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Complexation of dihydropyridine derivatives with cyclodextrins and 2-hydroxypropyl-β-cyclodextrin in solution

B.W. Müller [1] and E. Albers [2]

[1] *Department of Pharmaceutics, Christian Albrecht University, Gutenbergstr. 76–78, D-2300 Kiel 1 (Germany)* and [2] *Department of Pharmaceutical Development, Byk Gulden Research Centre, D-7750 Konstanz (Germany)*

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Summary

Structure-interaction relationships in the inclusion complexation of cyclodextrins (CD) and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) with homologous derivatives of 1,4-dihydropyridines (DHPs) were investigated on the basis of phase solubility analysis, stabilization effects, and ^{1}H-NMR spectroscopy. Furthermore, the influence of 2-HP-β-CD on the adsorption of DHPs into the administration set and the compatibility of the complex with excipients for intravenous administration was examined. The interaction of DHPs with CD decreased in the order β-CD > 2-HP-β-CD > γ-CD > α-CD. The solubilization of DHP derivatives with 2-HP-β-CD was strongly influenced by the pH and the counterion indicating a considerable influence of the degree of dissociation of the guest molecule on complex formation. DHP derivatives with aromatic substitution were much more strongly solubilized than alkyl esters such as nitrendipine. It was found that the intensity of interaction of 2-HP-β-CD with DHPs depends on the substituents in the aromatic side chain decreasing in the order chlorobenzyl substitution, linear monoaryl structures, and geminal diaryl structures. Aromatically substituted 4-(3-nitrophenyl)-DHP derivatives were found to be photolabile, however, no photostabilization was observed with 2-HP-β-CD. Concerning the chemical stability of DHP derivatives, 2-HP-β-CD exerted destabilizing effects. By means of ^{1}H-NMR chemical shift measurement with the respective aromatic amines, the inclusion of the aromatic ester side chain within the cavity of 2-HP-β-CD was elucidated in aqueous solution. The adsorption of DHPs on hydrophobic PVC administration tubes was significantly reduced due to complexation with 2-HP-β-CD. The addition of human albumin led to complete recovery of the drug. The compatibility of the complexes with solutions for infusion was generally improved compared with conventionally solubilized systems, and increased in the order modified gelatin, bovine serum, physiological NaCl, dextran, and carbohydrates such as hexoses or sugar alcohols.

Introduction

Chemically modified cyclodextrins are molecular encapsulation agents with considerably in-

Correspondence: B.W. Müller, Dept of Pharmaceutics, Christian Albrecht University, Gutenbergstr. 76–78, D-2300 Kiel 1, Germany.

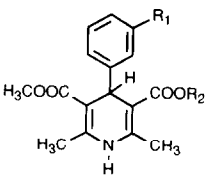
creased water solubility compared to the parent cyclodextrins. The pharmaceutical acceptance and application of cyclodextrin derivatives as new parenteral drug carriers are decisively focussed on 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) owing to its biological compatibility and lack of toxicity as a result of its intrinsically amorphous character. The properties of 2-HP-β-CD have been summarized in serveral reports (Pitha et al., 1986; Pitha, 1987; Szejtli, 1988; Yoahida et al., 1988; Brewster et al., 1989; Duchène and Wouessidjewe, 1990). Complexation generally leads to an alteration of the physicochemical properties of the guest molecule. The potent solubilizing properties of 2-HP-β-CD, as described previously (Müller and Brauns, 1985), are often accompanied by stabilizing effects, if the labile part of the guest molecule is included within the cyclodextrin cavity (Loftsson et al., 1989). The investigation of new drug molecules is often limited by their poor solubility. By means of inclusion complexation with 2-HP-β-CD, the saturation solubility of the drug is enhanced to the solubility of the complex, ideally resulting in therapeutically useful solutions for parenteral administration. Many drug molecules are known to form inclusion complexes with cyclodextrins and it seems that the only condition that has to be fulfilled is the proper fit of the guest molecule in the cyclodextrin cavity.

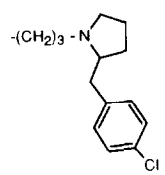
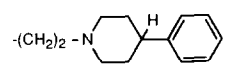
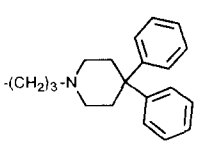
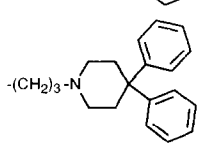
The DHP esters are calcium antagonists having the familiar negatively inotropic and vasodilative effects. Their extreme lipophilicity leads to a number of undesirable physicochemical properties, such as very poor solubility in water, a strong tendency to be adsorbed on glass and various plastics as well as incompatibility reactions with various excipients of pharmaceutical preparations. Difficulties also result from the sensitivity of dilute solutions to oxidation, especially photooxidation. In particular, the photolability of nifedipine has so far prevented its use in the form of parenteral preparations. The most serious biopharmaceutical drawbacks of the new investigational DHP derivatives are irritating effects after intravenous application which prevented the drug from toxicological and pharmacological characterization, as well as low bioavailability. In this respect, DHP derivatives appear to be ideal model drugs for the investigation of the effects and usefulness of 2-HP-β-CD within the preclinical development of parenteral formulations. According to our previous report (Müller and Albers, 1991), the parenteral application of 2-HP-β-CD showed crucial advantages such as lack of precipitation of the drug and consequently the avoidance of irritations caused by the drug itself, whereas the pharmacokinetic parameters of the guest molecule as well as the onset and course of pharmacological effects remained unaltered. Furthermore, hemolytic side effects have been completely avoided.

