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A physico-chemical study on the interaction between papaverine and natural and modified β-cyclodextrins

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Abstract

The interaction of the alkaloid drug papaverine (PAP) with different cyclodextrins (CyDs) was investigated. Freeze-drying method was used to prepare solid complexes that were characterized in the solid state using differential scanning calorimetry (DSC) and X-ray diffractometry. Circular dichroism spectroscopy (CD) and ^{1}H-NMR was used in an aqueous solution to obtain information about the inclusion mode of PAP into the cavity of CyDs. β-cyclodextrin (β-CyD) and dimethyl-β-cyclodextrin (DM-β-CyD) are able to include the drug but in all cases the stability constant values are very small, indicating a weak host–guest interaction. No exact information exists about the interaction of PAP with the internal cavity of hydroxypropyl-β-cyclodextrin (HP-β-CyD). Solubility studies in the presence of all CyDs were performed using Higuchi and Connors methods at 37°C and pH 7.4. The influence of complexation on the diffusion rate of PAP through artificial membranes was evaluated using dialysis bags. Little influence was observed in the presence of all macrocycles. © 1998 Elsevier Science B.V.

Keywords: Papaverine; β-cyclodextrin; Modified β-cyclodextrins; Characterization of the complexes; Solubility studies; Diffusion studies

1. Introduction

Papaverine (PAP) is a benzylisoquinoline alkaloid obtained from opium that shows a direct spasmolytic effect on the smooth muscles of the bronchi, gastrointestinal tracts, ureters and biliary system (Coleman and Farmer, 1971; Kadlee and Bauer, 1971; Martin et al., 1993) and a pronounced relaxant effect on blood vessels including coronary, cerebral, pulmonary and peripheral arteries (Tadashi et al., 1992; Takaaki et al., 1993). 'In vitro' studies demonstrated that cyclic AMP is implicated as a mediator of the PAP pharmacological effectiveness. The drug is able to inhibit the cyclic nucleotide phosphodiesterase (Berndt et al., 1976), thus increasing levels of intracellular

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cyclic AMP and inhibit the calmodulin that stimulates cyclic AMP-phosphodiesterase activity (Mannhold, 1988).

Because of its relaxant activity, PAP can be effectively used in cardiovascular insufficiency, cholecystolithiasis, gastrointestinal spasms and psoriasis. In recent years, PAP has been effective in the treatment of male impotency (De Bruin et al., 1991), it improves penis erection by increasing local circulation. In a study conducted by Voss and Eichler (1989) on topical or intra-urethral treatment of impotency, PAP was the preferred vasodilator compared to other vasodilators or alpha-blockers. In the topical formulation they used DMSO as the penetration enhancer.

Significant results were obtained by El Rashidy (1992), demonstrating the increase 'in vitro' of the flux of PAP through the whole glans penis skin when hydroxypropyl-β-cyclodextrin (HP-β-CyD) is used as penetration enhancer.

Usually, cyclodextrins (CyDs) are used as complexing agents of non-polar drugs, to modify some physico-chemical properties of these molecules (Uekama and Otagiri, 1987; Puglisi et al., 1996a; Ventura et al., 1997). After administration of the complexes, an increase of bioavailability, ascribed generally to the enhancement of solubility and dissolution rate of the complexed drug (Duchêne, 1987; Puglisi et al., 1995) is observed. However, it seems that this is not the only mechanism involved in drug absorption. In fact, it was recently demonstrated that CyDs were able to increase the drug bioavailability despite the formation of an inclusion complex. For example, dimethyl-β-cyclodextrin (DM-β-CyD) does not include insulin but it is able to improve nasal and rectal absorption of this hormone (Watanabe et al., 1992a,b). CyDs are able to include some components of biological membranes (phospholipids and cholesterol) altering their permeability (Ohtani et al., 1989; Puglisi et al., 1996b) and hence influencing the flux of various drugs. Therefore, CyDs can act on drugs absorption by means of two mechanisms: (i) (indirectly) influencing physico-chemical properties of the drugs and/or (ii) (directly) influencing the biomembrane permeability.

