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Inclusion complexation of amide-typed local anaesthetics with β-cyclodextrin and its derivatives. II. Evaluation of affinity constants and in vitro transfer rate constants

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Abstract

The inclusion complex-forming abilities of five local anaesthetics of the amide-type (LAs), bupivacaine (BVC), etidocaine (EDC), lidocaine (LDC), mepivacaine (MVC) and prilocaine (PLC), with three cyclodextrins (CDs), β-cyclodextrin (βCD) and its alkylated derivatives 2-hydroxypropyl-β-cyclodextrin (HPβCD) and heptakis (2,6-di-*o*-methyl)-β-cyclodextrin (DMβCD), were studied in aqueous solution at 25°C and 37°C using the solubility method of Higuchi and Connors (1965) (*Adv. Anal. Chem. Instr.*, 4 (1965) 117–212) based on changes in the solubility of substrates (LAs) upon the addition of ligands (CDs). The interaction was quantified for each LA-CD system by determination of the stability constant, from the slope of the phase-solubility diagram. This second part of a study dealing with improvement in LA biopharmaceutics provided more evidence about LA-CD complexation. The solubility increase of the LAs in the presence of CDs was in the rank order DMβCD > HPβCD ≫ βCD; BVC showed the greatest stability constant values of all LAs tested, for all CDs and there was an influence of the temperature upon the complexation, only with βCD. Then, the effect of DMβCD and HPβCD on transfer of BVC from an aqueous to an organic phase was investigated with a two-phase system, water with methylene chloride or *n*-octanol. The BVC-CDs complexation provided modifications in first-order transfer rate constants compared with BVC alone, showing a decrease in the transfer rate of BVC between the two phases.

Keywords: Local anaesthetics; Cyclodextrins; Inclusion compound; Phase-solubility analysis; Stability constant; Two-phase system; Transfer rate constant

1. Introduction

The β-cyclodextrins are α-1,4-linked cyclic oligosaccharides composed of seven D-glucopyranose units (Duchêne, 1987), known for their great

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potential as drug carriers, because they are able to form reversible non-covalent inclusion complexes with various drugs of appropriate size and polarity (Uekama and Otagiri, 1987; Brewster et al., 1989), thus improving their solubility, stability or bioavailability (Duchêne et al., 1985). In vivo, such physicochemical modifications can affect the diffusional properties of the reversibly complexed drug ruled by mass action kinetics, leading to a time-dependent liberation of the included molecule into the surrounding biophase (Szejtli, 1982). Thus, complexation with torus-shaped cyclodextrins (CDs) as excipients in drug formulation and drug delivery systems is currently very active (Hassan et al., 1990) and may present an interest with local anaesthetic drugs (LAs). Indeed, these lipophilic drugs are more and more used for regional anaesthesia despite their short half-lives and their side-effects induced by systemic entrance. In a previous paper, qualitative evidences of the complexation between LAs of the amide type and CDs were obtained by spectral methods, i.e. NMR and IR, and by thermal analysis data from DSC (Dollo et al., 1996). To investigate the complexation thoroughly, the aim of the current study was to characterize in vitro the interaction process between five LA drugs of the amide type, i.e. bupivacaine (BVC), etidocaine (EDC), lidocaine (LDC), mepivacaine (MVC) and prilocaine (PLC) and three CDs among the most used in the pharmaceutical field, i.e. βCD and two derivatives (with improved solubilizing effect and lower parenteral toxicity in comparison with the parent βCD), heptakis (2,6-di-*o*-methyl)-β-cyclodextrin (DMβCD) and 2-hydroxypropyl-β-cyclodextrin (HPβCD). For that purpose, the intensity of the interaction was investigated by phase-solubility analysis for two temperatures. Then, after we had taken into account the stability constants (K_s) values obtained, we attempted to mimic in vivo transfer by using an in vitro two-phase system, aqueous liquid phase-organic liquid phase (methylene chloride or *n*-octanol) initially described by Uekama as a three-phase system including one dissolution stage (Uekama et al., 1983), and modified as Frijlink did for complexes already in solution (Frijlink et al., 1989). We choose to study the effect of DMβCD and HPβCD on the interfacial transfer of BVC from the aqueous to the organic phase. During this last stage prior to in vivo biopharmaceutic investigation, our goal was to determine one LA-CD couple in order to improve the biopharmaceutics of these LAs.

