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The effects of cyclodextrins on drug absorption. I. In vitro observations

H.W. Frijlink, A.J.M. Schoonen and C.F. Lerk

Department of Pharmaceutical Technology and Dispensing, University of Groningen, Groningen (The Netherlands)

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Summary

A two-phase transfer system, water with organic solvent, was used to investigate the effect of cyclodextrins on transport of dissolved lipophilic drugs from the aqueous to the organic layer. Four model drugs, diazepam, medazepam, *n*-butyl-*p*-aminobenzoate and *n*-pentyl-*p*-aminobenzoate were used. Stability constants of different cyclodextrin complexes were determined by a phase solubility technique or by a spectral displacement technique. It was found that the first-order transport rate constants of the drugs were hardly affected by complexation with β-cyclodextrin. This phenomenon could be explained by displacement of the drug from the cyclodextrin complex by organic solvent near the interface. This process results in a unique system which has a low concentration of free drug in the bulk of the aqueous layer, whereas near the interface a relatively high concentration of free drug exists. It could be demonstrated that the properties of the organic solvent also influenced the transport rate of complexed drug.

Introduction

Cyclodextrins are toroidal oligosaccharides known to form inclusion complexes in aqueous solutions with various types of organic substances, among which many hydrophobic drugs. The solubility of these drugs is often increased by this complexation (Lach and Pauli, 1966).

The complexation depends largely on the dimensions of the cyclodextrins and the particular sterical arrangement of the functional groups of the molecules, which leads to a relatively hydrophilic outside and a hydrophobic cavity of the molecule. The apolar cavity is that part of the molecule able to bind guest molecules by hydrophobic forces (Szejtli, 1982).

The use of cyclodextrins in drug formulations has been given increased attention lately, and many papers describing improved bioavailability have been published (Pitha et al., 1983; Jones et al., 1984). Many of these deal with the use of solid or semi-solid dosage forms, and solid drug-cyclodextrin complexes. The effects of cyclodextrin complexation on the absorption of dissolved drugs, however, remains largely unclear, and there seems to be a lack of experimental data concerning this topic. The only knowledge available comes from computer simulation studies (Habon et al., 1984), or stems from experiments in which solid dosage

Correspondence: H.W. Frijlink, Department of Pharmaceutical Technology and Dispensing, University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands.

forms were administered (e.g. Uekama et al., 1983a) therefore the results are difficult to interpret.

To study the effects of several auxiliary substances on drug transport from the gastrointestinal tract to the circulation, many in vitro systems, simulating this transport, have been developed (Howard et al., 1969; Nook et al., 1987; Vromans and Moolenaar, 1985). These in vitro models, however, have hardly been used for studying the effects of cyclodextrins on drug absorption. The only study describing these effects was performed by Uekama (1983b), using a 3-phase system (solid–aqueous–organic) for studying drug release from tablets of cyclodextrin complexes. The obtained data should, however, be treated with caution because these results do not enable one to make a distinction between effects of cyclodextrins on dissolution and on the phase transfer of dissolved drug(–complex). Several studies on the membrane permeation of drug–cyclodextrin complexes were also performed (e.g. Otagiri et al., 1983), but they are of little value because the drug will in vivo not be absorbed in the complexed form (Frijlink et al., 1987a).

In this paper the results of an in vitro study performed with a 2-phase transfer system (aqueous–organic) are described. The study deals with the effects of cyclodextrin complexation on the phase transfer of dissolved drugs, using 4 model substances: diazepam, medazepam, *n*-butyl-*p*-aminobenzoate, and *n*-pentyl-*p*-aminobenzoate. Some preliminary explanations about the behaviour of the complex near the interface are given. In the second part of this study the in vivo effects of cyclodextrins on drug absorption will be presented.

Materials and Methods

Materials

β-Cyclodextrin was kindly supplied by AVEBE, Veendam, The Netherlands. The sodium 4-(4-hydroxy-1-naphthylazo)-1-naphthalene sulfonate (HNS) was a gift from Dr. Matsui, Shimane University. Japan. Medazepam was a gift from Hoffmann- la Roche, Basel, Switzerland. The diazepam was obtained from Centrachemie, Etten-Leur, The Netherlands, and the *n*-butyl-*p*-aminobenzoate (*n*-butyl-PABA) from Sigma, St. Louis, U.S.A. The *n*-pentyl-*p*-aminobenzoate (*n*-pentyl-PABA) was synthesised by the method described by Kadaba et al., and recrystallised before use. The water used throughout the study was deionized and distilled. All other chemicals used were of analytical grade.