In this paper we investigate the interaction of PAP with different CyDs (natural β-CyD, DM-β-CyD and HP-β-CyD) to evaluate their influence on physico-chemical properties of the drug. The freeze-dried samples were investigated in the solid state by differential scanning calorimetry (DSC) and X-ray diffractometry, to verify the existence of an interaction between the two components. Circular dichroism (CD) and ^{1}H-NMR spectroscopy were used to obtain information about the existence of the complexes in solution. The influence of CyDs on water solubility of PAP was studied in a buffer solution of pH 7.4 and stability constants were determined from the solubility phase diagrams. Diffusion of PAP in the presence of different CyDs through an artificial membrane was evaluated by dialysis with respect to free drug.

2. Materials and methods

2.1. Chemicals

Papaverine hydrochloride (PAP) was obtained from Sigma (St. Louis). β-Cyclodextrin (β-CyD) and hydroxypropyl-β-cyclodextrin (HP-β-CyD), with 0.6 degree of average substitution, were kindly provided by Roquette Italia (Cassano Spinola, Italy). Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) was supplied by Nikon Skoduhhin Kako (Tokyo, Japan).

All other chemicals and solvents were analytical reagent grade. Deionized, double distilled water was used.

2.2. Preparation of solid inclusion complexes of PAP with CyDs

The inclusion complexes of PAP and different CyDs were prepared in 1:1 and 1:2 mole ratio by freeze-drying method.

CyDs ($4 \cdot 10^{-4}$ mol) were solubilized at room temperature in the minimum water amount and added to solid PAP ($2 \cdot 10^{-4}$ or $4 \cdot 10^{-4}$ mol). The suspensions were solubilized in a few minutes. The solutions obtained were stirred for 2 h and then freeze-dried using a Modulyo 4K (Edwards).

2.3. Determination of water solubility of free and complexed PAP

Free PAP or freeze-drying samples in 1:2 mole ratio were suspended in buffer solution pH 7.4 and stirred at 37 ± 0.5°C for 8 h. After centrifugation (Beckman mod J2-21) at 5000 r/m for 15 min the supernatant solutions were separated and assayed spectrophotometrically at 238 nm (UV/Vis Uvikon spectrophotometer, Kontron) to determine drug concentration.

2.4. Differential scanning calorimetry (DSC)

DSC scans were recorded on a Mettler DSC 12E, equipped with a Haake thermocryostate, mod. D8-G. A Mettler TA89E and FP89 system software was used for data acquisition. Each sample (inclusion complexes, physical mixtures in the same molar ratio and pure components) was scanned at a speed of 10°C/min, in the temperature range between 30 and 300°C, using nitrogen as purging gas.

2.5. X-ray diffractometry

X-ray of the samples were recorded using a PW 1050 Powder Diffractometer (Philips) under the following condition: Ni-filtered Cu radiation ($\lambda =$ 1.5418 Å); tube settings 40 KV, 20 mA; angular speed 1° 2 θ/min; 0–0.1–1 slits.

2.6. Circular dichroism spectroscopy (CD)

CD spectra of PAP alone and in the presence of different CyDs (molar ratio 1:2) were performed in buffer solution (pH 1.1 and 7.4) on a Jasco J-600D recording spectropolarimeter.

2.7. ^{1}H-nuclear magnetic resonance (^{1}H-NMR)

^{1}H-NMR spectra of free PAP or in the presence of β-CyD and DM-β-CyD were carried out on Varian Gemini 300 spectrometer at a probe temperature of 303 K. The chemical shift at 4.8 ppm due to residual solvents (H_2O and HDO) was used as internal reference. Equimolar deuterated aqueous solutions of PAP and DM-β-CyD were mixed in a 1:2 molar ratio in NMR tubes (final concentrations were: $2.66 \cdot 10^{-3}$ mmoles and $3.32 \cdot 10^{-3}$ mmoles for PAP and CyDs respectively in 0.7 ml of D_2O) and equilibrated overnight before the analysis. Pure components were analyzed in the same concentration used for the mixtures.

2.8. Solubility studies

According to Higuchi and Connors (1965), excess amounts of PAP were added to buffer solutions (pH 7.4) containing various concentrations of different CyDs. Each sample was accurately sheltered from the light and shaken at 37 ± 0.5°C until the equilibrium was reached (2 days). Then, an aliquot (5 ml) of each suspension was centrifuged. The supernatant was diluted and analyzed spectrophotometrically. Apparent 1:1 stability constants (K_c) were determined from the straight portion of the phase solubility diagrams according to Higuchi and Connors equation's (1965).

