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Interactions between cyclodextrins and pilocarpine – as an example of a hydrophilic drug

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Abstract

Investigations of interactions between cyclodextrins (CyDs) and pilocarpine (P) were carried out by means of several common methods. Subjects of interest were aqueous solutions and solid dispersions of the drug and various cyclodextrins. The solid dispersions were prepared by spray-drying and by lyophilization of the aqueous solutions. These products and the equivalent physical mixtures were compared by scanning electron microscopy (SEM), differential thermal analysis (DTA), powder X-ray diffractometry (XRD) and Fourier transformation infrared (FTIR) spectroscopy. Methods for investigations of the aqueous solutions were ^{1}H-NMR, ^{13}C-NMR, correlation, rotating frame Overhauser effect and nuclear Overhauser effect spectroscopy, reversed phase high performance liquid chromatography (HPLC), UV-spectroscopy and conductometry. The results obtained may be interpreted as an inclusion of pilocarpine in cyclodextrins. In vitro and in vivo studies yielded results of a modified release and a slightly higher bioavailability of pilocarpine in the presence of the tested cyclodextrins. Therefore we assume that the use of cyclodextrins in ophthalmic preparations could be advantageous not only for lipophilic drugs but also for hydrophilic drugs.

Keywords: Pilocarpine; Cyclodextrin; Solid dispersion; Inclusion complex; Physicochemical properties; In vitro release; Physiological compatibility; Miotic bioavailability

1. Introduction

Cyclodextrins (CyDs) are usually capable of forming inclusion complexes with lipophilic drugs by taking up a whole molecule or some part of it into the cavity. Therefore they are used for increasing the solubility, stability and bioavailability of mainly lipophilic drugs. Interactions between CyDs and hydrophilic drugs have scarcely been investigated up to now (Uekama et al., 1989; Puglisi et al., 1990). Such an interaction could

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result in a retarding effect of the associated drug. In this study our aim was to investigate whether hydrophilic drugs such as pilocarpine (P) form associates or even complexes with CyDs as well and if it could be advantageous to use them in ophthalmic preparations of P.

P has long been used in the therapy of glaucoma, especially in aqueous solutions, but because of the high drainage rate only a small amount of the applied quantity of the drug produces the therapeutic response. The usual aqueous solutions have to be taken four times a day because their activity lasts no longer than 5 or 6 h. This fact does not promote the compliance of the patients. Therefore, many efforts have been made to increase the efficacy of the therapy.

In addition to a lot of alternative delivery systems aqueous solutions with viscosity enhancers like cellulose derivatives, povidone, polyvinyl alcohol or polyacrylic acid were produced (Keipert, 1994). More viscous eye drops may offer a chance of improving the bioavailability of the drug and at the same time of reducing the systemic side effects. Another possibility of influencing the bioavailability of P could be the use of CyDs. Though they do not usually show any irritation of the mucous membrane their ocular use has found little attention so far (Uekama et al., 1990).

The purpose of this work was the preparation of complexes of P and selected CyDs in solution as well as in solid state, and the physicochemical characterization of these products. Furthermore, determination of the in vitro release was necessary for a possible use of these formulations and the miosis test on rabbit eyes should give first information about the bioavailability of P.

Table 1
Contents and physicochemical parameters of the aqueous eye drop solutions

	Solution 1	Solution 2	Solution 3
P-HCl (%)	2.0	2.0	—
α-CyD (%)	—	8.0	8.0
NaCl (%)	0.4	0.2	0.7
n_{25}	1.334	1.342	1.346
η (mPa s)	0.947	1.118	1.159
pH	4.3	4.2	6.7
Osmolality (mosmol/kg)	293	313	323

2. Materials and methods

2.1. Chemicals

P was obtained from Ankerpharm Rudolstadt (Germany) and P-hydrochloride (P-HCl) from Merck (Germany). α-CyD from SERVA Feinbiochemica (Germany) and β-CyD from Chinoin (Hungary) were used for the aqueous solutions and α- and β-CyD from Merck (Germany) for the solid dispersions. γ-CyD, hydroxypropyl-β-cyclodextrin (HP-β-CyD) with a degree of substitution of 1.0 and hydroxyethyl-β-CyD (HE-β-CyD) with a degree of substitution of 0.9 were supplied by Wacker Chemie GmbH (Germany). All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Preparation of the solid dispersions

2.2.1.1. Spray-drying. P-HCl and the appropriate CyD were dissolved in double-distilled water in molar ratios of 1:1, 2:1 and 3:1, and spray dried in a laboratory spray dryer (Büchi 190 mini spray dryer, Büchi AG, Switzerland). The inlet temperature was between 165 and 175°C and the outlet temperature was between 85 and 100°C. The products were stored under a light screen and in a dry place.