Determination of the stability constants of the complexes

The stability constants (K_s) of the 4 model substances were determined using the phase solubility method of Higuchi and Connors (1965). An excess amount of drug was weighed into water containing different concentrations of β-cyclodextrin, and shaken at 37 °C until equilibrium was reached. The solutions were filtered (0.2 μm) and the concentration of dissolved drug was determined by UV-absorption spectroscopy, using a Beckman model 25 UV-vis spectrophotometer. Stability constants were calculated from the initial straight line portions of the phase solubility diagrams, using the equation:

$$K_s = \frac{\text{slope}}{S_0\ (1 - \text{slope})}$$

in which S_0 is the solubility without β-cyclodextrin (β-CD). The initial free fractions of the drugs, in transport experiments, were calculated using this stability constants in solving the cubic equation obtained upon rearranging the basic equation:

$$K_s = \frac{[D\beta\text{-CD}]}{[D] \cdot [\beta\text{-CD}]}$$

to:

$$D = \left[-(M - N + K^{-1}) + \sqrt{(M - N + K^{-1})^2 + 4NK^{-1}} \right] [2]^{-1}$$

In this equation $M = [D\beta\text{-CD}] + [\beta\text{-CD}]$ and $N = [D\beta\text{-CD}] + [D]$. To determine stability constants of organic solvents a spectral displacement tech-

nique described by Selvidge and Eftink (1986) was used. Phenolphthalein and sodium 4-(4-hydroxy-1-naphthylazo)-1-naphthalenesulfonate were used as chromophoric ligand. Stability constants of the chromophores themselves were determined by adding increasing amounts of β-cyclodextrin to solutions of the dyes. The phenolphthalein was used in a sodium carbonate buffer pH = 10.8 (4×10^{-3} M) at a final concentration of 2.5×10^{-5} M. Total concentration of β-cyclodextrin ($[\beta\text{-CD}]_0$) varied from 0 to 5×10^{-4} M. The 55- nm absorbances were measured at 37 °C. The stability constant (K_1) was then calculated from the differences in absorbance (δA) due to β-cyclodextrin complexation.

First a double reciprocal plot of $1/\delta A$ vs $1/[\beta\text{-CD}]_0$ was constructed. The δA_{max} (the theoretical absorbance when all ligand is complexed) could then be estimated by extrapolation to the y-axis. The fraction of the chromophoric ligand (v) complexed by β-cyclodextrin can now be calculated through the following equations:

$$v = \frac{[L\beta\text{-CD}]}{[L]_0} = \frac{K_1[\beta\text{-CD}]}{1 + K_1[\beta\text{-CD}]} = \frac{\delta A}{\delta A_{max}}$$

In this equation $[L\beta\text{-CD}]$ is the concentration of the complex, whereas $[\beta\text{-CD}]$ and $[L]_0$ denote the concentration of non-complexed β-cyclodextrin and the total concentration of phenolphthaleïn respectively. The free concentration β-cyclodextrin was calculated as: $[\beta\text{-CD}] = [\beta\text{-CD}]_0 - v[L]_0$. So from a double reciprocal plot of $1/v$ versus $1/[\beta\text{-CD}]$ the value of the stability constant of phenolphthalein β-cyclodextrin complex could be determined.

The stability constant of HNS was determined in the same way using a solution of 4×10^{-5} M HNS in citrate-phosphate buffer pH = 6.4 (8.5×10^{-4} M). β-Cyclodextrin concentrations were over a range from 0 to 5×10^{-4} M, the absorbances at 483 nm were measured.

To determine stability constants of the organic solvents, small amounts of them were added to a buffered 2.5×10^{-5} M phenolphthaleïn solution, with 2.7×10^{-5} M β-cyclodextrin, and the A_{550} was determined at 37 °C. The same procedure was used with HNS (4×10^{-5} M), at a β-cyclodextrin concentration of 1×10^{-4} M, measuring A_{483}. The concentrations of the organic solvents varied over a range from 2.5×10^{-4} to 7×10^{-4} M. The absorbances were always measured immediately after the addition and mixing of the organic solvents. Stability constants (K_2) were calculated from degree of displacement of the chromophores by organic solvents, using the equation:

$$K_2 = \left\{[\beta\text{-CD}]_0 - \frac{v}{K_1(1-v)} - v[L]_0\right\} \times \left\{\frac{v}{K_1(1-v)} \times \left([O]_0 - [\beta\text{-CD}]_0 + \frac{v}{K_1(1-v)} + v[L]_0\right)\right\}^{-1}$$

In this equation $v = \delta A/\delta A_{max}$ in which δA_{max} is the one calculated in the determination of the stability constant of the chromophoric ligand–β-cyclodextrin complex. $[O]_0$ denotes the total concentration of organic solvent in solution.

Distribution studies in n-octanol/water systems

Partition coefficients were determined by using 25.0 ml water, with the drugs ($0.65–1.90 \times 10^{-4}$ M) (and β-cyclodextrin) dissolved in it, and 1.0 ml octanol saturated with water. Both phases were shaken vigorously for 2 h at 37 °C. After separation of the phases by centrifugation, the concentration in both phases was determined by UV-absorption measurements. The partition coefficients (PC) were calculated from the concentrations in the aqueous (C_a) and organic (C_o) layer ($PC = C_o/C_a$). The amount of β-cyclodextrin used in the experiments was always so that a ratio drug : β-cyclodextrin of 1 : 40 was obtained. It should be noted that the aqueous phase was not saturated with octanol before the experiment, because octanol could interfere with the complexation of the drugs by β-cyclodextrin.

