high cyclodextrin concentrations.	(Legendre et al. 1995; Arima et al. 1996; Vitória et al. 1997; Arima et al. 1998; Babu and Pandit 2004)
Cyclodextrins can only act as enhancers from an aqueous exterior.	(Preiss et al. 1994; Arima et al. 1998; Uekama et al. 1998; Loftsson and Masson 2001)
Hydrophilic cyclodextrins reduced the amount of drug released from w/o cream but enhanced the release from o/w cream. When applied to excised human skin cyclodextrins enhanced drug delivery from o/w cream through the skin.	(Preiss et al. 1994; Preiss et al. 1995)
Cyclodextrins and conventional penetration enhancers, like fatty acids, or mechanical enhancers, like iontophoresis, can have additive or synergistic effect on drug delivery through biological membranes.	(Adachi et al. 1992; Adachi et al. 1993; Loftsson et al. 1998; Sinha et al. 2003)
Cyclodextrins can, at least in theory, enhance drug bioavailability by stabilization of drug molecules at the biomembrane surface.	(Irie et al. 1992)
Number of studies using various biomembranes, and under several different experimental conditions, have shown that excess cyclodextrin, i.e. more than needed to solubilize a given lipophilic drug in an aqueous vehicle, results in decreased drug penetration through the membrane. Maximum enhancement is obtained when just enough cyclodextrin is used to solubilize the lipophilic drug in the aqueous vehicle.	(Cho et al. 1995; Jarho et al. 1996; Kublik et al. 1996; Chang and Banga 1998; Bary et al. 2000; Zuo et al. 2000; Loftsson and Masson 2001; Felton et al. 2002; Babu and Pandit 2004; Richter and Keipert 2004; Manosroi et al. 2005) Cho et al. 1995; Jarho et al. 1996; Kublik et al. 1996; Chang and Banga 1998; Bary et al. 2000; Zuo et al. 2000; Loftsson and Masson 2001; Felton et al. 2002; Babu and Pandit 2004; Richter and Keipert 2004; Manosroi et al. 2005)
In oral drug delivery, greatest bioavailability enhancements are, in general, obtained for Class II (high permeability, low solubility) drugs. No or very insignificant enhancements are frequently obtained for Class I (high permeability, high solubility) and Class III (high solubility; low permeability) drugs.	(Loftsson et al. 2004a)
For comparison: conventional penetration enhancers, like fatty acids and fatty alcohols, improve transmembrane drug permeability by penetrating into the membrane to reversibly decrease its barrier function.	(Tsutsumi et al. 2002; Williams and Barry 2004)

pear to be similar to those of semi-permeable membranes with a MWCO much greater than 2000 (the MW of the drug/cyclodextrin complex) (Másson et al. 2002).

Hairless mouse skin is much more permeable than human skin (i.e. gives larger P_M than human skin) and, under certain conditions (i.e. when $P_M > P_{Aq}$) drug permeation through the skin can be hampered by drug permeation through an external aqueous diffusion layer. Under such conditions cyclodextrins can act as penetration enhancers for lipophilic water-insoluble drugs. However, excess cyclodextrin will reduce the apparent partition coefficient (K in Eq. 2) of the drug from the aqueous exterior to the lipophilic membrane (Másson et al. 2005). Thus, excess cyclodextrin will lower the value of P_M leading to reduced drug permeation.

Many biological membranes have an aqueous mucus layer or other aqueous layers (e.g. tear film or saliva) adjacent to the membrane surface. These aqueous layers can contain proteins and polysaccharides (e.g. mucins) that bind water molecules into gel-like structures of relatively high viscosity (η) and, since P_{Aq} decreased with increasing η (Eqs. (2) and (7)), these layers are more effective diffusion barriers (i.e. lower P_{Aq}) than pure unstirred water layers. Also, according to Eq. (2) P_{Aq} decreases with increasing thickness of the aqueous layer (h). Under these conditions P_M is frequently larger than P_{Aq} and cyclodextrins can act as penetration enhancers for lipophilic water-insoluble drugs. In general, since P_M decreases with increasing MW of the drug (i.e. increasing r in Eq. (7)) cyclodextrins are more effective enhancers for low MW drugs (MW less than 500) than for high MW drugs.