2. Materials and methods

2.1. Materials

βCD (KLEPTOSE®, ref. no. 472408, mol. wt. = 1135 g/mol) was kindly supplied by Roquette Frères (Lestrem, France), DMβCD (ref. no. 03483/01, mol. wt. = 1331 g/mol) was purchased from AVEBE (Veendam, The Netherlands), HPβCD used for phase solubility analysis (ENCAPSIN®, ref. no. 30.221.54, M.S. = 0.47, mol. wt. = 1330 g/mol) was purchased from Janssen Biotech (Olen, Belgium) while HPβCD used for two-phase transfer study (LAB 1456, ref. 490295, M.S. = 0.59, mol. wt. = 1374 g/mol) was kindly supplied by Roquette Frères (Lestrem, France). All CDs were used as received after considering their water content, determined by the Karl Fisher method with a Methrom E408 A apparatus and found to be 11.34%, 4.58%, 2% and 0.85% for βCD, HPβCD from Janssen, HPβCD from Roquette and DMβCD, respectively (each value is the mean of three determinations).

All LAs (Fig. 1) were supplied by Laboratoire Astra (Nanterre, France): among them, only PLC (mol. wt. 220.21) was received in its active base form while all others, BVC (mol. wt. 288.43), EDC (mol. wt. 276.42), LDC (mol. wt. 234.33) and MVC (mol. wt. 246.34) were supplied in their hydrochloride form. All bases were obtained by precipitation from an alkaline (3% aqueous NH_4OH solution) saturated solution of the corresponding hydrochlorides. The precipitates were rinsed by distilled water until a neutral pH filtrate was obtained. The bases were then dried (+ 40°C) before their purity was compared with hydrochloride standards by HPLC. All other reagents and solvents (E. Merck, Darmstadt, Germany) were of analytical grade. Freshly prepared distilled water was used as medium throughout the study.

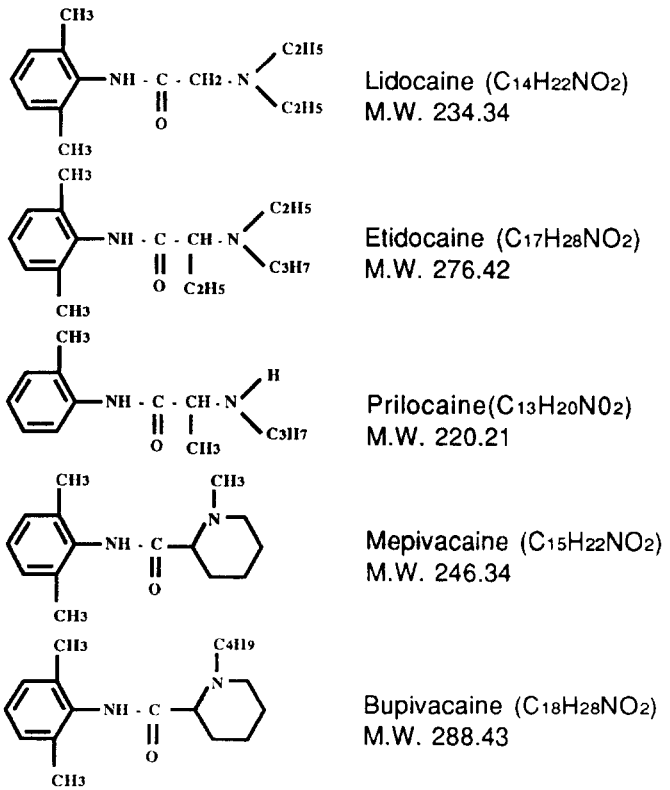

Fig. 1. Chemical structures of the amide-typed LAs studied and their molecular weights.