Determination of transport rate using a two-phase transfer system

The two-phase transfer system used for determination of transport rates of the drugs, from

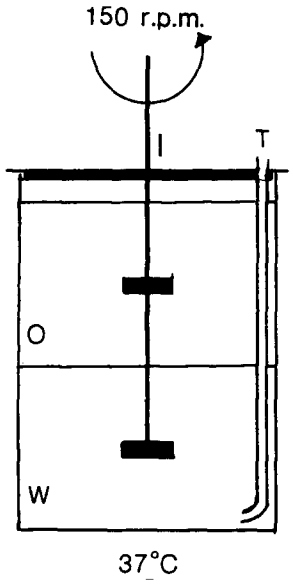

Fig. 1. Two-phase transfer system. I = impeller; T = sample tube; O = organic phase; W = aqueous phase.

different β-cyclodextrin drug complexes, is depicted in Fig. 1. It consists of a beaker thermostated at 37 °C with an impeller in the centre rotating at a speed of 150 rpm. Both phases had a volume of 200 ml. Drugs (and β-cyclodextrin) were dissolved in water at appropriate concentrations before the experiments. *n*-Octanol, chloroform, or cyclohexane, saturated with water before the experiment, were used as organic phases. Samples of 1.0 ml were taken from the aqueous phase at appropriate times, during 2 h. Concentration of the total drug (free and complexed) in these samples was determined by UV absorption measurements. The β-cyclodextrin concentration was determined using the HPLC-method described earlier (Frijlink et al., 1987b).

Observed first-order transport rate constants (K_{obs}) were calculated by performing a linear regression on the logarithm of drug concentration against time. All experiments were performed in 3-fold, and the given results are the mean of the 3 experiments.

To study processes near the interface more closely several observations were done in a non-stirred system. A solution of 5×10^{-5} M phenolphthaleïn in 8×10^{-3} M sodium carbonate buffer (pH = 10.8) was prepared, and decolored by 1×10^{-3} M β-cyclodextrin. The reference solution was prepared by diluting the phenolphthalein solution with buffer until it had the same colour as the β-cyclodextrin containing solution. Upon both solutions (50 ml) 15 ml of octanol saturated with water was poured carefully, and the colour intensity of the system was observed.

Results and Discussion

Stability constants of the complexes

Table 1 presents the stability constants of β-cyclodextrin complexes, as they were obtained using different methods. The benzodiazepine β-cyclodextrin system showed solubility curves of the A_1-type, and the PABA-ester β-cyclodextrin systems of the B_s-type. Stability constants are lower than those found by Andersen and Bundgaard (1982) and Uekama et al. (1983c); this difference can be explained for by the higher temperature used. The use of a spectral displacement technique was necessary for determination of the stability constants of organic solvents. The phase solubility method could not be applied to these solvents because they could not be determined by simple UV-absorption measurements. Constants found for *n*-butyl-PABA using the 3

TABLE 1

Stability constants of different β-cyclodextrin complexes at 37 °C

	K_s (M^{-1}) P.S. [a]	K_s (M^{-1}) S.D. Ph. [b]	K_s (M^{-1}) S.D. HNS [c]
Diazepam	179		
Medazepam	794		
n-Butyl-PABA	911	892	1031
n-Pentyl-PABA	1320		
Chloroform			151
Cyclohexane		1351	1422
n-Octanol		1667	1646
Phenolphthalein		19157	
HNS			2195

[a] Stability constant determined by the phase solubility method.
[b] Stability constant determined by the spectral displacement method using phenolphthaleïn as chromophoric ligand.
[c] Stability constant determined by the spectral displacement method using HNS as chromophoric ligand.

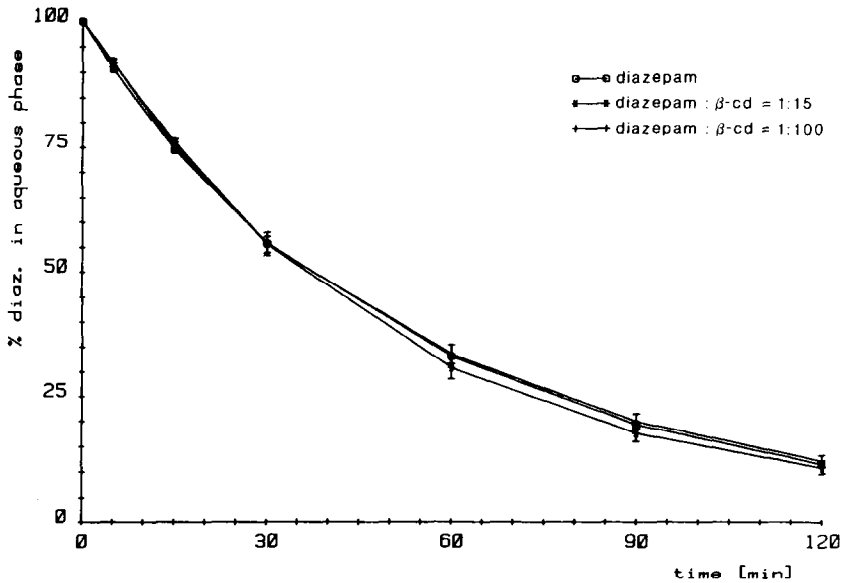

Fig. 2. Experimental plot showing the effect of β-cyclodextrin on transport of diazepam from water to *n*-octanol.