Finally, since drug/cyclodextrin complexes are unable to permeate lipophilic membranes, and since excess amounts of cyclodextrins reduce the drug partition coefficient from the aqueous exterior into the membrane, cyclodextrins can reduce delivery of water-soluble drugs through lipophilic membranes, resulting in reduced bioavailability. The conclusions are summarized in Table 4.

Table 3: Effect of cyclodextrin solubilization of drug permeability through various membranes

Membrane	Type of barrier	Character	Diffusion layer thickness (ìm)	Effect of increasing cyclodextrin concentration	
				Saturated drug solutions	Unsaturated drug solutions with excess cyclodextrin
Semi-permeable cellophane	Size exclusion, membrane controlled	Porous with MWCO 500	~0	No effect	Decreased permeability
Semi-permeable cellophane	Size exclusion, membrane controlled, diffusion controlled	Porous with MWCO 12–14,000	Membrane thickness	Increased permeability	No effect
Fish skin	Aqueous, diffusion controlled	Porous with MWCO $\gg$ 2000	$\gg$0	Increased permeability	No effect
Hairless mouse skin*, stirred donor phase	Lipophilic, no aqueous diffusion layer	Lipophilic membrane controlled	~ 0	No effect	Decreased permeability
Hairless mouse skin*, unstirred donor phase	Lipophilic with aqueous diffusion layer	Aqueous diffusion and lipophilic membrane controlled	>1	Increased permeability	Decreased permeability
Buccal and sublingual	Lipophilic with aqueous diffusion layer	Aqueous diffusion and lipophilic membrane controlled	70–100	Increased permeability	Decreased permeability
Eye cornea	Lipophilic with aqueous diffusion layer	Aqueous diffusion and lipophilic membrane controlled	~8	Increased permeability	Decreased permeability
Nasal mucosa	Lipophilic with aqueous diffusion layer	Aqueous diffusion and lipophilic membrane controlled	~50	Increased permeability	Decreased permeability
Intestinal mucosa	Lipophilic with aqueous diffusion layer	Aqueous diffusion and lipophilic membrane controlled	30–100	Increased permeability	Decreased permeability
Lung mucosa	Lipophilic with aqueous diffusion layer	Aqueous diffusion and lipophilic membrane controlled	~ 5	Increased permeability	Decreased permeability

* Permeability determined *in vitro* in Franz-type or side-bi-side diffusion cells
Assuming a hydrophilic cyclodextrin ($\log K_{o/w} < -3$) and a lipophilic water-insoluble drug ($\log K_{o/w}$ between 1 and 5, molecular weight less than 500 Dalton, and aqueous solubility less than 1 mg/ml)
Based on the following references: (Cho et al. 1995; Jarho et al. 1996; Kublik et al. 1996; Chang and Banga 1998; Lennernäs 1998; Loftsson and Järvinen 1999; Masson et al. 1999; Bary et al. 2000; Zuo et al. 2000; Loftsson and Masson 2001; Washington et al. 2001; Felton et al. 2002; Loftsson and Stefánsson 2002; Loftsson et al. 2003b; Babu and Pandit 2004; Felton et al. 2004; Loftsson et al. 2004a; Richter and Keipert 2004; Loftsson et al. 2005a; Manosroi et al. 2005)

Table 4: When do cyclodextrins act as penetration or absorption enhancers?

Conditions	Effect
No complexation	If the drug does not form a cyclodextrin complex then cyclodextrins will have no effect.
Water-soluble drugs that form cyclodextrin complexes	Formation of a cyclodextrin complex will decrease drug permeability through biological membranes leading to reduced bioavailability.
Lipophilic water-insoluble drugs that form water-soluble cyclodextrin complexes	Cyclodextrins will enhance drug permeation through the membrane if, and only if, P_M is greater than P_{Aq}, or in other words, when permeation through the aqueous layer is the rate-determining step in the overall membrane permeation. Cyclodextrins are more effective enhancers for small MW drugs (have larger P_M) than for high MW dugs. Since P_{Aq} decreases with increasing h, the effectiveness of cyclodextrins will increase with increasing thickness (h) of the unstirred water layer. Since P_{Aq} decreases with increasing η, the effectiveness of cyclodextrins will increase with increasing viscosity (η) of the unstirred water layer.

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