2.2.1.2. Lyophilization. The same solutions as for the spray-drying were used, kept at a temperature of −38 to −42°C for 24 h and then lyophilized at −42°C for another 24 h (Christ-Loc-1m Alpha 1–4, Christ, Germany). Storage conditions see above. Reversed phase high performance liquid chromatography (HPLC) was employed for both preparation methods for the determination of P and of possible degradation products of it according to the method described elsewhere (Wiesend, 1988).

Table 2
Changes of the chemical shifts of the H-atoms of the tested CyDs caused by P or P-HCl

CyD-Proton	$\Delta\delta = \delta$CyD $-\delta$CyD + P-HCl (ppm)			$\Delta\delta = \delta$CyD $-\delta$CyD + P (ppm)	
	α-CyD	β-CyD	γ-CyD	α-CyD	β-CyD
H1	0.06	0.04	0.01	0.0	−0.03
H2	0.03	0.0	0.01	0.0	−0.03
H3	0.09	0.04	0.01	0.04	0.08
H4	—	0.0	0.0	—	−0.01
H5	0.07	0.03	0.02	0.03	0.03

2.2.2. ^1H- and ^{13}C-nuclear magnetic resonance spectroscopy (^1H-, ^{13}C-NMR spectroscopy)

^{1}H-NMR measurements were carried out with solutions containing P or P-HCl and α-, β- or γ-CyD dissolved in a molar ratio of 1:1 in 1 ml D_2O and with solutions of P-HCl and α-CyD in a molar ratio of 1:1 and addition of 7 mg or 14 mg NaCl dissolved in 1 ml D_2O (Bruker 300 MHz-spectrometer, Bruker, Germany). Solutions of P-HCl and α-CyD in a molar ratio of 1:1 and 7 mg NaCl in 1 ml D_2O were used for ^{13}C-NMR measurements (75.4 MHz-spectrometer, Bruker, Germany). Sodium-3-trimethylsilylproprionate was used as internal reference.

2.2.3. Reversed phase high performance liquid chromatography (RP-HPLC)

Chromatographic experiments were performed using an HPLC unit equipped with an L-6000 pump, L-4250 UV-VIS detector (216 nm) and a D-2500 Chromato-Integrator (Merck and Hitachi, Japan). Stainless steel columns (125 × 4 mm) packed with 5-μm LiChrosorb RP-18 end-capped (Merck, Germany) were employed. The mobile phase consisted of an 0.7% aqueous solution of NaCl containing various concentrations of α-, β-, γ-CyD or glucose. The pH of 5.0 was adjusted with 0.1 molar hydrochloric acid. These conditions turned out to be optimal for a suitable retention time and, furthermore, they are usual for the formulation of pilocarpine eye drops. For the sample solutions P, P-HCl, isopilocarpine (Ip), isopilocarpic acid (Ipa) and pilocarpic acid (Pa) were dissolved to a concentration of 0.50 mg/ml in distilled water. All these solutions were filtered through an 0.22 μm membrane filter. The injection volume was 10 μl. The flow rate was 2 ml/min for P and Ip and 0.5 ml/min for Pa and Ipa.

Equation for the calculation of the stability constant, modified from Uekama et al. (1978):

Table 3
Shifts of the protons (ppm) of P-HCl in D_2O

Proton	With α-CyD	With β-CyD	With γ-CyD	With 0.7% NaCl and α-CyD	With 1.4% NaCl and α-CyD
H1	0.30	0.13	0.07	0.29	0.28
H2	0.31	0.12	0.05	0.26	0.28
H3	0.32	0.14	0.06	0.28	0.27
H4	0.33	0.13	0.06	0.27	0.26
H5	0.28	0.11	0.05	0.27	0.25
H6	0.31	0.11	0.05	0.25	0.26
H7–H9	0.30	0.12	0.05	0.26	0.25
H10/H11	0.34	0.15	0.06	0.29	0.28
H12	0.31	0.17	0.08	0.35	0.37
H13	0.31	0.21	0.11	0.44	0.48
H14–H16	0.31	—	—	0.30	0.31