TABLE 1

Chemical structures of the 1,4-dihydroxypyridine derivatives I–IV

General structure:



DHP	R_1	R_2
Nitrendipine	$-NO_2$	$-CH_2-CH_3$
I	$-NO_2$	
II	$-NO_2$	
III	$-OCF_2-CHF_2$	
IV	$-NO_2$	

The purpose of this study is to elucidate the structure-interaction relationships between 2-HP-β-CD and various 4-phenyl-1,4-dihydropyridine (DHP) derivatives, in which the ester functions contain alkyl and aromatic structures with basic nitrogen. The chemical structures of the investigated DHP esters **I–IV** are given in Table 1. Furthermore, the influence of 2-HP-β-CD on certain drug inherent problems mentioned above was investigated.

Physically stable solutions have been obtained by hydrotropic solubilization of DHPs with sodium benzoate, but there was no accompanying photostabilization of the active ingredient (Jain et al., 1988). Preclinical studies with new DHP derivatives **I–IV** were primarily based on systems with cosolvents. Physical and chemical stable solutions were obtainable only with 1,2-propylene glycol (1,2-PG) at concentrations above 30% at pH 4, together with ascorbic acid/Na_2EDTA. However, in vivo experiments showed that these solutions were unacceptable for intravenous administration because dilution of the cosolvent-solubilized drug led to its precipitation. The widespread occurrence of these drug inherent drawbacks is mainly caused by the extensive hydrophobicity of new drug molecules necessarily leading to substantial accumulation in various human tissues. Furthermore, growing objections arise concerning conventional solubilizing agents such as Cremophor RH 40 and 1,2-PG, which stimulated our search for effective parenteral drug formulations with biologically tolerated cyclodextrin derivatives as parenteral drug carrier.

Materials and Methods

Nitrendipine (NT), nimodipine (NM), and nisoldipine (NS) were obtained from Bayer AG, Wuppertal, Germany. The dihydropyridine derivatives listed in Table 1 and the respective amines are research substances produced by Byk Golden Pharmazeutika, Konstanz, Germany. The DHP esters were investigated in the form of the racemates. In the case of the DHP ester **IV**, the two enantiomers **IV**(+) and **IV**(−) were available. Both the DHP ester and the amines were used as hydrochlorides. The unsubstituted cyclodextrins (CDs) and 2-hydroxypropyl-β-cyclodextrin (2-HP-β-CD) with a molar degree of substitution (MS) equal to 0.52 were supplied by Chinoin, Budapest, Hungary. The following water contents were determined with a Karl-Fischer apparatus (Metrohm AG, Herisau, Switzerland) and taken into account: α-CD, 2.6%; β-CD, 10.9%; γ-CD, 9.4%; 2-HP-β-CD, 3.1%. All compounds were fully characterized by spectroscopic analysis prior to use (UV-Vis; ^{1}H-NMR); all other materials and solvents were of analytical reagent grade.

Phase solubility studies were carried out as described by Higuchi and Connors (1965). Excess amounts of the DHPs (100–200 mg/vial) were weighed into 50 ml brown glass bottles for two series of measurements in each case, and 10 ml portions of aqueous cyclodextrin solutions of various concentrations were added. The cyclodextrin solutions were prepared using freshly distilled water. Argon was passed through the preparations to avoid photodegradation during equilibration. In the case of the solubility isotherms, the pH was adjusted to 5.8 with 0.05 M potassium phosphate buffer, and the pH profiles were additionally measured in citric acid/NaOH/HCl buffer (Titrisol®, Merck, Darmstadt, Germany). The pH measurements were carried out with a Microprocessor pH Meter 761 (Knick, Berlin, Germany) with automatic temperature compensation < 1 K and measurement error $< 0.01\%$. Sufficient wettability of the hydrophobic compounds with the aqueous phase was attained by sonicating the suspensions for 4 min at room temperature under a nitrogen atmosphere, while longer exposure was avoided due to heating and possible contact with oxygen. The tightly stoppered bottles were agitated in a shaking thermostat (MS 20 S, Lauda, Lauda-Königshofen, Germany) at 25 ± 0.01 °C and treated at intervals in an ultrasonic bath to achieve redispersion of the sediment. When the formation of the complex had reached equilibrium (≈ 7 days), the suspensions were filtered through 0.22 μm membrane filters (Millex®-GS, Millipore Corp., Bedford, MA, U.S.A.), and the absolute quantity of dissolved DHP in the clear filtrate was determined.

Because of the strong membrane adsorption of the DHPs, the first portion of the filtrate was discarded. All percentages given for solutions are w/v. The analyses were carried out by high-performance liquid chromatography using an HPLC instrument equipped with a UV-Vis detector (Merck/Hitachi, Darmstadt, Germany). A 250 × 4.6 mm i.d. stainless-steel column, packed with 5 μm particles of ODS Hypersil® (Shandon, Runcorn, U.K.) was used. In the case of NS and NM, a Spherisorb® 5 Phenyl column was used, thermostated at 30 °C. Samples were applied via autoinjection (autosampler, Merck). The mobile phase consisted of isocratic methanol (Lichrosolv®, Merck) and water at a flow rate of 1 ml min^{-1}. The eluent ratios, given as methanol: water (v/v), and detection wavelengths in nm were as follows: (DHP ester **I**) 89:11, 222.4; (DHP ester **II**) 89:11, 237.7; (DHP ester **III**) 89:11, 205.6; (DHP ester **IV**(+) and (−)) 93:7, 224.5; NT 85:15, 235.6. In the case of NS and NM, gradient elution was used (acetonitrile/0.01 M buffer, pH 7.4); the detection wavelength was 236 nm.