2.2. Phase-solubility studies

Solubility measurements were determined according to the method of Higuchi and Connors (1965). Excess amounts of the drug to be tested were weighted into 1 ml screw-cap polypropylene tubes (30 mg for BVC and EDC; 60 mg for MVC and LDC; 100 mg for PLC), to which were added aqueous solutions containing various concentrations of CDs, ranging from 0% to 50% (w/v) for DMβCD and HPβCD, 0% to 2.4% (w/v) for βCD. The suspensions formed were then rotated on a top to bottom shaker, thermostatically controlled at 25 ± 0.1°C and 37 ± 0.1°C. After solubility equilibrium for 24 h (further studies for up to 72 h did not show any difference), an aliquot was filtered through a cotton filter, appropriately diluted with the mobile phase (in a 5:1000 ratio) and total concentration of drug in the filtrate was analysed by HPLC. The experiment was carried out in triplicate for each LA-CD couple tested for both temperatures studied. The stability constant (K_s) was then calculated from the initial linear portion of the phase solubility diagrams (reporting drug concentration vs. CD concentration), assuming that a 1:1 stoichiometric ratio complex was formed at the initial step (slope smaller than 1 for all systems) according to Eq. (1) (Higuchi and Connors, 1965):

$$K_s = \text{slope}/s_o(1 - \text{slope}) \quad (1)$$

where s_o is the drug solubility in water.

2.3. Interfacial transfer

The two-phase stirred transfer device used is shown in Fig. 2. It consisted of a beaker thermostated at 37°C with a water bath, one glass bladed impeller in the centre of the upper phase (water when methylene chloride was used as organic phase, *n*-octanol otherwise) with a rotation speed of 100 rev./min, a magnetized agitator in the bottom phase giving an opposite 100 rev./min rotation in order to reduce the vortex effect near the interface, improving in that way the passage into the organic layer. The volume of the organic phase was 200 ml, equilibrated for 2 h at 37°C with 450 ml of water. At the beginning of the experiment, a solution of water containing 5 mg BVC (with or without CDs at appropriate ratio) was poured directly into the aqueous phase with a glass funnel, giving a final volume of 500 ml for the aqueous phase. Samples of 250 μl were collected from the aqueous phase at appropriate times, during 90 min and total concentration of BVC in the samples (free and complexed) was analysed by HPLC. The model system chosen was employed to measure the transfer rate constants of BVC alone or in the presence of CDs from the aqueous (water) to the organic phase (*n*-octanol or methylene chloride). The first-order transfer rate constants (K_1) were obtained from the slopes of the linear regressions on the logarithm of total BVC concentration in the aqueous phase against time.

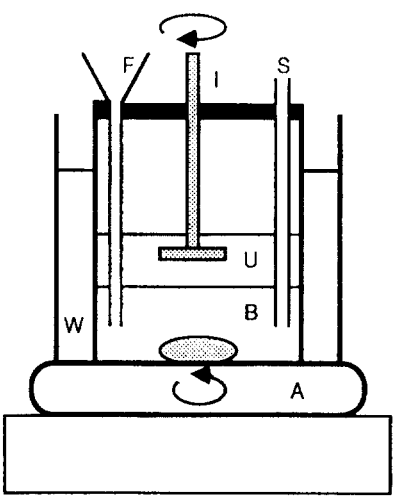

Fig. 2. Two-phases transfer system. U, upper phase (*n*-octanol for *n*-octanol/water system or water for methylene chloride/water system); B, bottom phase (methylene chloride or water); I, glass-bladed impeller; F, funnel; S, sample collector; A, magnetized agitator; W, thermostated water bath.