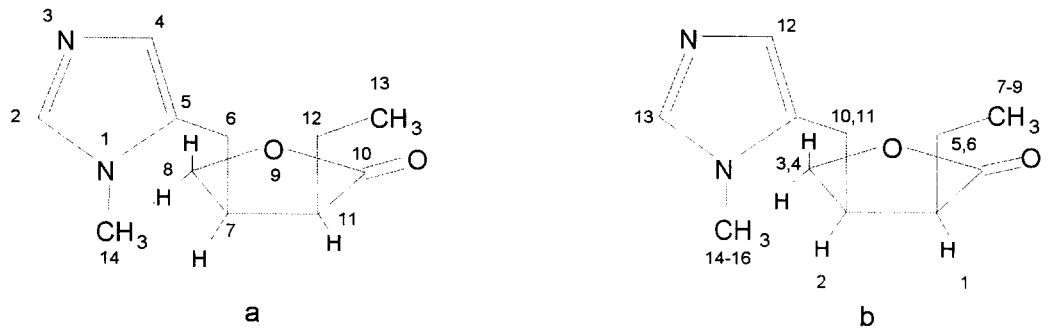

Fig. 1. Numbering of the C- (a) and H-atoms (b) of pilocarpine for the NMR interpretation.

$$\frac{[CyD]}{T_0 - T_{obs}} = \frac{1}{T_0 - T_c}[CyD] + \frac{1}{K(T_0 - T_c)}$$

with the concentration of CyD, T_0, the retention time without CyD, and T_{obs}, the retention time with a defined amount of CyD. The retention time of the complex is termed T_c.

2.2.4. UV spectroscopy

Samples containing 20 mg/ml P-HCl and various concentrations of α-, β- or HP-β-CyD in distilled water were investigated with a Uvikon 940 (β- and HP-β-CyD) (Kontron Instruments, Switzerland) and a Specord M 40 (α-CyD) (Carl Zeiss, Germany). The spectra were recorded with a reference solution of the same CyD concentration as in the sample.

2.2.5. Conductometry

The conductance measurement of solutions consisting of 0.002% P-HCl and increasing amounts of α-, β-, γ- or HP-β-CyD in distilled water was carried out with a KM2 (Kombinat Medizin- und Labortechnik, Germany). The measuring cell with the fixed Pt-electrodes was set at 25°C. The measuring cell constant was 0.19839 cm^{-1}.

2.2.6. Scanning electron microscopy (SEM)

The spray-dried (SD) products of P-HCl, α-CyD and the SD dispersion of P-HCl and α-CyD, in a molar ratio of 1:1, were fixed with lite tabs (∅ 12 mm) (Plano, W. Plannet GmbH, Germany) to aluminium discs. To improve the conductivity the samples were sputtered with Au in an atmosphere of ionized argon (Surface Preparator PEC-A2, Shimadzu, Japan). The prepared samples were then placed into the microscope (Stereoscan S 360, Leica, Germany).

2.2.7. Fourier transformation infrared (FTIR) spectroscopy

The samples of the solid dispersions and the physical mixtures of P-HCl and α-, β-, γ-, HE-β- and HP-β-CyD in molar ratios of 1:1, 2:1 and 3:1 were mixed with KBr and pressed to a small tablet which was mounted in the infrared beam (Genesis FTIR, Mattson-Unicam, Germany).

2.2.8. Differential thermal analysis (DTA)

The pure substances, the solid dispersions and the physical mixtures in a molar ratio of 1:1 and

Table 4
Chemical shifts (ppm) of the C-atoms of α-CyD and P-HCl in an 0.7% NaCl solution in D_2O

C-atoms	α-CyD	C-atoms	P-HCl
C1	1.41	C2	1.62
C2/C5	1.42	C4	1.14
C3	1.40	C5	2.26
C4	1.49	C6	1.97
C6	1.53	C7	1.90
		C8	1.99
		C10	2.13
		C11	1.90
		C12	1.91
		C13	1.97
		C14	2.20