The drug adsorption of solutions comprising 144 μg ml^{-1} DHP ester **IV**(−) on intravenous PVC application systems was investigated using an Original-Infusomat® tube (2.5 m length, 3 mm i.d., 4.1 mm o.d., 17.67 cm^3 residual volume; Braun Melsungen AG, Melsungen, Germany). Formulations with 10% 1,2-PG and 2-HP-β-CD in 0.02 M potassium phosphate buffer, respectively, were passed through the administration set using a peristaltic pump at a constant flow rate of 1 ml min^{-1}. All tubes of the apparatus were saturated with DHP before the application system was set online. The effect of albumin on the adsorption of DHP ester **IV**(−) on the application system was additionally investigated by adding 20% human albumin (Immuno GmbH, Heidelberg, Germany) to the preparation with 1,2-PG. Furthermore, the effect of albumin solution previously passed through the administration set was studied. The effluent volume was collected into HPLC autosampler vials in fractions of 2 min and the remaining concentration of DHP ester **IV**(−) was determined as described previously.

Compatibility studies were carried out with solutions containing 2 mg ml^{-1} DHP ester **IV**(−) in 10% 2-HP-β-CD, referred to as FIa, and 30% 1,2-PG (FIb), respectively, as well as 1.33 mg ml^{-1} DHP ester **IV**(−) in 10% 2-HP-β-CD (FII). Constant volumes of the stirred solutions FIa and FIb were mixed with progressive amounts of distilled water, physiological NaCl, and bovine serum, until turbidity effects or crystallization occurred. The particle size of the precipitates was determined with an Autosizer apparatus (Autosizer 2c Malvern, Mütek, Herrsching, Germany). Incompatibility reactions were quantitatively measured with formulation FII. Constant volumes of the complex were diluted 1:10 with the following solutions under investigation: distilled water; fructose, xylitol, sorbitol, glucose, dextran (all Boehringer Mannheim GmbH, Mannheim, Germany); oxypoly-gelatin (Gelifundol®, Biotest Pharma GmbH, Frankfurt, Germany), and bovine serum. After the solutions had been allowed to stand for 2 h, they were filtered through 0.22 μm membrane filters and analyzed for remaining DHP ester **IV**(−) by means of HPLC. In the DHP stability samples, the DHP was present at a concentration of 2 mg ml^{-1} in isotonic (adjusted with sorbitol) pH 5.5 potassium phosphate buffer containing 30% of 1,2-PG (BASF AG, Ludwigshafen, Germany) or 10% of 2-HP-β-CD. The solutions were aseptically filled into 10 ml clear glass ampoules, with bubbling of nitrogen, and stored in thermostatically controlled ovens at various temperatures (30, 40, 50, and 70 °C). Groups of three ampoules were selected at appropriate intervals, and their undecomposed DHP contents were determined.

Photostability studies were carried out with 7.75×10^{-5} M solutions of DHP ester **I** in 50 vol.% aqueous acetonitrile (1) and in 1.55×10^{-2} M aqueous 2-HP-β-CD solution (2), corresponding to a 200-fold excess. The aqueous component contained sodium dihydrogen phosphate at a concentration of 0.01 M. The solutions, in tightly closed quartz cells having a layer thickness of 50 mm (100 QS, Hellma, Müllheim, Germany), were exposed to UV radiation for 4 h with water cooling and under a constant radiation intensity in a Suntest CPS exposure apparatus (Heraeus,

Hanau, Germany), and the UV-Vis spectra were recorded by means of a Lambda 5 UV-Vis twin-beam spectrophotometer (Perkin-Elmer, Überlingen, Germany).

The ^{1}H-NMR measurements were performed with a pulsed-FT high-resolution spectrometer (Bruker Instruments Inc., Billerica, MA, U.S.A.), equipped with an Aspect 2000 microcomputer, operating at 250 MHz. The spectra were recorded at 298 K in 5 mm o.d. spinning tubes (Norell Inc., Landisville, NJ, U.S.A.) in D_2O (99.8%, Merck) providing the field-frequency lock signal. The data acquisition occurred with 256 scans. Prior to use, 2-HP-β-CD (MS 0.51) was dissolved in distilled water, filtered through 0.22 μm membrane filters, freeze-dried and stored over P_2O_5 in vacuo. The guest concentration was 0.005 M; the cyclodextrin concentration was varied over the range 0.0005–0.15 M ($n = 15$) to achieve molar guest/host ratios of 0.1:30 using microlitre syringes (Unimetrics®, Shorewood, IL, U.S.A.). Chemical shifts were referred to an external sodium trimethylsilylpropionate-d_4 reference signal using the absolute scale for calibration (0 ppm = 2816.32 Hz). Chemical shift changes were calculated according to $\Delta\delta = \delta(\text{complex}) - \delta(\text{free})$.

Results and Discussion

The pH dependence of the complexation of the basic DHP derivatives with 2-HP-β-CD was investigated on the basis of solubility/pH profiles. Fig. 1 shows the solubility profile of DHP ester **I** in the presence and absence of 10% 2-HP-β-CD as well as the influence of the buffer ion. In the phase solubility test with DHP derivatives, the limiting solubilities were considerably influenced by the counterion. The solubility was found to be highest in phosphate buffers, and was considerably reduced in systems containing chloride or citrate, owing to the lower solubility products of the salts formed. The same dependence of the counterion was found for the amount solubilized with 2-HP-β-CD. In Fig. 1, the pH profiles in citrate buffer for both the pure buffer solutions and the complex evidently run below the curves determined in phosphate buffer solution, respectively. Hence, the following investigations were carried out with preference for the latter medium. The solubility of DHP ester **I** increased sharply in the acidic range. In the case of the uncomplexed ester, the solubility jump did not occur at the pK_a (pH 9),but was displaced by several pH units in the acidic direction. The phosphate must therefore be significantly more soluble than the free base. The same pH dependence was observed with 2-HP-β-CD, and the solubility jump for the complex was not altered, but occurred at considerably higher concentrations. The solubilizing effect clearly increased in the acidic range. A disproportionately large solubility increase in the lower pH range was also found for the complex of DHP ester **IV**(+), which displayed lower complex concentrations than in the case of DHP ester **I**. The pH jump occurred at pH 5 and no displacement occurred with respect to the free drug. The increase in the limiting solubility of the drug in the acidic range is accompanied by improvement in the solubilizing properties of 2-HP-β-CD. An increase in complex formation with increasing pH has been reported for anionic guest molecules, specifically *N*-phenylanthranilic acids (Otagiri et al., 1976). The degree of dissociation thus has a