methods show that there is a good correlation between them. However, on the other hand, it should be noted that the use of the spectral displacement technique is certainly not the method of first choice when stability constants of substances possessing a low stability constant and a low aqueous solubility have to be determined, because then differences in absorption will be too small to allow for a reliable determination. The phase solubility method provides a much better determination in these cases. The stability constant of medazepam could not be determined by

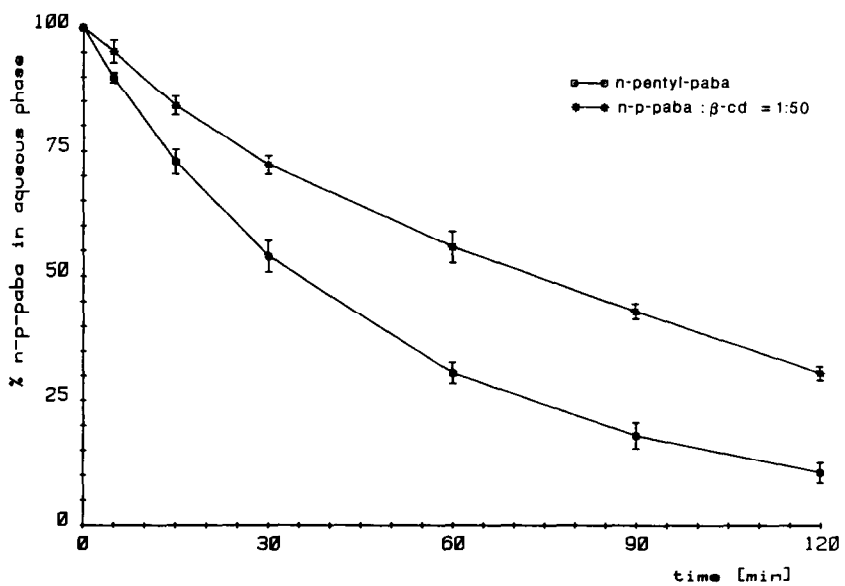

Fig. 3. Experimental plot showing the effect of β-cyclodextrin on transport of *n*-pentyl-PABA from water to *n*-octanol.

the spectral displacement technique because this drug has an absorption at 483 nm and 550 nm itself.

Differences in the stability constants of the organic solvents determined with phenolphthalein or HNS as chromophoric ligand are only small. However, due to the fact that the stability constant of β-cyclodextrin HNS complex is much smaller than that of β-cyclodextrin–phenolphthalein complex, the relative differences in absorbance due to addition of the organic solvents are larger with HNS; therefore stability constants obtained using HNS as chromophoric ligand are considered to be more reliable. The stability constant of chloroform–β-cyclodextrin complex could not be determined in a reliable way using phenolphthalein, because the difference in the stability constants was so large that upon addition of chloroform changes in absorption were too small to allow a determination of the stability constant in an accurate way.

Another problem in determining the stability constants of organic solvents was the fact that UV-absorption measurements had to be performed immediately after addition and mixing of the solvent. Otherwise evaporation of the solvent would occur.

Transport experiments

Figs. 2 and 3 illustrate curves which are obtained as a result of drug transport from aqueous to an organic phase. Table 2 presents the resulting transport constants (K_{obs}) using a *n*-octanol–water system. When no cyclodextrins are used all rate constants are of the same order of magnitude. It was found by several authors (Flyn et al., 1974; Byron et al., 1980; Van de Waterbeemd et al., 1980; De Haan et al., 1983; De Meere and Tomlinson, 1984) that with highly lipophilic drugs (large partition coefficients) the observed transport rates are practically equal to the transport of the solute from water to the organic phase. This rate is determined by a diffusion step through the stagnant aqueous layer adjacent to the interface. In this layer a concentration drop occurs, by virtue of which the drug is transported to the interface (Fig. 4). Therefore observed transport rates of both benzodiazepines and both PABA-esters are practically the same (diffusion rates of the drugs are only slightly different). Further on in this discussion the observed transport rates are assumed to be the rate of drug transport from the aqueous to an organic phase.

TABLE 2

First-order rate constants (k_{obs}) of drug transport from the aqueous to the n-octanol phase, and the calculated initial free fractions

	Ratio Drug : β-CD	Free fraction	k_{obs} (min^{-1})
Diazepam	–	1.00	0.0176
	1 : 15	0.4886	0.0180
	1 : 100	0.4835	0.0177
Medazepam	–	1.00	0.0177
	1 : 20	0.1796	0.0155
	1 : 40	0.1765	0.0145
	1 : 80	0.1749	0.0126
n-butyl-PABA	–	1.00	0.0192
	1 : 10	0.1664	0.0161
	1 : 40	0.1575	0.0144
n-pentyl-PABA	–	1.00	0.0188
	1 : 50	0.1139	0.0096

Concentration β-cyclodextrin = 6.0×10^{-3} M. Concentration of the drugs varies by the ratio.