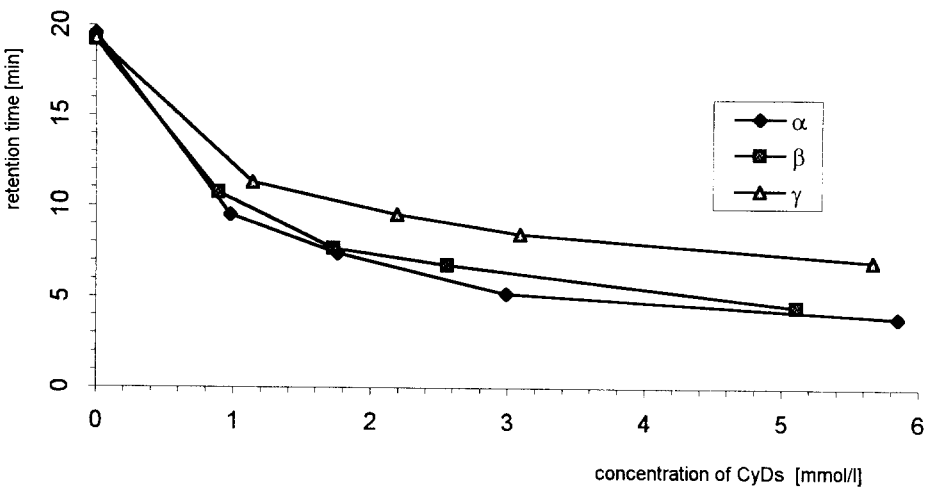

Fig. 2. Retention times of pilocarpine influenced by cyclodextrins (standard deviation ± 0.07–0.1 (min)).

3:1 of P-HCl, α-, β- and γ-CyD were blended with Al_2O_3 in a ratio of 1:1 and then filled into the probe system (mini Pt-cup and Pt-cup). Al_2O_3 was used to avoid a foaming of the samples, especially of the spray-dried. The measurement was performed under argon atmosphere at temperatures of 25–300°C (STA 429, Netzsch, Germany).

2.2.9. X-ray diffractometry

The X-ray diffraction (XRD) pattern were measured using Ni filtered Cu-K_α radiation at a scanning rate of 0.05° 2Θ/5 s. The patterns for each sample were obtained at a 2Θ range of 4–64°. The pure substances, the dried powders and the physical mixtures of P-HCl and α-, β- or γ-CyD were examined densely packed in a flat sample holder (Horizontalzählrohrgoniometer HZG 4C, Freiberger Präzisionsmechanik, Germany).

2.2.10. Eye drop solutions

2.2.10.1. Physiological compatibility. The hen's egg-chorioallantoic membrane (HET-CAM) test (Luepke, 1985) was used to estimate the irritation potential of the native and hydroxyalkylated CyDs (Confarma, Germany). An aqueous solution of 1.7% of β-CyD was chosen as example for the native CyDs and a 5% aqueous solution of HP-β-CyD represented the hydroxyalkylated β-CyDs.

The DRAIZE test as described elsewhere (Pergande et al., 1990) was carried out with the solutions tested on the rabbit's eye in the in vivo study.

Pilot compatibility tests on the human eye with an 8% aqueous solution of α-CD and a 12.5% aqueous solution of HP-β-CD were also carried out.

2.2.10.2. In vitro release. For the investigation of the in vitro release rates a membrane permeation model and method was used as described elsewhere (Keipert and Schulz, 1994). A multi-layer membrane consisting of two Nephrophan® membranes (Filmfabrik Wolfen, Germany) with a membrane filter (regenerated cellulose, 0.22 μm, Sartorius, Germany) between them, soaked with 2-octyldodecanol, simulated a hydrophilic–lipophilic–hydrophilic barrier. The amount of re-

Table 5
Calculated stability constants K (mol^{-1})

	α-CyD	β-CyD	γ-CyD
P	1300	1100	1000
Ip	1500	1200	1000
Pa	1300	2000	1300
Ipa	1400	2500	1200