2.4. Quantitative determinations

The quantitative determinations were performed on a reverse-phase high-performance liquid chromatographic (HPLC) system according to Le Guevello et al. (1993). It consisted of a Waters Model 6000A pump (Waters Assoc., Milford, MA, USA) equipped with a Waters Model WISP 710 B automatic injector, an LDC Milton Roy Model Spectromonitor 3100 variable-wavelength UV detector (LDC Milton Roy, Riviera Beach, FL, USA), and a Delsi Model Enica 21 integrator (Delsi, Suresnes, France). The analytical chromatography column was a Waters Model μBondapak C_{18} (250 × 4 mm i.d.; particle size 10 mm) thermostated at 30°C. The mobile phase was a mixture of acetonitrile and 0.01 M sodium dihydrogenphosphate solution (20:80) for the quantitative determination of lidocaine, mepivacaine and prilocaine, (30:70) for the quantitative determination of bupivacaine and etidocaine, to shorten the retention time of these more lipophilic drugs. In both cases the pH of the aqueous solution was brought to 2.1 after addition of 15 N phosphoric acid. For the quantitative determination of all LAs, the flow rate was 1 ml/min and the detector was operated at 205 nm, excepted for prilocaine where it was set at 262 nm (λ_2 max).

2.5. Statistical analysis

All results were expressed as mean ± S.D. The effect of temperature upon complexation was studied using the *t*-test for comparison of slopes. To compare the transfer rate of BVC alone or complexed with CDs, statistical analysis was performed using one-way analysis of variance (ANOVA) (all values were normally distributed). If a significant difference was found, Fisher's PLSD test was used to identify which groups were different. $P < 0.05$ was considered statistically significant.

Table 1
Type of solubility curves, slopes obtained (according to Higuchi and Connors, 1965) and increase of solubility of local anaesthetics (under base form) with βCD, DMβCD and HPβCD, in water at 25°C and 37°C

LA	CD	Type of curve		Slope of curve[a]		Solubility increase (s_t/s_o[b])	
		25°C	37°C	25°C	37°C	25°C	37°C
Bupivacaine (BVC)	βCD	Al	Al	0.038	0.030	2.9	2.8
	DMβCD	Ap	Ap	0.081	0.080	147.7	151.6
	HPβCD	Al	Al	0.032	0.034	32.8	44.4
Etidocaine (EDC)	βCD	Al	Al	0.010	0.0084	1.8	1.6
	DMβCD	Ap	Ap	0.019	0.019	106.2	87.0
	HPβCD	Al	Al	0.006	0.006	10.1	9.0
Lidocaine (LDC)	βCD	Al	Al	0.30	0.26	1.4	1.4
	DMβCD	Al	Al	0.37	0.40	10.2	11.8
	HPβCD	Al	Al	0.24	0.25	6.4	7.2
Mepivacaine (MVC)	βCD	Al	Al	0.33	0.33	1.7	1.8
	DMβCD	Al	Al	0.33	0.34	14.3	15.6
	HPβCD	Al	Al	0.27	0.27	11.1	12.0
Prilocaine (PLC)	βCD	Al	Al	0.53	0.66	1.4	1.5
	DMβCD	Al	Al	0.58	0.60	9.7	10.3
	HPβCD	Al	Al	0.46	0.45	6.6	7.0

[a]Mean of three determinations.
[b]s_t = solubility of LA base in 0.021 M βCD, 0.442 M DMβCD and 0.385 M HPβCD solutions (mean of three determinations), s_o = water solubility of LA base (mean of eight determinations).

3. Results and discussion

3.1. Phase-solubility studies

The complexing capacity of each CD with BVC, EDC, LDC, MVC and PLC in aqueous solution at 25°C and 37°C was quantified using the solubility method. The equilibrium phase-solubility diagrams obtained for LAs with the CDs are presented in Figs. 3–6 (error bars are not shown, the magnitude of standard deviation is lower than 5% for each point).

Table 1 shows the types of solubility curves for LA-CD systems, all classified as type A phase diagram, implicating formation of soluble complexes, according to Higuchi and Connors (1965). βCD and HPβCD exhibited Al type diagrams with all LAs studied, showing linear increase for LAs solubilities as a function of CDs concentration, suggesting that all the complexes formed were of the first-order in CD (i.e. LA-CD, LA_2-CD, LA_3-CD,..., LA_m-CD). Figs. 3 and 4 show examples of Al phase-solubility diagrams obtained with βCD and HPβCD. However, complexes obtained with DMβCD were of two types, i.e. PLC, LDC and MVC exhibited Al type phase diagrams (Fig. 5) while BVC and EDC exhibited Ap type phase behaviour (Fig. 6), the positive deviation from linearity showing that the complexes formed were present to a higher order than 1 in CD. Also from Table 1, increased LA solubilities obtained with the addition of CDs at 25°C and 37°C, are evaluated by the s_t/s_o ratio. This solubility enhancement is considered to be mainly due to the formation of inclusion complexes