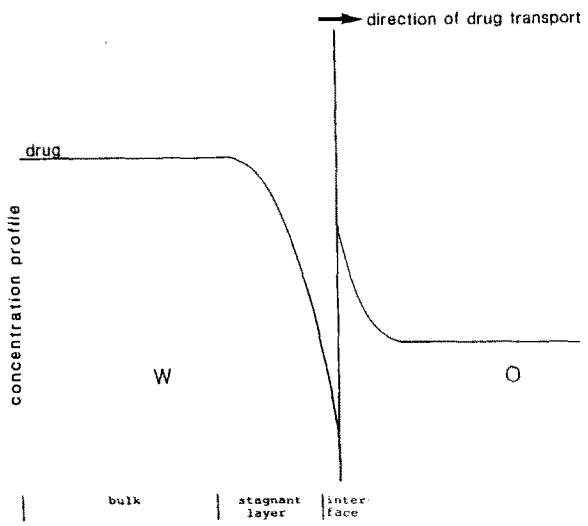

Fig. 4. Model of drug concentration profile near the interface without cyclodextrin.

Effects of β-cyclodextrin on transport

When β-cyclodextrin was added to the aqueous phase, transport rates were expected to decrease, due to the fact that β-cyclodextrin and β-cyclodextrin–drug complex were found not to enter the organic phase. According to classical two-film theory, only the free fraction of drug was thought to be the driving force in mass transport to the organic layer.

As is shown in Table 2, however, no or only a relatively slight decrease in transport rate was observed after addition of β-cyclodextrin. When compared with the initial free fractions of the drugs it is clear that the observed decrease in transport rates is much smaller than was expected. So there had to be another process influencing the transport of complexed drug. This process could be a displacement of drug from the β-cyclodextrin complex, near the interface, by organic solvent entering the aqueous phase. A strong indication for the occurrence of this process was found when *n*-octanol was poured on a solution of phenolphthalein (pH = 10.8), decolored by complexation with β-cyclodextrin. Fig. 5 clearly shows the pink layer near the interface which occurred in the solution decolored by β-cyclodextrin. This layer of increased concentration of free phenolphthalein can only be explained by displacement of phenolphthalein from the complex by octanol.

This model (Figs. 6 and 7) explains the observed phenomena mentioned above, because in this model the free fraction of drug near the interface will be determined by the degree up to which an organic solvent will be able to displace drug from the complex. This process will depend upon:

- the ratio drug : β-cyclodextrin;
- the stability constant of the drug–β-cyclodextrin complex;