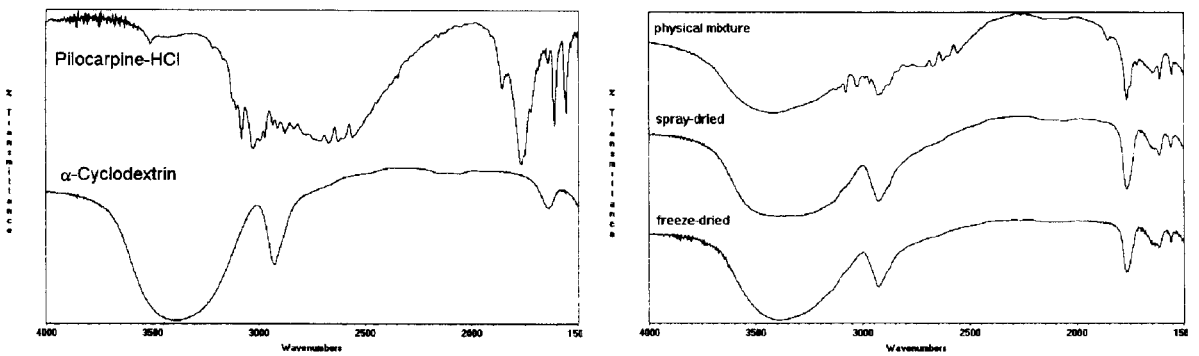

Fig. 3. FT-IR spectra of P-HCl, α-CyD 1:1 mol/mol physical mixture and the solid dispersions.

leased P was determined spectrofotometrically at 216 nm (UV-2101 PC, Shimadzu, Japan) and the quantity of α-CyD which passed the membrane was measured by HPLC (see Section 2.2.3.) with a differential refractometer as detector (RI 71, Merck/Hitachi, Japan). The mobile phase consisted of 67% acetonitrile and 33% double-distilled water. A Bischoff nucleosil-NH_2 5 μm column (4 mm × 250 mm) was employed (Bishoff, Germany).

2.2.10.3. In vivo tests. The contents and the physicochemical parameters of three aqueous solutions determined after filtration through an 0.22 μm membrane filter are listed in Table 1. Osmolalities were determined with a half-micro osmometer type Dig. L (Knauer, Germany), the viscosities with a viscosity measuring unit AVS 350, thermostat CT 1450 (Schott, Germany), the refraction indices with an Abbé-refractometer, Zeiss (Germany) and the pH values with a pH-meter 522 (WTW, Germany).

The in vivo study of the miosis was carried out with four female and three male New Zealand white albino rabbits with a weight of 2–2.5 kg. The rabbits were housed singly in cages under standard laboratory conditions: 12 h dark/12 h light cycle. The rabbits had free access to food and water. The experiments conformed to the German law for the prevention of cruelty to animals. Approximately 50 μl of solutions 1, 2 and 3 (Table 1) were instilled into the conjunctival sac of the right eye. The left eye was used as control. The pupillary diameter was measured with a stencil at 10, 20, 30, 40, 50 and 60 min and then every 30 min until the starting diameter was again reached. The reading precision was 0.5 mm. Pharmacokinetic parameters were calculated with the PC software TOPBAS 2.0a.

3. Results and discussion

3.1. Aqueous solutions

3.1.1. ^{1}H-NMR- and ^{13}C-NMR-spectroscopy

The NMR-techniques are commonly used to make inclusion complexes of CyDs evident. A great advantage is that all interacting species are observed simultaneously and that NMR deals with nuclei. Chemical shifts of the H3- and H5-atoms of the CyDs are considered to verify a real inclusion complex whereas chemical shifts of the other protons, which are located on the outer-sphere of the CyDs just indicate a simple association complex.

Changes of the shifts of the ppm data for the H3- and H5-atoms of α- and β-CyD under the influence of P or P-HCl, especially for α-CyD and P-HCl, have been determined (Table 2). The ppm data of γ-CyD were influenced less by P and P-HCl. The ppm shifts are low, but associate formation can be assumed.

Chemical shifts of the H-atoms of the guest molecule are generally indicative of complex formation as well. Therefore the ppm-shifts of the