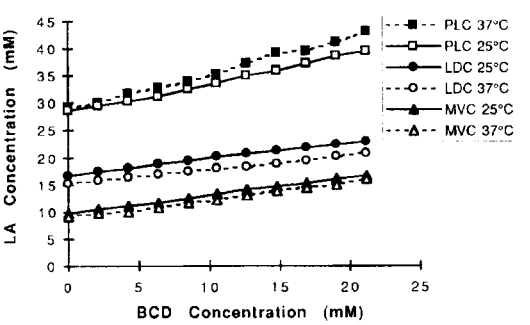

Fig. 3. Phase solubility diagrams for LDC, MVC and PLC with βCD at 25°C and 37°C. Each data point is the mean of three determinations.

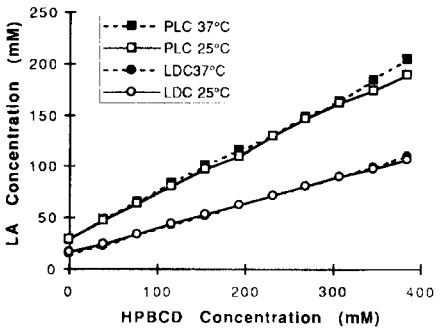

Fig. 4. Phase solubility diagrams for LDC and PLC with HPβCD at 25°C and 37°C. Each data point is the mean of three determinations.

(Higuchi and Connors, 1965). LA solubility obtained with alkylated CDs were much greater than those obtained with the parent βCD with the same guest molecules, in the rank order of DMβCD $\gg$ HPβCD $>$ βCD. This may be attributed to the different physicochemical properties of the alkylated CDs as compared with natural βCD. DMβCD was the most effective in markedly increasing the solubility of BVC and EDC because of their Ap type solubility curve. For example, the 25°C solubilities of BVC in 0.021 M βCD, 0.385 M HPβCD and 0.442 M DMβCD aqueous solutions were about 3, 33 and 148 times, respectively, higher than BVC alone (3.52×10^{-4} M).

There was an influence of the temperature upon solubility of LAs, observed only with βCD for all

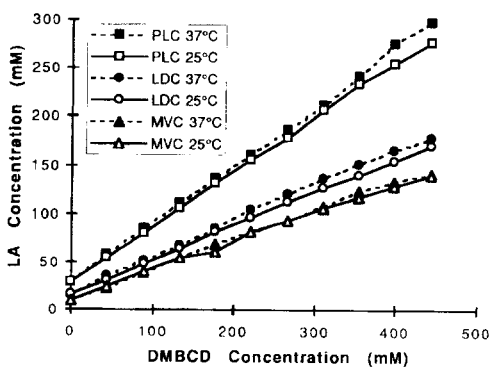

Fig. 5. Phase solubility diagrams for LDC, MVC and PLC with DMβCD at 25°C and 37°C. Each data point is the mean of three determinations.

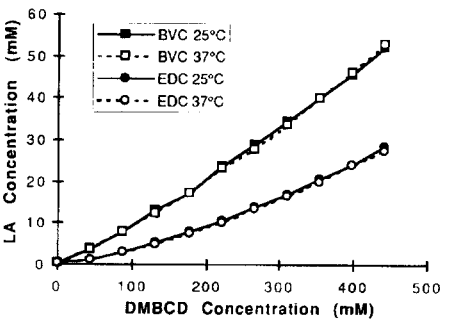

Fig. 6. Phase solubility diagrams for BVC and EDC with DMβCD at 25°C and 37°C. Each data point is the mean of three determinations.