2.9. Determination of the partition coefficient

The partition coefficient of PAP alone or in the presence of various CyDs were determined used octanol as lipophylic phase and buffer solutions at pH 1.1 and 7.4 as hydrophylic phase. Due to low solubility of PAP in pH 7.4 buffer solution, different aliquot of PAP ($2.66 \cdot 10^{-4}$ and $26.6 \cdot 10^{-3}$ mmoles for pH 7.4 and 1.1, respectively) was solubilized in 10 ml of buffer solution containing or not the CyDs ($5.32 \cdot 10^{-4}$ and $53.2 \cdot 10^{-3}$ mmoles) and added to 10 ml of organic phase. The two phases presaturated each other. The samples were sheltered from the light, immersed in a thermostated water bath at 37 ± 0.5°C and mixed thoroughly by shaking for at least 24 h. The phases were separated by centrifugation at 5000 r/m for 15 min. Aliquots were removed from the upper and lower phases and then assayed spectrophotometrically. The partition coefficient was expressed as the logarithm of the ratio of the amount of PAP in the organic phase to that in the aqueous phase (log P).

2.10. Diffusion studies trough dialysis membranes

A 10 ml aliquot of saturated buffer solutions (pH 7.4) of PAP alone or of 1:2 lyophilized complexes were poured into a preswelled dialysis bags (benzoylated cellulose, cut off 2.000; Sigma, St. Louis). The dialysis bags were immersed in the receiver compartment containing 50 ml of buffer solution pH 7.4. At fixed time intervals (15, 30, 60, 90, 120, 150, 180 and 240 min) 1 ml of dialyzed solution was analyzed spectrophotometrically to determine PAP concentration. The medium was reconstituted with 1 ml of fresh buffer solution.

3. Results and discussion

To verify the existence in the solid state of the interaction between PAP and various CyDs, each sample was analyzed by DSC and X-ray diffractometry.

The calorimetric curves obtained for PAP-DM-β-CyD system are shown in Fig. 1. The disappearance of fusion peak of PAP at 228°C in the thermograms of the samples (both 1:1 and 1:2 mole ratio) indicates the presence of an interaction between the two components (Cabral-Marques et al., 1990). In both samples one or two exothermic peaks are present, evidence of their amorphous nature (El Gendy et al., 1986; Hanawa et al., 1993), probably due to the preparation method.

X-ray diffraction pattern (Fig. 2) of the 1:2 PAP-DM-β-CyD product also shows the amorphous state of the sample. In fact, the disappearance of PAP signals while present in the physical mixture shows the existence of a new solid phase having a lower crystallinity than the drug and CyDs.

DSC scans and X-ray diffraction patterns of other PAP-CyDs samples were not reported because similar results were obtained.

It is know (Djedaïni and Perly, 1991) that solid state characterization gives information about the interaction between drug and CyDs that could interest only the external surface of the CyDs, hence, the guest molecule could be accommodated not in the cavity of CyD but externally, between two or more CyD molecules.

To clarify the existence of the complexes, other techniques can be used such as CD and ^{1}H-NMR spectroscopy.

CD spectra of PAP in the presence or absence of different CyDs, at pH 7.4, are reported in Fig. 3.

A positive CD band was observed at 238 nm for PAP alone. In the presence of β-CyD and particularly DM-β-CyD, this band increases its intensity as a result of perturbation of the electronic transition of the drug caused by the asymmetric cavity of CyDs following complexation (Hirayama and Uekama, 1987). Due to the intensity of CD, bands indicate the strength of host-guest interaction, the relatively small increase of