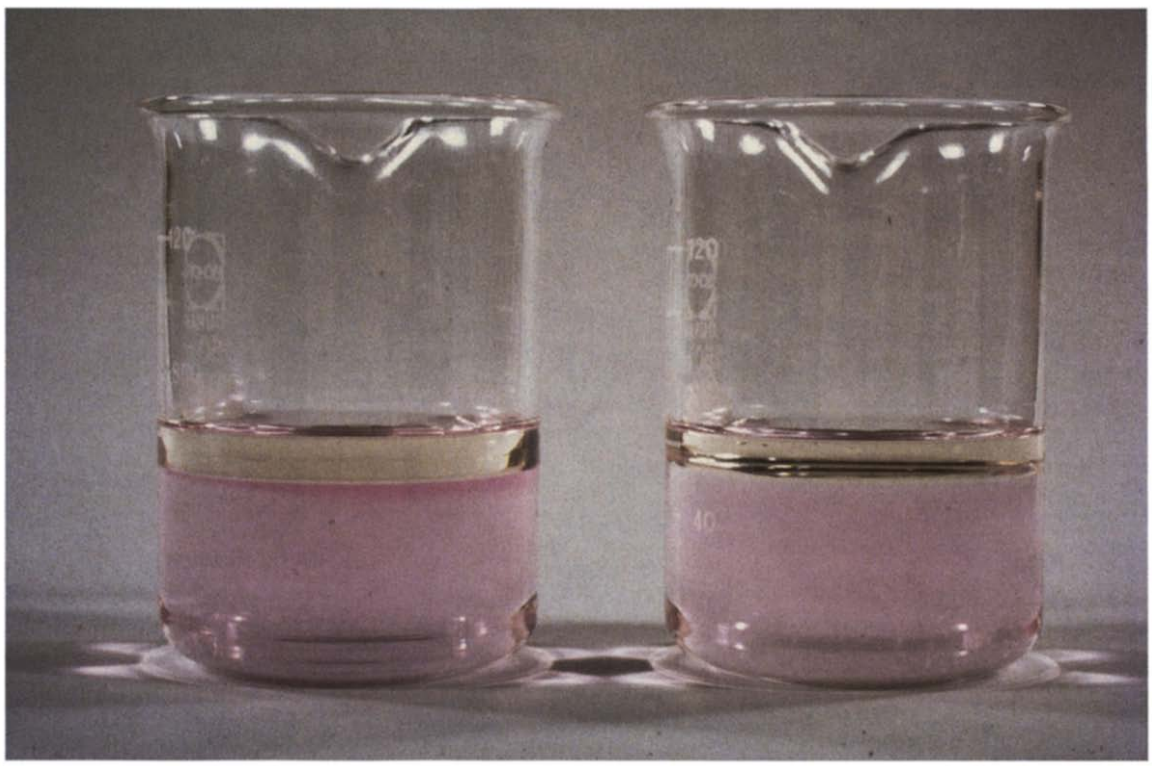

Fig. 5. Photograph of phenolphthalein solutions 15 min after addition of *n*-octanol. In the left beaker with cyclodextrin a layer of increased free phenolphthalein (pink layer) is clearly visible. The beaker on the right side contains the reference solution without cyclodextrin which is decolored by dilution.

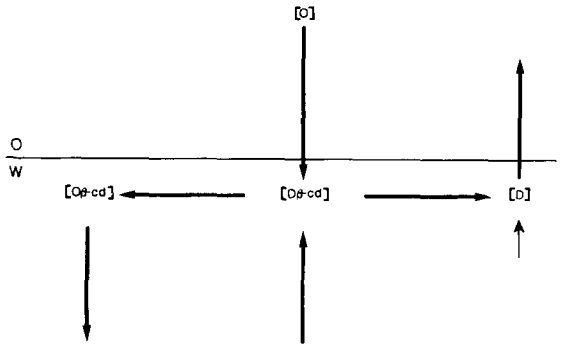

Fig. 6. Scheme for transport mechanism of complexed drug from the aqueous to an organic phase.

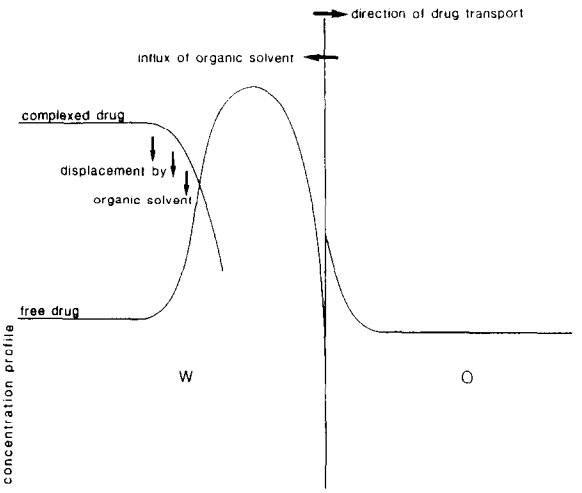

Fig. 7. Model of the free and complexed drug concentration profile near an interface as a result of displacement of drug from the drug–β-cyclodextrin complex by an organic solvent.

- the concentration of drug–β-cyclodextrin complex in the aqueous layer;
- the stability constant of the organic solvent–β-cyclodextrin complex; and
- the solubility of the organic solvent in the aqueous layer.

In order to verify this model further, several other experiments were designed. In Table 3 and Fig. 8 the effect of concentration of drug–β-cyclodextrin complex in the aqueous layer on transport rate is depicted. The ratio of drug : β-cyclodextrin was kept at 1 : 40 during all experiments but the absolute amounts of drug and β-cyclodextrin were changed. The biphasic curves which were found can be explained easily by the given model. In the first (horizontal) part of the curve the concentration of the complex is low and the organic solvent penetrating the aqueous layer will be able to displace almost all drug from the β-cyclodextrin complex near the interface, so the free fraction of the drug near the interface will be practically equal to the total (complexed and free) amount of drug available, and thus will the observed transport rate remain constant.

A decrease in transport rate observed in the second part of the curve can be explained by the

TABLE 3

Effect of concentration of β-cyclodextrin and drug on transport rate of drug from the aqueous to the n-octanol phase, at a fixed drug : β-cyclodextrin ratio of 1 : 40

Drug	Drug conc. (M)	β-CD conc. (M)	k_{obs} (min^{-1})	Φ (M/min)
Medazepam	5.00×10^{-5}	–	0.0177	
	2.22×10^{-5}	8.88×10^{-4}	0.0177	3.93×10^{-7}
	3.70×10^{-5}	1.48×10^{-3}	0.0177	6.55×10^{-7}
	7.38×10^{-5}	2.95×10^{-3}	0.0159	11.73×10^{-7}
	1.49×10^{-4}	5.96×10^{-3}	0.0143	21.31×10^{-7}
n-butyl-PABA	1.00×10^{-4}	–	0.0192	
	1.81×10^{-5}	7.25×10^{-4}	0.0192	3.48×10^{-7}
	2.58×10^{-5}	1.03×10^{-3}	0.0191	4.93×10^{-7}
	5.18×10^{-5}	2.07×10^{-3}	0.0169	8.75×10^{-7}
	1.04×10^{-4}	4.14×10^{-3}	0.0156	16.22×10^{-7}
	1.55×10^{-4}	6.21×10^{-3}	0.0144	22.32×10^{-7}