LAs studied, except for MVC where the isotherms did not show any significative difference in the slope as a function of temperature. Concerning the remaining four, only PLC showed a positive effect of the temperature (slope increasing from 0.53 at 25°C to 0.66 at 37°C, $P < 0.001$), while the slope decreased for LDC (from 0.30 at 25°C to 0.26 at 37°C, $P < 0.001$), EDC (from 0.010 at 25°C to 0.0084 at 37°, $P < 0.001$) and BVC (from 0.038 at 25°C to 0.030 at 37°C, $P < 0.001$). This temperature-dependent solubilization was also obtained for hydrocortisone by Chun and Yun (1993), where a positive temperature effect was observed from 20°C to 37°C for α, β, γ-CD and DMβCD systems, resulting in an increase in the slope of the solubility curve related to the liberation of water molecules bound in the cavity. Hoshino also found that increase in temperature enhances solubility of carbamazepine, dexamethasone and griseofulvin in aqueous solutions of hydroxypropylcyclodextrins, counterbalancing the simultaneous dissociation of the complexes formed, as seen by lower K_s values obtained (Hoshino et al., 1993). With HPβCD and DMβCD, however, similar solubilization patterns for both temperatures did not show any significant difference in the solubility of LAs as shown in Table 1. Menard also observed a similar phenomenon for diazepam, ibuprofen and hydrochlorothiazide systems with βCD, i.e. a solubilization power essentially temperature-independent (Menard et al., 1990). With these different results, it seems that the chemical structure of

Table 2
Summary of apparent stability constants K_s (M^{-1}) of LA-CD complexes determined by the solubility method in water at 25°C and 37°C (mean ± S.D., $n = 3$)

CD	T (°C)	Local anaesthetic drug				
		Bupivacaine (BVC)	Etidocaine (EDC)	Lidocaine (LDC)	Mepivacaine (MVC)	Prilocaine (PLC)
βCD	25	112.0 ± 6.3	38.4 ± 1.6	25.8 ± 0.6	50.9 ± 1.3	96.0 ± 1.1
	37	87.5 ± 1.0	27.1 ± 1.7	23.6 ± 1.2	53.4 ± 2.0	68.0 ± 3.3
DMβCD	25	244.6 ± 8.7	73.3 ± 1.5	35.1 ± 1.5	50.3 ± 0.3	48.8 ± 1.1
	37	265.9 ± 8.5	61.5 ± 1.6	43.6 ± 0.7	57.5 ± 1.9	52.0 ± 2.4
HPβCD	25	95.1 ± 0.8	24.2 ± 0.8	19.9 ± 0.3	38,0 ± 1.1	30.0 ± 0.2
	37	101.2 ± 0.4	20.5 ± 0.3	23.1 ± 0.4	41.6 ± 1.3	28.9 ± 1.2

the drug may determine the predominant factor contributing to the driving forces for cyclodextrin complexation, among hydrophobic interactions, hydrogen bonding, Van der Waals and London dispersion forces, release of high energy water molecules from the cavity of the CD and release of strain energy in the macromolecular ring of the CD (Nakajima et al., 1984; Menard et al., 1990).

Table 2 summarizes the apparent stability constant (K_s) values of each LA-CD inclusion compound, obtained from Eq. (1) as a measure of the magnitude of the complexation. High values for K_s reflect favourable positioning of the drug inside the cavity of the CD molecule (Duchêne, 1987). K_s values were estimated from the linear portion of the phase solubility diagrams. Excepted for PLC, the higher LA-CD complex K_s value was obtained with DMβCD, i.e. the best solubilizing CD. βCD and its alkylated derivatives formed more or less stable inclusion complexes with LAs, weak values of K_s were obtained (between 20 and 50 M^{-1}) for most LA-CD complexes. However, K_s values found for BVC were between 90 and 265 M^{-1} suggesting stronger interaction. By way of comparison, the K_s value for BVC hydrochloride with HPβCD at 25°C was found to be 2.02 M^{-1}, showing that LAs under their hydrophobic base form were leading to stronger complexes, as shown by their much higher K_s values. Low K_s values for drug-CD complexes are commonly found which allow complex formation as well as improved biopharmaceutical properties. Indeed, Uekama and Otagiri (1987) mentioned K_s values for piroxicam systems with βCD and DMβCD (i.e. 90 and 200 M^{-1}, respectively), comparable with our data and Hassan also found a K_s value for the famotidine-βCD complex to be just below 80 M^{-1} (Hassan et al., 1990). We observed that K_s values for LA-HPβCD complexes were always between the K_s values of βCD and DMβCD, showing that CD nature also influenced the stability constant. Among the CDs tested, DMβCD showed the best complexing abilities, due to the methylation of hydroxyl residues. In fact, this procured an inhibition of the hydrogen bonds responsible for the weak aqueous solubility of βCD as well as an expansion of the hydrophobic cavity by capping, procuring greater surface for complexation (Brewster et al., 1989) and so an increase of the substrate binding by hydrophobic bounds (Green and Guillory, 1989).