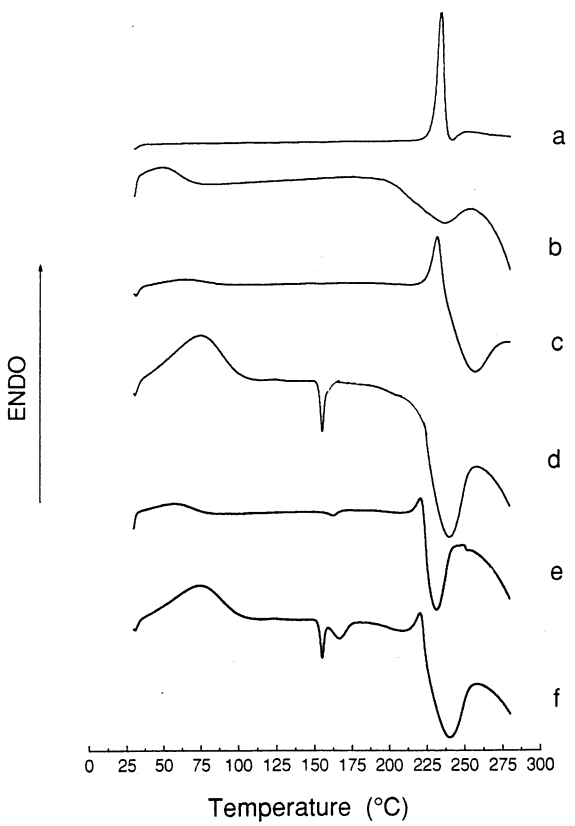


Fig. 1. DSC thermograms of PAP-DM-β-CyD system. (a) PAP; (b) DM-β-CyD; (c) PAP-DM-β-CyD 1:1 physical mixture; (d) PAP-DM-β-CyD 1:1 complex; (e) PAP-DM-β-CyD 1:2 physical mixture; (f) PAP-DM-β-CyD 1:2 complex.

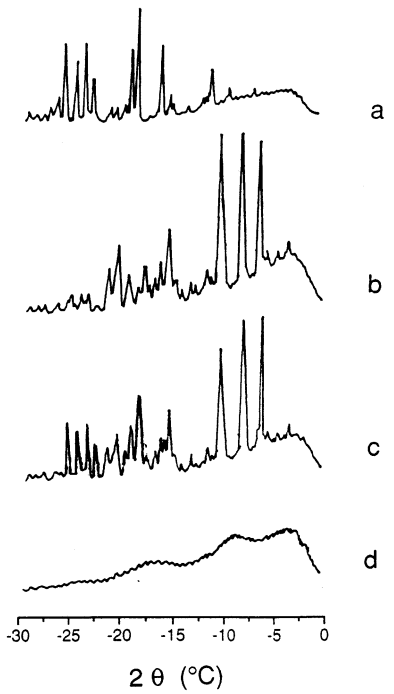


Fig. 2. X-ray diffraction pattern of PAP-DM-β-CyD system. (a) PAP; (b) DM-β-CyD; (c) PAP-DM-β-CyD 1:2 physical mixture; (d) PAP-DM-β-CyD 1:2 complex.

intensity observed in our case shows that only a weak interaction exists between PAP and CyDs, particularly with β-CyD. Apparently, no effect was produced by HP-β-CyD.

At pH 1.1 there is very little interaction between CyDs and the drug, in fact CD band of PAP was very weakly influenced. In this condition PAP is in its protonated form, then, highly solvated from water molecules, showing lower affinity for apolar CyD cavity (spectra not reported).

The greater attraction of CyDs for PAP in a more lipophylic form is clearly demonstrated by the coefficient partition values (log P) evaluated at pH 1.1 and 7.4 for free PAP or in the presence of different CyDs (Table 1). Log P values observed at pH 1.1 in the presence of CyDs are in all cases negative and comparable to log P of free PAP. At pH 7.4 a positive log P value was observed for free PAP that decreases in the presence of the CyDs. At this pH value the drug is present as free

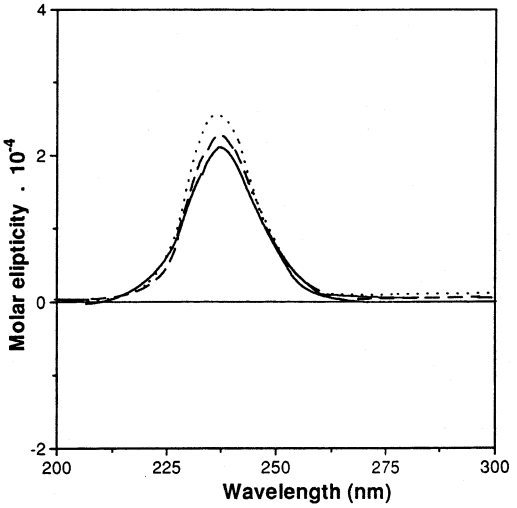


Fig. 3. CD spectra of free PAP and in the presence of different CyDs at pH 7.4.; —, PAP; – – –, PAP + β-CyD; ..., PAP + DM-β-CyD.

base and CyDs present in the aqueous phase are able to complex or to interact with the drug, reducing its partition in the organic phase.