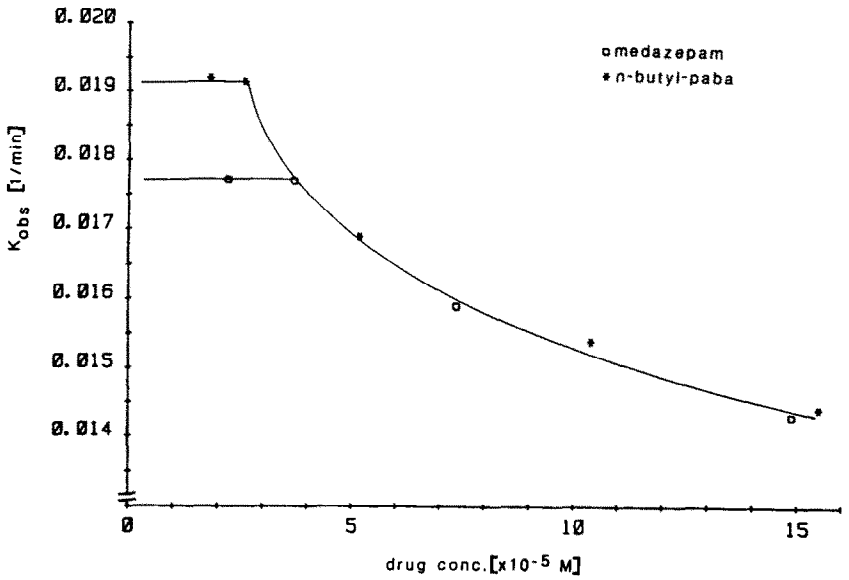

Fig. 8. Experimental plot presenting the effect of drug–β-cyclodextrin complex concentration on the observed transport rate constant in a *n*-octanol–water system. For the medazepam and *n*-butyl-PABA complexes with β-cyclodextrin at a ratio of 1 : 40 in all experiments.

fact that in this range the concentrations of the complex are so high that the organic solvent is not able to displace all drug from the complex. So the influx of *n*-octanol into the aqueous layer will become rate-limiting. A substantial amount of the drug remains now in the complexed form, and a

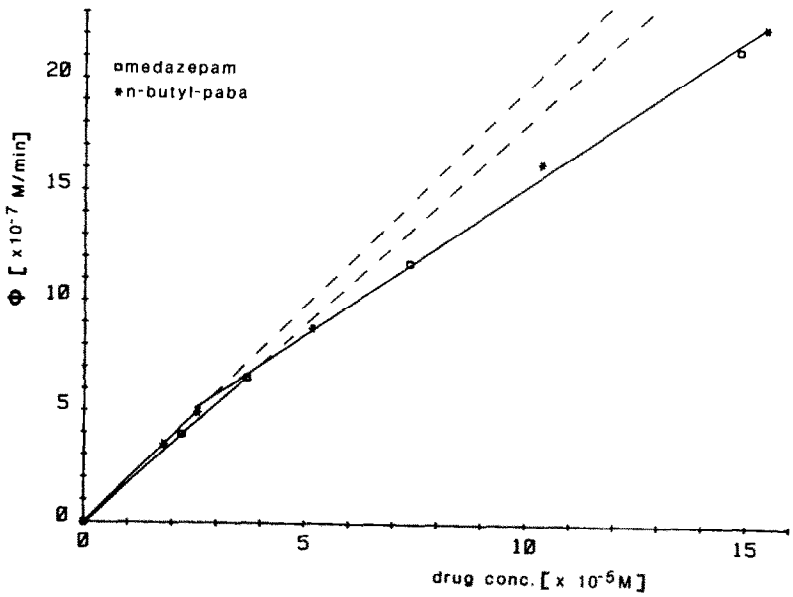

Fig. 9. Experimental plot of the initial mass flux versus the complex concentration in the *n*-octanol–water system. For medazepam and *n*-butyl-PABA complexes with β-cyclodextrin at a ratio of 1 : 40 in all experiments. The dotted lines are extrapolations of the first part of the curves.

decrease in transport rate constant is observed. As stability constants of the substances are of the same order of magnitude, rate constants will be equal in this part. This is also seen when the initial mass flux (Φ), which is defined as the product of the initial drug concentration and the rate constant, is studied (Fig. 9). Again it is seen that in the first part almost all drug is displaced from the complex and a direct relation between drug concentration and mass flux is observed. In the second part, however, the slope of the line is decreased because not all complex is dissociated before it reaches the interface. The fact that there is not a more pronounced decline in the slope of the lines indicates that the increasing amount of complex entering the stagnant layer has an increasing ability to counterbalance the decrease of the free fraction in this layer. This phenomenon can be described as a "material depot effect" of β-cyclodextrin–drug complex in the stagnant aqueous layer. As soon as some free drug is transported to the organic layer, equilibrium will be restored by dissociation of some of the complex, a fast process. In this way a decrease in free drug concentration adjacent to the interface is kept relatively small compared to a system without β-cyclodextrin, so overall transport rates will decrease less than expected.