3.2. Interfacial transfer

We used an in vitro phase transfer model as a way to simulate the effects of CDs on the in vivo BVC membrane transfer process. The biological activity of a drug depends on its concentration in the receptor compartment, which strongly depends on the transfer rate between both administration and receptor compartments. Transfer rate studies often involves biphasic models, water-organic solvent, and during the interfacial transfer of a drug, changes of concentration between the aqueous and the organic phase are following the equation: $dC_{org}/dt = K_1C_{aq} - K_2C_{org}$, where C_{aq} and C_{org} represent the concentration of the drug in the aqueous and organic phases, respectively,

K_1 and K_2 represent forward (from aqueous to organic phase) and reverse transfer rate constants, respectively. Kubinyi found a relationship between K_1, K_2 and the partition coefficient (P) of a substance, simplified for first-order kinetics, giving the following equation: $P = K_1/K_2$ (Kubinyi, 1977). That is to say, for highly lipophilic drugs (high values for P), $K_1 \gg K_2$, the transfer rate constant K_1 can be considered as the transfer of the drug from the aqueous to the organic phase. Fig. 7 schematizes the transfer mechanism of BVC from the aqueous to the organic phase. By altering the apparent solubility and partition coefficient of a substance, the complexation influences the transport from the aqueous to the organic phase (Flynn et al., 1974). Depending on the relative magnitudes of the stability constant of the inclusion compound (K_s), diffusivity of free and complexed drug in each phase and P of free and complexed drug between both phases, complexation will not change, will reduce or increase the transfer rate of the drugs (Flynn et al., 1974). According to the two-film theory, we will consider the assumption that when dissociation of the complex occurs in the aqueous phase, only the free fraction of BVC (the driving force in mass transport) can diffuse in the organic phase, depending on the P value. We then followed BVC concentration in the aqueous phase as a function of time. By plotting the logarithmic curve of BVC remaining in the aqueous phase as a function of time, we had an easy access to the first-order transfer rate constant K_1 as well as the half-live ($T_{1/2}$) of the phenomenon. Fig. 8 shows the experimental effect of HPβCD and DMβCD on transfer of BVC

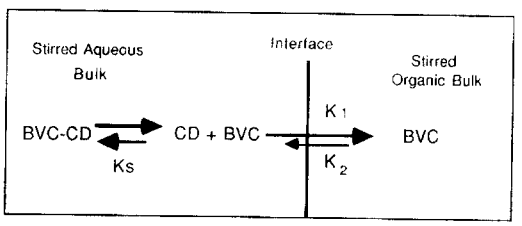

Fig. 7. Model for the transfer mechanism of complexed BVC from the aqueous to the organic phase (*n*-octanol or methylene chloride). K_s represents the stability constant of the BVC-CD complex, K_1 and K_2 represent forward and reverse rate constants from aqueous to organic phase, respectively.

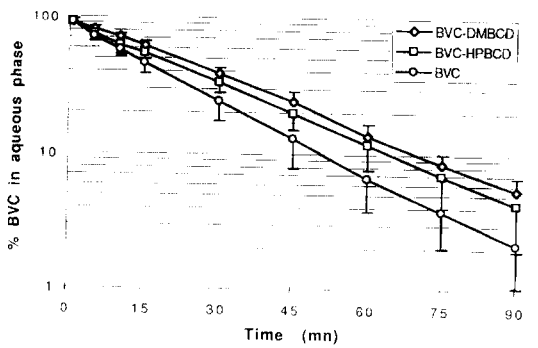

Fig. 8. Logarithmic curves, showing the effect of HPβCD and DMβCD on transfer of BVC from water to methylene chloride, in function of time. Each point represents the mean ± S.D. (n = 12 for BVC alone; n = 9 for BVC-DMβCD at a 1:10 ratio; n = 7 for BVC-HPβCD at a 1:10 ratio).