^{1}H-NMR spectroscopy assures the existence of the complexes in solution and also gives information about the inclusion mode of PAP.

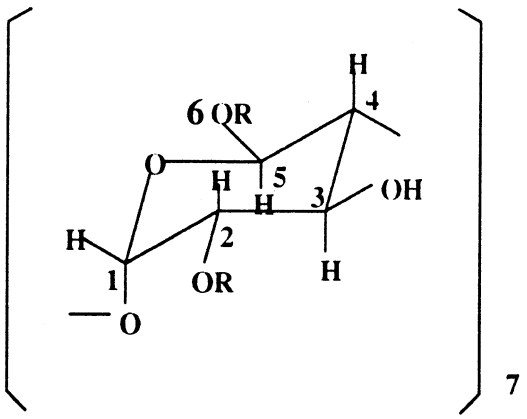
The shifts of β-CyD and DM-β-CyD protons in the presence of the drug are showed in Table 2.

The upfield shifts observed for H-3 and H-5 protons of DM-β-CyD, localized at the internal surface of the Cyd cavity, is evidence of the presence of host-guest interaction. These shifts, in fact, are probably due to anisotropic ring-current produced by aromatic rings of PAP (Djedaïni et al., 1990). The consistent shifts observed indicate that PAP deeply penetrates into DM-β-CyD cavity. Moreover, because of the higher shielding

Table 1
Log P values of PAP alone or in the presence of different CyDs at pH 1.1 and 7.4

Compounds	pH 1.1	pH 7.4
PAP	−0.485	1.996
PAP-β-CyD	−0.490	1.032
PAP-HP-β-CyD	−0.480	1.320
PAP-DM-β-CyD	−0.523	0.688

Table 2
^{1}H-chemical shifts of free CyDs and in the presence of PAP



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R = H β-CyDR = CH_3 DM-β-CyD

Protons	β-CyD	PAP-β-CyD complex	$\Delta\delta$ [a]	DM-β-CyD	PAP-DM-β-CyD complex	$\Delta\delta$ [a]
H-1	5.040	5.035	−0.005	5.222	5.204	−0.018
H-2	3.555	3.551	−0.004	—	—	—
H-3	3.939	3.929	−0.010	3.954	3.909	−0.045
H-4	3.617	3.614	−0.003	—	—	—
H-5	3.823	3.813	−0.010	3.861	3.836	−0.025
H-6	3.851	3.840	−0.011	3.713	3.699	−0.014
H-2′	—	—	—	3.534	3.524	−0.010
H-6′	—	—	—	3.373	3.362	−0.011

[a] $\Delta\delta$, $\delta_{complex}$-δ_{free}

effect on H-3 proton with respect to H-5 it can be hypothesized that PAP penetrate into the cavity from the secondary rim of the macrocycle.

In the case of β-CyD the presence of PAP produces only a small shift of the H-3, H-5 and H-6 protons, however because other protons, localized on the external surface of the macrocycles, are less influenced, we can conclude that an interaction, although weak, occurs between PAP and the β-CyD cavity. The shift of H-6 proton suggests a probable penetration of the drug from the primary rim of this CyD.

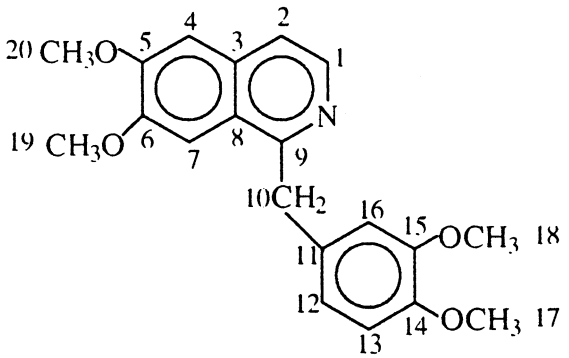
Unfortunately, it is not possible to have definite information about the inclusion of PAP into HP-β-CyD cavity, because the signals of the internal protons of this macrocycle are overlapped to those of hydroxypropyl group. We can only observe the shifts of the drug protons which give information about the existence of an interaction in solution but not about the inclusion.