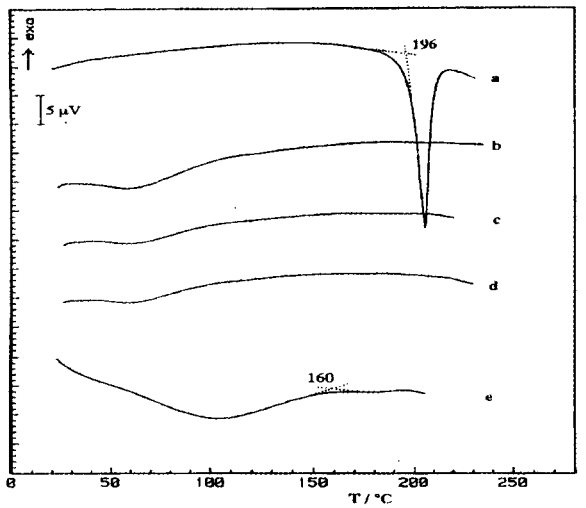

Fig. 4. DTA thermograms of P-HCl (a), α-CyD SD, P-HCl/α-CyD SD (c), P-HCl/α-CyD FD (d) and P-HCl/α-CyD PM (e) in molar ratio of 1:1.

P-protons have been determined with α-, β- and γ-CyD (Böhm, 1991) and, to obtain additional information about the binding behaviour, also in solutions containing 0.7 or 1.4% NaCl, comparable with ophthalmic preparations, and α-CyD (Table 3, Fig. 1) (Hanke, 1993). All H-atoms are affected by the CyDs, a clear graduation α- > β- > γ-CyD can be seen. A favoured orientation of P in the cavity of the CyDs can hardly be determined. Slight differences in the ppm values for H10/11 with α-CyD and for H12 and H13 with β-CyD are indicative of the preferred interaction of the imidazole ring of P. The addition of NaCl to the solution of P affects the H-atoms mentioned above such that their ppm values are shifted to the left.

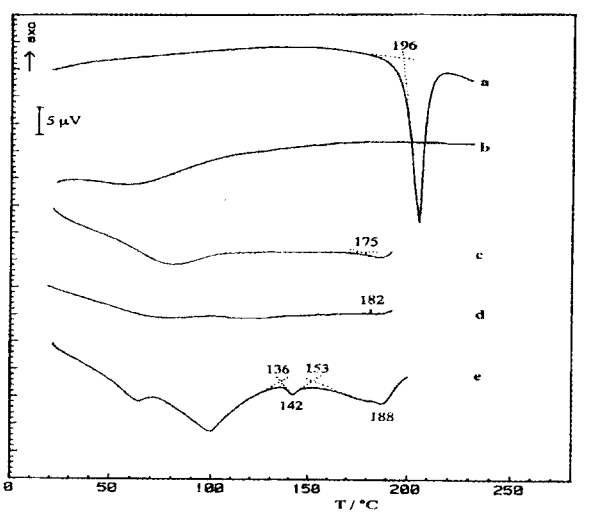

Fig. 5. DTA thermograms as in Fig. 4, but in molar ratio of 3:1.

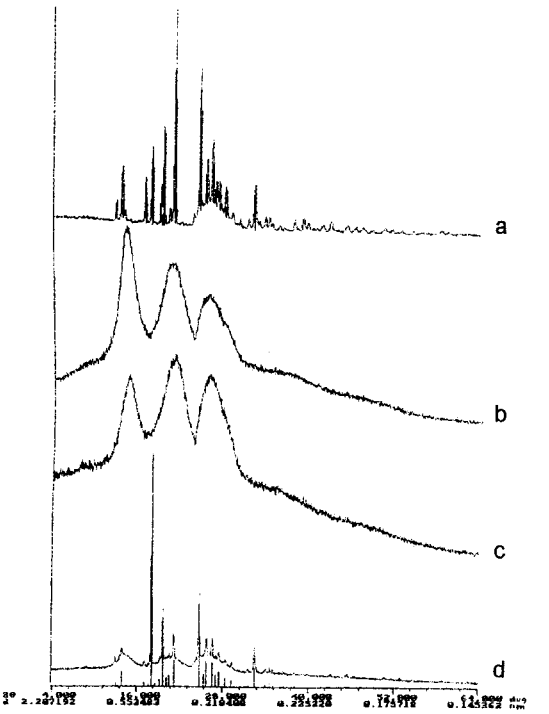

Fig. 6. Powder X-ray diffractograms of P-HCl SD (a), P-HCl/α-CyD SD (b), P-HCl/α-CyD FD (c) and P-HCl/α-CyD PM (d) in molar ratio of 1:1.