To show that organic solvents play an important role in the whole transport process, experiments with organic solvents other than octanol were performed. Table 4 contains the results of these experiments. As described before, the stability constant of the organic solvent–β-cyclodextrin complex, as well as the solubility of the organic solvent in the aqueous layer will influence transport rate. When cyclohexane is compared to *n*-octanol, the stability constants of the complex differ only slightly but the solubility of cyclohexane in the aqueous phase is far less (5.6×10^{-3} M against 4.0×10^{-3} M for *n*-octanol). Therefore a larger decrease in transport rate is observed using cyclohexane instead of *n*-octanol. Chloroform has a smaller stability constant than octanol but an aqueous solubility which is much higher (6.4×10^{-2} M), and a large decrease in transport rate due to the small stability constant is not observed because the large amount of chloroform entering the aqueous layer is still able to displace almost all drug from the complex (solubility data from Smith et al., 1975).

In the above-described model concentrations of different components can be depicted schematically as in Fig. 8. However, it was found for diazepam and medazepam that, even when total drug concentrations were above the solubility in

TABLE 4

Effect of the organic solvent on drug transport rate from aqueous to organic layer

	β-CD conc. [M]	Ratio drug : β-CD		
n-butyl-PABA conc. (M)			*n*-octanol k_{obs} (min^{-1})	cyclohexane k_{obs} (min^{-1})
5.2×10^{-5}	–	–	0.0192	0.0158
1.3×10^{-4}	1.3×10^{-3}	1 : 10	0.0192	0.0125
2.9×10^{-5}	1.4×10^{-3}	1 : 50	0.0184	0.0095
Medazepam conc. (M)			n-octanol k_{obs} (min^{-1})	Cyclohexane k_{obs} (min^{-1})
2.2×10^{-5}	–	–	0.0177	0.0155
2.2×10^{-5}	8.8×10^{-4}	1 : 40	0.0177	0.0121
Medazepam conc. (M)			n-octanol k_{obs} (min^{-1})	chloroform k_{obs} (min^{-1})
3.7×10^{-5}	–	–	0.0177	0.0154
3.7×10^{-5}	1.48×10^{-3}	1 : 40	0.0177	0.0151
3.7×10^{-5}	2.96×10^{-3}	1 : 80	0.0150	0.0147

TABLE 5

n-Octanol–water partition coefficients of model substances without and with β-cyclodextrin (at a ratio of drug:β-cyclodextrin of 1:40)

	Partition coefficients o/w	
	Without β-CD	With β-CD
diazepam	473	472
medazepam	> 2000	> 2000
n-butyl-PABA	776	456
n-pentyl-PABA	1516	1396

pure water, no or only a slight decrease in transport rate was observed. This indicates that near the interface free concentration of drug is above its saturation concentration in pure water, and crystallization of the drug had to be expected. That no crystallization is observed in these experiments can be explained by the slow rate of crystallization as compared to transport rate. The displaced drug molecules will thus be transported to the organic layer before crystallization occurs. This was confirmed by an experiment in which only a few drops of *n*-octanol were added to the aqueous solution instead of 200 ml, containing 2×10^{-4} M medazepam and 3×10^{-3} M β-cyclodextrin. After 18 h medazepam crystals were observed in the aqueous solution. Medazepam displaced by *n*-octanol in this case could not be transported to an organic layer, and precipitated. Crystals were also observed at the interface when a very high concentration of β-cyclodextrin was used, but in this case they were solid complexes of the organic solvent and β-cyclodextrin.

Partition coefficients given in Table 5 clearly show that none of the observed decreases in transport rate were due to changes in the partition coefficient upon addition of β-cyclodextrin. Although partition coefficients are decreasing, values are still so high that theoretically the transport process of drug is still controlled by diffusion through the stagnant aqueous layer.

Conclusions

The above-described experiments show that addition of β-cyclodextrin to a two-phase solvent system with lipophilic drugs results in a unique system. In the bulk of the aqueous layer the concentration of the free drug will be low, due to complexation of drug by β-cyclodextrin. Near the interface, however, free drug concentrations will be relatively high, due to displacement of the drug from its complex by the organic solvent. Mass transport of drug from the aqueous to the organic layer will thus be mainly determined by the total (complexed and free) drug concentration in the aqueous phase and not only by the free fraction.

As the organic solvent enters the aqueous layer the dissolution-enhancing activity of cyclodextrins towards the drug will decrease, due to competition between solvent and drug for complexation. The partition coefficient will thus increase only slightly upon addition of cyclodextrins. Their use in extraction processes may therefore be advantageous over many other dissolution enhancers.

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