The shifts observed for the protons of the drug are shown in Table 3.

In the presence of both β-CyDs and DM-β-CyDs, all PAP protons were shifted downfield except H-12 and H-13 that were shifted upfield. The proton shifts are higher for DM-β-CyD with respect to β-CyD, confirming a weak interaction of the drug with the latter.

According to Ganza-Gonzalez et al. (1994) the upfield shifts of the proton drug are due to the association with the oxygen atoms of the CyD, rich in π electrons, while the downfield shifts are probably due to a variation of local polarity (Djedaïni et al., 1990) or at a deshielding effect

Table 3
^{1}H-chemical shifts of PAP, both free and in the presence of different CyDs



Protons	PAP	PAP-β-CyD complex	$\Delta\delta$ [a]	PAP-HP-β-CyD complex	$\Delta\delta$ [a]	PAP-DM-β-CyD complex	$\Delta\delta$ [a]
H-1	8.114	8.126	0.012	8.123	0.009	8.144	0.030
H-2	7.928	7.943	0.015	7.928	0.000	7.969	0.041
H-4	7.416	7.432	0.016	7.415	−0.001	7.456	0.040
H-7	7.513	7.523	0.010	7.509	−0.004	7.542	0.029
H-10	4.663	4.684	0.021	4.664	0.001	4.713	0.050
H-12	6.808	6.795	−0.013	6.787	−0.021	6.791	−0.017
H-13	6.924	6.921	−0.003	6.915	−0.009	6.911	−0.013
H-16	6.968	6.989	0.021	6.984	0.016	7.025	0.057
CH_3-17	3.741	3.752	0.011	3.751	0.010	3.744	0.003
CH_3-18	3.744	3.752	0.008	3.742	−0.002	3.773	0.029
CH_3-19	3.905	3.909	0.004	3.903	−0.002	3.915	0.010
CH_3-20	3.993	4.001	0.008	3.993	0.000	4.011	0.018

[a] $\Delta\delta$, $\delta_{complex}$-δ_{free}

due to Van der Waals forces between the drug and carbohydrate chains (Zhang et al., 1990). Similar magnitude shifts are observed for all protons of PAP and this suggests that both isoquinoline group and benzene ring could interact with the internal surface of CyDs. However, because all external protons of CyDs were also influenced in the presence of PAP, we cannot exclude that the drug interacts both with the internal and external surface of the macrocycles.

In the presence of HP-β-CyD only a few protons are weakly shifted upfield (H-12 and H-13) or downfield (H-1, H-16 and H-17), showing that an interaction with this CyD in solution exists.

Two-dimensional NMR studies and molecular graphic are in progress to gain more information about PAP- HP-β-CyD interaction and to confirm our hypothesis about PAP-β-CyD and PAP-DM-β-CyD inclusion complexes.

3.1. Solubility studies

The phase solubility diagrams obtained at pH 7.4 and 37°C are shown in Fig. 4. A difference in the solubility curves are observed. A linear increase of the PAP solubility was observed as a function of DM-β-CyD concentration. This curve can be classified as A_L type and because the slope value is less than unity (data not reported) it is possible to hypothesize a complex with 1:1 stoichiometry (Higuchi and Connors, 1965).

The PAP-β-CyD system shows a Bs type solubility curve. The initial rising portion was followed by a plateau, indicating the limited solubility of the complex that precipitated at β-CyD concentration higher than $14 \cdot 10^{-3}$ mol.

Because the increase of water solubility of a drug by adding a legant is not necessarily related to the formation of a host–guest complexes, solubility studies were also performed in the presence of HP-β-CyD. A soluble system was formed and the isotherm presents a positive curvature (Ap type curve), indicating the existence of an interaction between PAP and HP-β-CyD with an order higher than 1:1. It is probable that more than one CyD molecule interacts with the PAP during solubilization process. In the first part of the plot the curve can be considered of A_L type and in this range it is possible to hypothesize a 1:1 interaction.