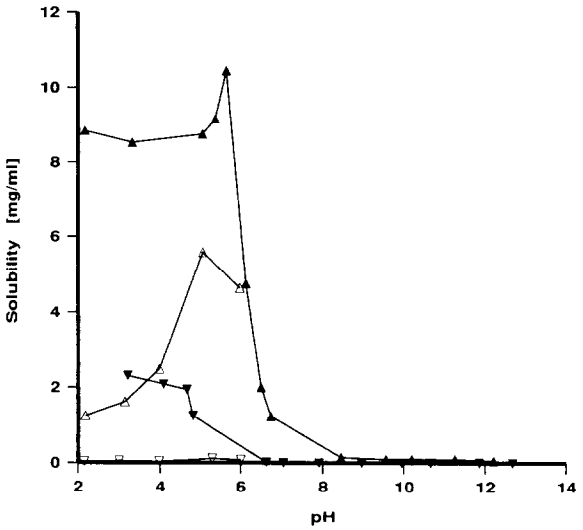


Fig. 1. pH profile of DHP ester **I** in 0.05 M phosphate buffer solutions (▼) and of its 2-HP-β-CD complex (▲) and in citric acid/NaOH/HCl buffer in the absence (▽) and presence (△) of 10% 2-HP-β-CD.

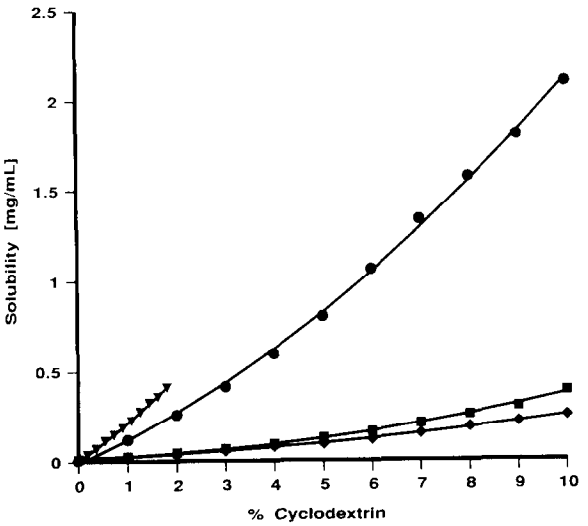


Fig. 2. Phase solubility diagram of DHP ester **IV**(+) in phosphate buffer at pH 5.8 and 25 °C with various cyclodextrins: α-CD (◆), β-CD (▼), γ-CD (■), 2-HP-β-CD (●).

decisive influence on the complexability of ionic guest molecules. Conclusions regarding the nature of the bonding forces can also be drawn from these results. Hydrogen bonding with the protonated amine functions of the aromatic ester side chain appears to make a contribution, in addition to the known hydrophobic interactions and van der Waals forces with lipophilic partial structures. Due to the pH dependence of the complexing abilities of 2-HP-β-CD with ionic drug molecules, the amount solubilized can be optimized, if the chemical stability of the drug is not affected.

The phase solubility behaviour of poorly soluble DHP esters with various cyclodextrins was investigated at pH 5.8, this value being chosen as a compromise between the high solubility of the complex and physiologically tolerable conditions. The increase in the saturation solubility of the DHP esters as a function of the cyclodextrin used was investigated for DHP ester **IV**(+) with four cyclodextrins. Fig. 2 shows the phase solubility diagrams recorded for unsubstituted cyclodextrins and for 2-HP-β-CD.

DHP ester **IV**(+) was solubilized by all of the cyclodextrins investigated, albeit to various degrees. The solubilization decreased in the order β-CD > 2-HP-β-CD > γ-CD > α-CD. The isotherms were examined on the basis of linear and exponential curve fits, according to which the correlation coefficients showed greater values for the exponential type. This would lead to the conclusion that exclusively soluble complexes deviating from 1 : 1 stoichiometry were formed with all cyclodextrins, i.e. A_p type solubility diagrams according to Higuchi and Connors (1965), if pH effects were excluded. However, investigation of the pH values in the filtrates of all the DHP esters showed pH changes up to one unit toward the acid side. The buffer capacity had therefore been exceeded, and the apparently exponential behaviour of the isotherms was actually due to pH effects. This interpretation is well supported by the strictly linear isotherms for nimodipine with several hydroxyalkylated β-CD derivatives in pure water as reported previously (Yoshida et al., 1990). Preparation of CD inclusion compounds with aromatic substituted DHP esters in solution or in the solid state should preferentially occur with β-CD or 2-HP-β-CD owing to their solubilizing effects being the strongest, whereas γ-CD and especially α-CD appear to be unsuitable for this purpose. The diameter of the cavity of α-CD permits only slight penetration of DHP ester **IV**(+), whereas in γ-CD the cavity diameter is already too large for effective interactions to develop. Although the geometric conditions are optimized in the case of β-CD, a clear disadvantage arises from the low solubility of β-CD itself. This restriction is avoided with 2-HP-β-CD, but because of the steric blockade of the cavity, the course of the isotherm is less steep for 2-HP-β-CD than for β-CD. In view of these results, solubilization by 2-HP-β-CD was monitored for various structural analogues of nitrendipine, i.e. 4-(3-nitrophenyl)-DHP derivatives, as listed in Table 1. The isotherms obtained had different gradients, depending on the structure of the ester side chain (Fig. 3). No differences in complex solubility were detected between the enantiomer DHP esters **IV**(+) and (−). It was fundamentally possible to distinguish between DHP esters having exclusively aliphatic 3,5-dicarboxylic acid ester groups and those with aromatic structures. Nitrendipine (NT), nisoldipine (NS), and nimodip-