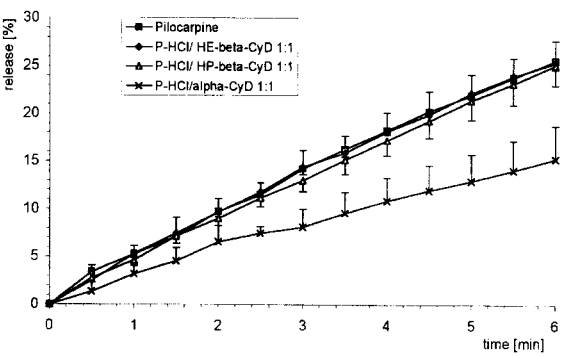

Fig. 7. Release of P from a 2.0% aqueous solution alone and with CyDs.

Table 6
Pharmacokinetic parameters of the miotic response

	Max. response (mm)	Time of max. response (min)	AUC (mm min)	Relative bioavailability
P-HCl	1.65	24.12	165.62	1.00
P-HCl/α-CyD (1:1)	1.81	41.52	218.41	1.32

Calculated with TOPFIT version TOPBAS 2.0-a.

^{13}C-NMR-spectra should also give indications of to which partial structure of the guest molecule reciprocates with the CyD. The ppm shifts of the C-atoms of P-HCl are diversely affected (Table 4) so that no part of the P molecule can be named as preferred. Further investigations using COSY, NOESY and ROESY admitted the possibility of associate formation, too (Hanke, 1993).

3.1.2. RP-HPLC

CyDs are nowadays used in HPLC to separate optical, geometrical and structural isomers as well as many non-isomeric compounds. Columns have therefore been developed which contain a stationary phase of bonded CyD. It is also possible to use a mobile phase containing CyD to modify the factor of distribution of a substance (Hilton and Armstrong, 1991).

The retention time of P is diminished in solutions containing CyD in comparison with the aqueous solution (Fig. 2). All the tested CyDs caused a significant retention time-shortening of pilocarpine. The retention times of Ip, Pa and Ipa are shortened as well. The complex stabilities (Table 5) which were calculated with the resulting retention times, permit the deduction of perceptible complex binding not only for P but also for Ip, Ipa and Pa. The stability constants of P or Ip are in the same range of α-CyD > β-CyD > γ-CyD as was found through NMR, whereas Ipa and Pa show an apparent preference of β-CyD which indicates another possibility of association. Tests with glucose in the mobile phase showed no influence on the retention time of P. Other factors which could affect the retention time have been denied by appropriate analysis (Hanke, 1993).

3.1.3. UV spectroscopy

An inclusion of a guest molecule in the cavity of the CyDs causes a decrease in its absorption and often isosbestic points are detected. P shows a maximum absorbance of the ultraviolet light at 214.6 nm. By means of UV spectroscopy a reduction in the extension of absorption of P in the order α- > β- > HP-β-CyD was found (Böhm, 1991).

3.1.4. Conductivity

The inclusion of a substance which is used as solution of its salt should yield conductivities which are decreased in comparison with the uncomplexed form. This assumption has been confirmed by measurements with P-HCl and various CyDs. A range of γ- < α- < β- = HP-β-CyD was found (Böhm, 1991).

3.2. Solid dispersions

3.2.1. SEM

The spray-dried products of α-CyD and of α-CyD/P-HCl in a molar ratio of 1:1 show the typical hollow spheres, whereas P-HCl has a crystalline shape after spray-drying. Consequently, it can be assumed that CyDs are disturbing the crystallization of P-HCl and that an inclusion of it could be possible.

3.2.2. FTIR-spectroscopy

Infrared spectroscopy is a method which does not always yield interpretable results, but in the case under review its use makes sense. The absorption bands of P-HCl in the area between the wave-numbers 3100 and 2550 cm^{-1} disappear in the spray-(SD) and freeze-dried (FD) preparations with α-CyD. Only the absorption band of the lactone ring at 1740 cm^{-1} verifies the presence of

P-HCl, whereas the physical mixtures produce spectra which are simple overlayings of the single spectra (Fig. 3). These results tend to prove the inclusion of the imidazole-part of the molecule. In molar ratios of 2:1 and 3:1 the absorption bands of P appear faintly. β-, γ-HE-β- and HP-β-CyD show the same influence on P but a range cannot be established.