from water to methylene chloride at 37°C. Results are presented in Table 3. Upon the addition of CDs on an aqueous solution of BVC (with a BVC/CD ratio of 1:10), we observed a decrease in first-order transfer rate constants up to 40% compared with BVC alone, i.e. from 0.046 min^{-1} with BVC alone to 0.033 min^{-1} with DMβCD, while the half-life of the transfer was increased up to 37%, from 15.7 min with βCD to 21.4 min with DMβCD. $T_{1/2}$ and K_1 values obtained with DMβCD and HPβCD were statistically different compared with BVC alone but not when compared together, despite the difference between both K_s (266 M^{-1} vs. 101 M^{-1} for DMβCD and HPβCD, respectively). Values obtained with *n*-octanol are presented Table 4. This time, no significant difference between half-lives and first-order transfer rate values was obtained with a BVC/CD ratio of 1:10. However, a BVC/CD ratio of 1:100 led to significant values, but only when compared with BVC alone (showing a decrease in first-order rate constant up to 37% with DMβCD correlated with an increasing half-life up to 39%). From these results, a decrease in transfer rates is observed showing that CDs are able to retain BVC in the aqueous phase, but the ratio BVC/CD needed is dependent on the organic solvent used. Frijlink explained this phenomenon by mean of displacement of the drug from the complex by the organic solvent, being a function of the stability constant of the complex formed between the or-

Table 3
Effect of CDs on BVC transfer from aqueous to methylene chloride layer. Results are expressed as mean ± S.D.

Compound	Ratio BVC/CD	n	$T_{1/2}$ (min)	K_1 (min^{-1})
BVC alone	–	12	15.66 ± 2.88	0.0458 ± 0.0094
BVC-DMβCD	1:10	9	21.40 ± 2.05*	0.0327 ± 0.0034*
BVC-HPβCD	1:10	7	20.08 ± 3.30*	0.0352 ± 0.0058*

*Statistically significant compared with BVC alone ($P < 0.05$).

Table 4
Effect of CDs on BVC transfer from aqueous to n-octanol layer. Results are expressed as mean ± S.D.

Compound	Ratio BVC/CD	n	$T_{1/2}$ (min)	K_1 (min^{-1})
BVC alone	–	6	24.67 ± 3.70	0.0287 ± 0.0044
DMβCD	1:10	4	22.66 ± 4.55*	0.0314 ± 0.0052*
HPβCD	1:10	4	23.81 ± 3.93*	0.0295 ± 0.0041*
DMβCD	1:100	3	33.86 ± 3.80**	0.0206 ± 0.0022**
HPβCD	1:100	3	31.83 ± 3.36**	0.0219 ± 0.0022**

*Non-significant compared with BVC alone.
**Statistically significant compared with BVC alone ($P < 0.05$)

ganic solvent and the CD (competition between drug and solvent for the CD), as well as the solubility of the solvent in the aqueous phase (Frijlink et al., 1989). However, an effect of CDs on the transfer between the two phases exists, that seems to be more marked with DMβCD than with HPβCD (in the same order as the K_s values), even if it was not statistically possible to affirm it.

4. Conclusion

Complexes between LAs and CDs exist in aqueous solution, since increasing concentrations of CDs result in an increase in the solubility of the LAs. There is an effect of the temperature on the complexation process between βCD and all LAs studied, except MVC. Among the CDs studied, DMβCD and HPβCD showed the best complexing abilities, forming the strongest complex with BVC. Furthermore, the formation of inclusion complexes between BVC and CDs can affect the in vitro transfer rate of BVC, which seems to deserve in vivo investigations. These preliminary results confirmed the possibility of using LA-CD complexes. In vivo studies with a BVC-HPβCD complex are in progress as well as in vitro studies, implicating several others CDs.

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