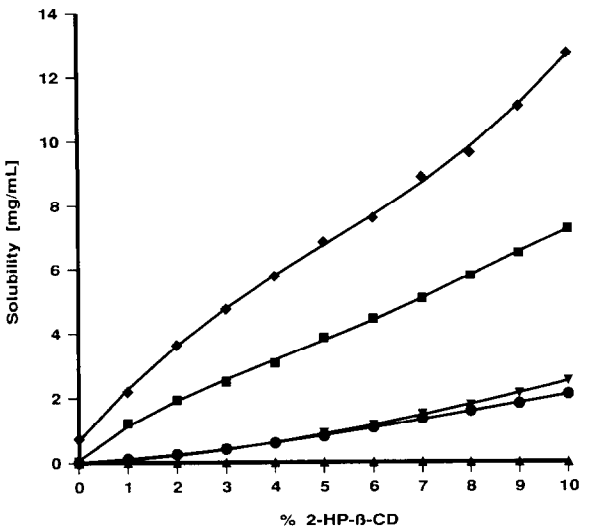


Fig. 3. Phase solubility diagram of DHP esters in phosphate buffer at pH 5.8 and 25 °C with 2-HP-β-CD: **I** (◆), **II** (■), **III** (▼), **IV**(+) (●), nitrendipine (▲).

ine (NM) were used as representatives of the first class. Compared with aromatic DHP derivatives, the solubility increase with 2-HP-β-CD was insignificant for these substances, and decreased in the order NS > NM > NT. In the case of the DHP alkyl esters, the inclusion ability of 2-HP-β-CD exceeds that of β-CD as demonstrated recently with NM (Yoshida et al., 1990). Effective solubilization by 2-HP-β-CD was possible for the DHP esters **I–IV** of the second class. Solubilization decreased in the order **I** > **II** > **III**, **IV**(+) = **IV**(−) > NT. From these results, it is possible to predict the probable solubilization of new calcium antagonists of the DHP series. The 2-(*N*-methyl-*N*-benzyl)aminoethyl ester nicardipine belongs to the second class, and is anticipated to exhibit high solubilization with 2-HP-β-CD in the acidic range.

From the results of the phase solubility experiments it is deduced that the structure-interaction relationship between 2-HP-β-CD and DHP esters is strictly limited to the ester structure of the DHPs. The influence of the structure of DHP esters on interactions with cyclodextrins is clearly demonstrated by the following deductions from the data in Fig. 3. Variation in the structure of the nitroaryl group, for example, between DHP esters **III** and **IV**, leads to isotherms that practically coincide. The complexing of the DHPs is not enantioselective with respect to the centre of chirality in the DHP, as can be concluded from the fact that the complex solubilities of the enantiomers **IV**(+) and **IV**(−) were identical. In general, enantiomers cannot be distinguished on the basis of solubility parameters, or of any other physicochemicial properties, but chiral pools that allow the differentiation of enantiomers are present in systems containing cyclodextrins. Inclusion of the DHP structure is also sterically hindered by the 2,6-dimethyl substitution of the dihydropyridine. To summarize, pending the results of the NMR studies, the following relationships between the structure of the aromatic side chain in DHPs and the interaction with 2-HP-β-CD were concluded: the optimum structural unit for interaction with 2-HP-β-CD is the freely rotating chlorobenzyl group; this is followed by linear monoaryl structures (DHP ester **II**) and geminal diaryl structures exhibiting mutual steric hindrance (DHP esters **III** and **IV**).

As has been pointed out in the Introduction, the DHP esters under investigation possess further undesirable physicochemical properties, of which the strong tendency to be adsorbed on synthetic material, e.g., PVC surfaces and membrane filters, as well as problems arising from incompatibility on combination with excipients hindered our previous work in this area. These drawbacks are well-known for certain drugs, e.g. diazepam or isosorbide dinitrate. Nakajima et al. (1987) found that 2-HP-β-CD exerted a considerable influence on the adsorption of isosorbide dinitrate into intravenous delivery systems. In this context, the combination of insulin solution with albumin is useful and suitable for reducing its loss into several synthetic materials. We therefore investigated the influence of 2-HP-β-CD on the adsorption of DHP ester **IV**(−) on hydrophobic surfaces, and the effect of albumin. Fig. 4 displays the adsorption profiles of DHP ester **IV**(−) on PVC administration sets from solutions comprising 1,2-PG and 2-HP-β-CD, and the effect of human albumin. With conventional solubilized systems, the initial concentration of the drug was significantly reduced to 83.4% within 6 min as

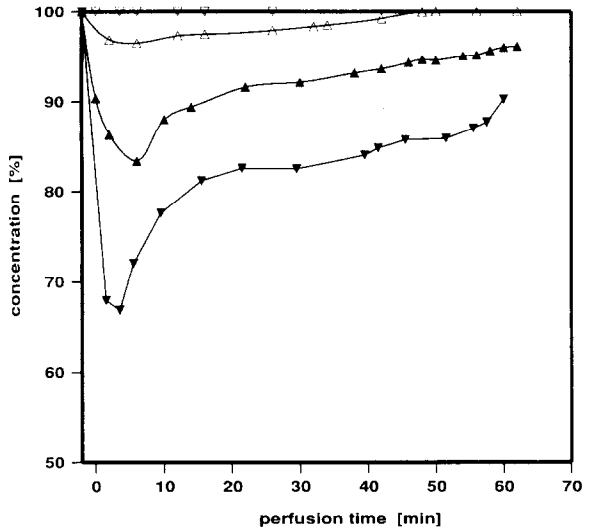


Fig. 4. Adsorption of DHP ester **IV**(−) on PVC administration set: recovery in % of the initial value, 144 μg ml^{-1} base, against the time of passage, in min, with 10% 1,2-PG (▲), 10% 2-HP-β-CD (△), 10% 1,2-PG with 20% human albumin (▽), and 10% 1,2-PG with preceding perfusion of the system with 20% human albumin (▼).