The stability constant of all systems, assuming a 1:1 stoichiometry, were as follows: 78, 93 and 154 M^{-1} respectively for PAP-HP-β-CyD, PAP-β-CyD and PAP-DM-β-CyD. In all cases the obtained values are very little, indicating a weak interaction between the two components and confirming the results obtained by CD and NMR spectroscopy.

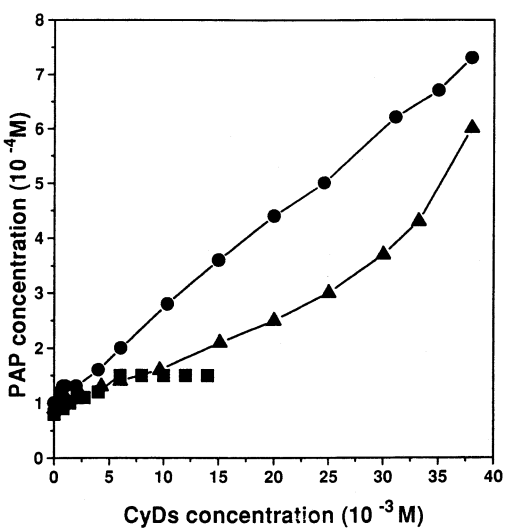


Fig. 4. Phase solubility diagrams of PAP in the presence of different CyDs at pH 7.4 and 37 ± 0.5°C. ■—■, PAP + β-CyD; ▲—▲, PAP + HP-β-CyD; ●—●, PAP + DM-β-CyD.

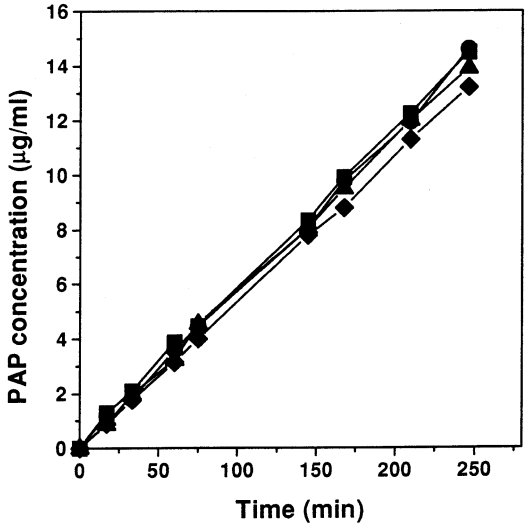


Fig. 5. Diffusion profiles of PAP in the absence and in the presence of various CyDs trough a dialysis bag. ●—●, free PAP; ■—■, PAP + β-CyD; ▲—▲, PAP + HP-β-CyD; ◆—◆, PAP + DM-β-CyD.

3.2. Diffusion studies

The influence of the interaction with CyDs on PAP diffusion through membranes, was evaluated by a dialysis experiment. Because of the artificial nature of the membrane used, the enhancer action exerted from CyDs on biological membranes can be excluded, thus limiting at the complexation and the eventual modification of the PAP flux.

The obtained results are shown in Fig. 5. Similar diffusion profiles are observed for PAP in the presence and absence of CyDs, probably as a result of a low increase of water solubility of the drug following complexation ($13.5 \cdot 10^{-4}$, $23.9 \cdot 10^{-4}$, $30.8 \cdot 10^{-4}$ and $48.8 \cdot 10^{-4}$ g/100 ml respectively for free PAP, PAP-β-CyD, PAP-HP-β-CyD and PAP-DM-β-CyD systems). On the other hand, although DM-β-CyD has more solubilizing effect on PAP, it shows lower diffusion profile, probably because the relatively higher K_c value of the complex reduces the amount of free PAP that can diffuse.

4. Conclusions

The results obtained by NMR studies proves the complexation of PAP into β-CyD and DM-β-CyD cavity. However, a weak interaction was observed in both cases. The drug is oriented in a different way into the two Cyd cavity and more deeply in DM-β-CyD compared to β-CyD. No sure evidence of the inclusion of the drug into HP-β-CyD cavity exist, we can only conclude that an interaction exists between the two components. All CyDs produce a small increase of water solubility of the drug, probably due to spatial disposition and dimension of the drug, the interaction strength is weak, in fact small K_c values were obtained from solubility phase diagrams. The diffusion of the drug through a dialysis bag is influenced only marginally by the presence of different CyDs, probably due to their poor complexant ability for the drug.

Acknowledgements

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