3.2.3. DTA

The solid dispersions of P-HCl and α-CyD or β-CyD were analysed by means of differential analysis to detect possible altered thermic properties with regard to the pure substances. P-HCl shows an endothermic peak with an onset temperature (T_{on}) of 196°C. In the thermogram of the spray-dried P-HCl, T_{on} is shifted slightly to a lower temperature of 184°C. Due to the smaller particle size and a lower sample volume the peak is broader and shorter.

α-CyD and β-CyD do not show any endothermic peak below 200°C. There is just the loss of water detectable between 50 and 100°C. By comparing the spectra of P-HCl and α-CyD as pure substances, solid dispersions and as a physical mixture (PM) in a molar ratio of 1:1 it is evident that the melting peak of P-HCl has disappeared in the spray-dried and freeze-dried preparations (Fig. 4). The thermogram of the PM indicates a small but clearly detectable endothermic effect with $T_{on} = 160$°C. Comparable results have been detected for β- and γ-CyD, too.

For demonstrating the strong influence of the CyDs on the crystallization of the P-HCl during the drying procedures, Fig. 5 compares the different solid dispersions of P-HCl and α-CyD in a molar ratio of 3:1. The amount of P-HCl in these products is 43%. Endothermic peaks are recognizable, but they are very small and shifted to lower temperatures. The findings of the DTA appear to prove the inclusion of P through α-CyD and β-CyD.

3.2.4. Powder X-ray diffractometry

After drying by the methods mentioned the cyclodextrins are more or less amorphous. P-HCl, as already shown by SEM, remains crystalline (Fig. 6). The diffraction patterns of the CyDs and their solid dispersions with P-HCl are similar. Under consideration of the results with the other methods this can be evaluated as an additional sign of complex formation. By comparison, the 1:1 mol/mol PM does not show any changes in the diffraction angles of P-HCl.

3.3. Eye drop solutions

3.3.1. Physiological compatibility

The surface activities of the native CyDs are very similar. β-CyD possesses the highest haemolytic effect of these. An 0.1% (w/v) solution of HP-β-CyD shows a lower surface tension than an 0.1% (w/v) solution of HE-β-CyD and has a greater haemolytic effect (Yoshida et al., 1988). That is why only these two substances were examined by the HET-CAM test. As expected, no indication of any irritation of the mucous membrane was found. An application on the cornea is therefore likely. Additionally, α-CyD (solution 3, Table 1) was examined prior to the miosis tests by a modified DRAIZE-test. This substance was classified as non-irritating.

3.3.2. In vitro release

Fig. 7 shows a release rate of P from a 2.0% aqueous solution of 25.6% after 6 h under the test conditions. Under addition of an equimolar amount of α-CyD the released quantity of P was found to be 15.3%. This result shows that α-CyD is able to modify the permeation of P. Described simply, the cornea consists of three consecutive parts: the lipophilic epithelium, the hydrophilic stroma and the lipophilic endothelium. This fact requires that a drug be lipophilic enough to enter the epithelium and the endothelium, but also that it be hydrophilic as well for passing the stroma. A decrease in the released amount of P has to be found in a higher lipophilicity because in the model used the first barrier is the hydrophilic Nephrophan®-membrane. This is advantageous for increased uptake in the cornea. Tests with the hydroxyalkylated CyDs did not alter the release of P.

3.3.3. In vivo tests

α-CyD increases the miotic side effect of P and this can serve as the criterion for bioavailability. Using solution 1 (see Table 1) as reference the 'relative bioavailability' was calculated to be 1.32 for solution 2. The time of maximum response was prolonged and the maximum response itself was increased by adding α-CD (Table 6). Commensurate with previous experiments a more intensive relation of the IOP will be expected (Keipert and Siebenbrodt, 1990). These results are in good correlation to the in vitro findings.

4. Summary

The association ability of the hydrophilic drug P and its hydrochloride with various CyDs and the physicochemical parameters of the solid dispersions and the aqueous solutions were determined. Thereby it was found that P itself, but also Ip, Pa and Ipa, are able to interact diversely with the tested CyDs. Because of the results of the NMR-, RP-HPLC- and FTIR-spectroscopy experiments it can be assumed that the inclusion of the imidazole ring of P will be given preference, but an association at the surface of the CyDs is probable as well. In vitro release rate studies showed a retarding effect of P in the presence of α-CyD and a comparable tendency was found by miosis testing in rabbits. With regard to the lowering of the intraocular pressure, the efficacy of the found interactions between P and the CyDs will be investigated by testings in human.

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