exemplified by the 1,2-PG formulation. This value proved to be relevant for pharmacological investigations. The adsorption of DHP ester **IV**(−) on hydrophobic surfaces is a time-dependent process accompanied by saturation effects. In the presence of 10% 2-HP-β-CD the adsorbed amount of DHP ester **IV**(−) was appreciably reduced up to 13.1% and complete elimination of the initial peak was observed. The recovery of the complexed drug approximated to the initial level within 50 min, whereas this was not observed with the cosolvent system. In terms of the underlying physicochemical principle, the sorption-desorption equilibrium of the guest molecule on hydrophobic surfaces is shifted as a result of complex formation in solution. Consequently, the adsorption-diminishing effect of 2-HP-β-CD can be ascribed to dynamic redistribution of the adsorbed drug molecules from the binding areas of the inner surface of the tube into the hydrophobic environment of the cyclodextrin cavities in solution. In practice, this is a crucial advantage justifying the application of CD complexes with highly biopotent drug molecules, e.g. peptide hormones. The effect of human albumin on the adsorption of DHP ester **IV**(−) was dependent on the mode of application. Complete recovery of the drug within the first few millilitres of the effluent was achievable when albumin was added directly to the solubilized systems providing better results than complex formation with 2-HP-β-CD. On the other hand, preceding perfusion and saturation of the administration system with human albumin negatively affected the loss of the drug, rising to 33.1% within 4 min. It appears from the data that the effect of human albumin on drug adsorption is governed by strong protein binding of DHP ester **IV**(−) which is quite conceivable on taking the hydrophobicity of the drug into account. Thus, free passage of the drug through the administration set is feasible with albumin present. On the other hand, if albumin is allowed to saturate all the adhesion areas prior to contact with the drug, the latter is evidently attached more extensively on the protein layer, which can be characterized in terms of coadsorption phenomena. With respect to the possible combination of soluble CD complexes with albumin, it is interesting to note that the extent of protein binding is determined by the stability constant K_c of the complex (Lin and Yang, 1987) and, accordingly, the adsorption-diminishing effect of albumin must be a function of K_c in such systems. To summarize, solutions comprising 2-HP-β-CD or human albumin display a considerable decrease or elimination in the adsorption of hydrophobic guest molecules on hydrophobic surface areas as compared to systems with conventional solubilizers. Although successfully applied in the case of insulin, the use of albumin should be considered carefully because of possible anaphylactic responses.

The susceptibility of aromatic substituted DHP esters to undergoing various incompatibility reactions was already apparent from the pH profiles, indicating negative effects of chloride and citrate on the solubility of the CD complexes. Furthermore, heat sterilisation of concentrated CD complexes in the presence of sorbitol led to irreversible precipitation of the active compound and was accompanied by loss amounting to 10.7%. On the other hand, hypo-osmotic complex solutions

allowed heat sterilisation in all cases. Since preclinical test doses of DHP ester **IV(−)** should be adjusted by combination of physiologically acceptable preformulations of the CD complex with suitable infusion solutions, we focussed our attention on the investigation of possible incompatibility reactions such as turbidity and precipitation with large-volume injection solutions intended for administration by intravenous infusion. Aqueous complex solutions with cyclodextrin derivatives are generally considered to withstand dilution without any precipitation. However, the results of the dilution studies listed in Table 2 reveal that this is not tenable in the case of DHP esters, although the dilution behaviour was significantly improved by complexation. The cosolvent system led to complete precipitation already with distilled water and became turbid even with slight amounts of sodium chloride or serum. These in vitro results reflect the observed irritant side effects of cosolvent-solubilized DHP ester **IV(−)** after intravenous administration in beagle dogs (Müller and Albers, 1991). On the other hand, the complex compatibility with regard to sodium chloride was improved by 11 orders of magnitude and initial turbidity could be avoided. The initial turbidity of the complex solution with serum was reversible with increasing amounts of serum and is due to the fact that complex binding of the DHP ester changes into protein binding with serum albumin. Since the dilution behaviour of injection solutions with serum is relevant for in vivo conditions, the average particle size of the precipitates was analysed. The particle size was found to be smaller for the complexes due to their finely divided dispersion in the medium. Furthermore, the significance of the compatibility of complex solutions intended for intravenous administration is obvious with respect to reproduction of the systemic bioavailability of the guest molecule. Thus, the compatibility of a given DHP test dose in the complexed state with several infusion solutions was quantified and the results are given in Table 2B. With dextran, modified gelatin, sodium chloride and bovine serum, spontaneously emerging, manifest incompatibility re-

TABLE 2

Incompatibility reactions of cosolvent-solubilized systems and cyclodextrin complex formulations with DHP ester IV(−) upon dilution with distilled water and several injection solutions intended for administration by intravenous infusion (vr, volume ratio 1,2-PG (2-HP-β-CD) solution : test media)

(A) Test media	2 mg ml^{-1} DHP ester **IV(−)** in	
	30% 1,2-PG	10% 2-HP-β-CD
(1) Distilled water	spontaneous precipitation	infinite dilution
(2) 0.9% sodium chloride injection	irreversible turbidity above vr = 1 : 0.1	irreversible turbidity above vr = 1 : 1.1
(3) Bovine serum	irreversible turbidity above vr = 1 : 1	initial turbidity, reversible above vr = 1 : 20
Average particle size	463 nm	317 nm

(B) Test media	1.33 mg ml^{-1} DHP ester **IV(−)** in 10% 2-HP-β-CD		
	Appearance		Content of DHP ester **IV(−)** after 2 h (%)
	Immediately	After 2 h	
(1) Distilled water	clear	clear	100.0
(2) 5% fructose	clear	clear	100.0
(3) 5% xylitol	clear	clear	100.0
(4) 5% sorbitol	clear	clear	100.0
(5) 5% glucose	clear	clear	100.0
(6) Dextran	initial turbidity	precipitation	91.6
(7) Oxypoly-gelatin	turbidity	precipitation	12.7
(8) 0.9% sodium chloride injection	intensive turbidity	intensive turbidity	80.7
(9) Bovine serum	turbidity	precipitation	58.8

actions accompanied by a significant loss of the active compound were ascertained. Dilution of the complex solution of the order 1 : 10 with oxypoly-gelatin being usually applied in the clinical field led to almost quantitative displacement of the solubilized DHP ester. Under the same conditions, incubation with bovine serum resulted in a 40% loss of the active compound. Similarly, the application of dextran or sodium chloride, e.g. by means of Ringer-lactate infusion, must be rejected. These results demonstrate appreciable incompatibility effects that need to be considered under clinical conditions with respect to intolerance reactions and the lack of precise administration of a certain dose. The results show that the only feasible and unobjectionable large-volume injection solutions for intravenous infusion are carbohydrates such as hexoses or sugar alcohols in the absence of sodium chloride which are characterized by complete cyclodextrin complex compatibility. However, owing to their desolvatation effect on the hydrophilic cyclodextrin periphery (Müller and Albers, 1991), water structure formers such as hexoses and sugar alcohols exhibit latent incompatibility effects that become manifest, for instance, during heat sterilisation. Thus, the combination of CD complexes with sugar alcohol infusions should only occur prior to application whereas fixed combinations in preformulations require aseptic treatment or preservation.

The chemical stability of the DHP derivatives is different from that of common esters. Because of the vinylogous carboxamide structure and the conjugation of the 3,5-carboxyl groups with the π-electron system of DHP, the DHP esters are resonance-stabilized and insensitive to alkaline hydrolysis. Decomposition in this case involves oxidative processes, such as pyridine formation and *N*-oxide formation in the side chain, and leads to different decomposition products. The photoreactivity of the DHP derivatives is less complicated than hydrolytic effects. Generally, the only photodecomposition products formed are the corresponding 4-(nitrosophenyl)pyridine derivatives. Physicochemical instability problems were encountered with the cyclodextrin formulations with DHP esters at higher temperatures; a comprehensive kinetic study was therefore pointless. Storage tests above 40 °C led to deposition of active substance on the ampoule glass, and to the formation of precipitates, which turned brown at 70 °C. The 1,2-PG solutions, on the other hand, showed no visible changes, and were also found to be more stable chemically (Table 3). The physical instability of the 2-HP-β-CD solution was associated with an accelerated decrease in the active content, and an increase in the number of secondary peaks in the HPLC was indicative of increased formation of decomposition products.

The destabilizing effects of 2-HP-β-CD on DHP esters can be attributed to the following reasons. As mentioned above, autoclaving experiments have shown that the stability of the complex is adversely affected by the sorbitol used to adjust the tonicity of the solution. Detailed investigations indicated that this is due to the effects of sorbitol on the structure of water (Müller and Albers, 1991). The complex formation constants of the DHPs with 2-HP-β-CD are low (200 l mol^{-1}, UV method), hence higher temperatures lead to considerable displacement of the complex formation equilibrium in the reverse direction.

TABLE 3

*Percentage decrease in the content of active substance in isotonic solutions of DHP ester **IV**(−) with 30% of 1,2-PG (1) and with 10% of 2-HP-β-CD (2) at various temperatures*

Time (days)	Temperature (°C)							
	30		40		50		70	
	1	2	1	2	1	2	1	2
30	99.9	99.4	101.1	98.6	101.1	94.8	98.8	75.4
120	100.5	97.9	98.8	94.1	98.7	85.0	91.2	51.8

TABLE 4

Absorption maxima and extinction values of 7.75×10^{-5} M DHP ester I before and after UV exposure for 4 h in aqueous acetonitrile solution and in 2-HP-β-CD solution

DHP ester	Aqueous solution containing	UV exposure	Absorption maxima (nm)	Extinction (A)
I	CH_3CN	–	353.9 (DHP)	0.502
			237.1	1.927
			220.6	2.163
I-pyridine	CH_3CN	–	< 219.4	–
I	CH_3CN	4 h	219.9	2.405
I	2-HP-β-CD	4 h	220.5	2.072
			202.1	2.407

Finally, the adhesion between the free base and the surface of the glass also contributes to the removal of the active substance from the complex formation equilibrium.

In addition to the chemical stability, the photostability of the DHP derivatives and the influence of 2-HP-β-CD were investigated. The 2-nitrophenyl derivatives of DHP, such as nifedipine, are extremely photolabile; special requirements are therefore necessary for the galenic form. 4-Nitrophenyl derivatives, on the other hand, are regarded as photostable (Berson and Brown, 1955). 3-Nitrophenyl derivatives differ in their photoreactive behaviour (Wu and Cai, 1986; Greiner et al., 1988). The results of light exposure studies on the 3-nitrophenyl-DHP derivative **I** in dilute aqueous solution are shown in Table 4.

The intensity of the DHP absorption maximum at 354 nm was found to decrease with increasing exposure time, and after 4 h the band could no longer be detected. A new maximum at 270 nm, which appeared only as a shoulder, can be attributed to the formation of nitrosophenyl products (Thoma and Klimek, 1985; Al-Turk et al., 1989). Aromatically substituted 4-(3-nitrophenyl) DHP derivatives must therefore also be regarded as photolabile, and are converted into the corresponding 4-(3-nitrosophenyl)pyridines by the action of light. Since the photochemical decomposition of DHP derivatives is associated with a considerable loss of pharmacological activity (Al-Turk et al., 1988), photostabilizing measures are essential. However, photostabilization could not be achieved by complexing with 2-HP-β-CD, since irradiation of DHP ester **I** in the presence of 2-HP-β-CD still led to a decrease in the intensity of the photochemically significant and hence stability-specific band. The results of the photostability study also exclude complexing of the DHP structure, since there was no detectable change in the photochemical reactivity. Photostabilization should result from the formation of intermolecular hydrogen bonds between 2-HP-β-CD and the 1,4 protons of DHP – which is sterically favoured

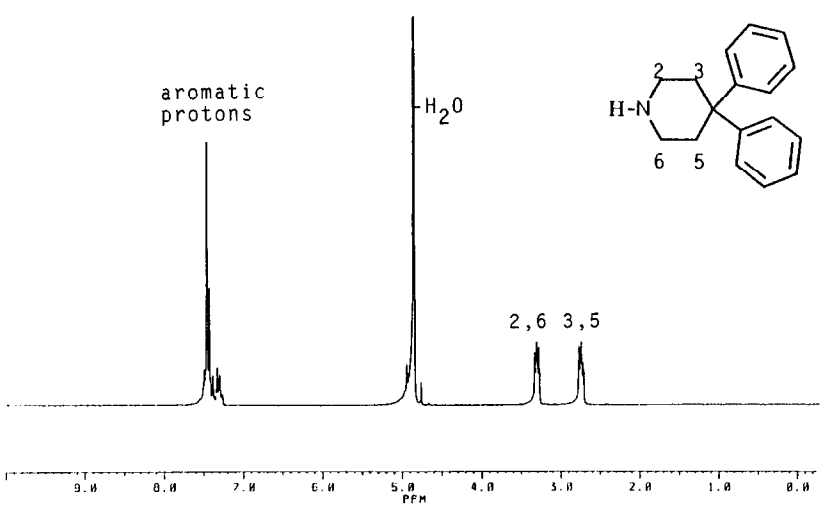


Fig. 5. ^{1}H-NMR spectrum of 5×10^{-3} M 4,4-diphenylpiperidine (DPP) in D_2O; 250 MHz; 2816.31 Hz = 0 ppm.

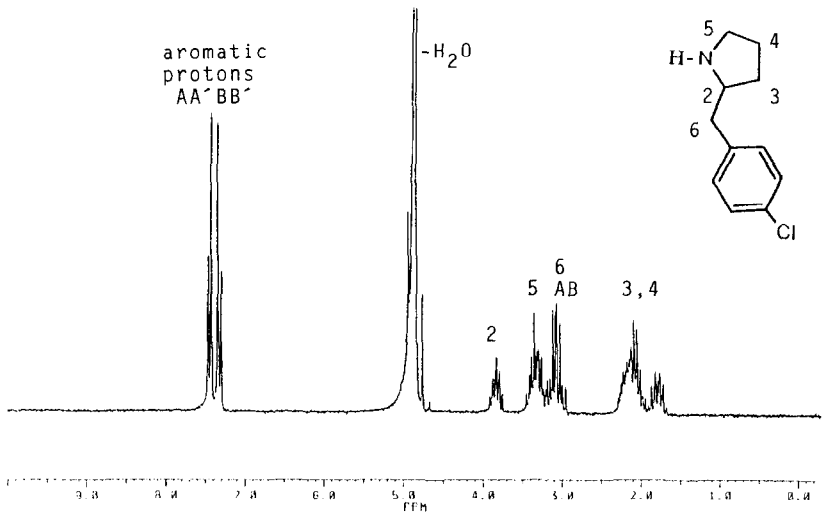


Fig. 6. ^{1}H-NMR spectrum of 5×10^{-3} M (−)-2-(4-chlorobenzyl)pyrrolidine (CBP) in D_2O; 250 MHz; 2816.31 Hz = 0 ppm.

by the inclusion of the aromatic ester side chain – since two DHP protons are removed together with oxygen in an intramolecular disproportionation which leads to formation of the aromatic nitrosopyridine. Thus, intermolecular hydrogen bonds with the 1,4 protons of DHP do not contribute to the binding mechanism with 2-HP-β-CD.

The interaction of 2-HP-β-CD with the DHP ester side chain was investigated by means of ^{1}H-NMR chemical shift measurements. A survey of applications of NMR for the structural determination of cyclodextrin inclusion compounds has previously been given (Miao, 1985; Yamamoto and Inoue, 1989). Owing to the poor water solubility of the DHP derivatives, the water-soluble amines corresponding to DHP ester **I**, (−)-2-(4-chlorobenzyl)pyrrolidine (CBP) and **III**, **IV**, 4,4-diphenylpiperidine (DPP), were directly treated

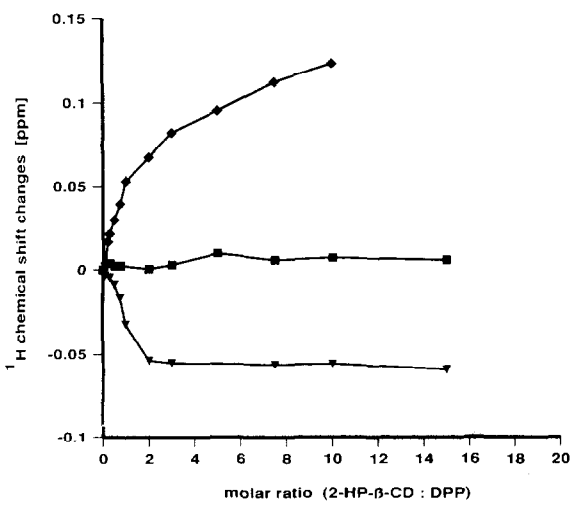


Fig. 7. Binding curve of 4,4-diphenylpiperidine with 2-HP-β-CD: piperidine protons 2,6 (♦), 3,5 (■); aromatic protons (▼). Negative shift values signify upfield shift.

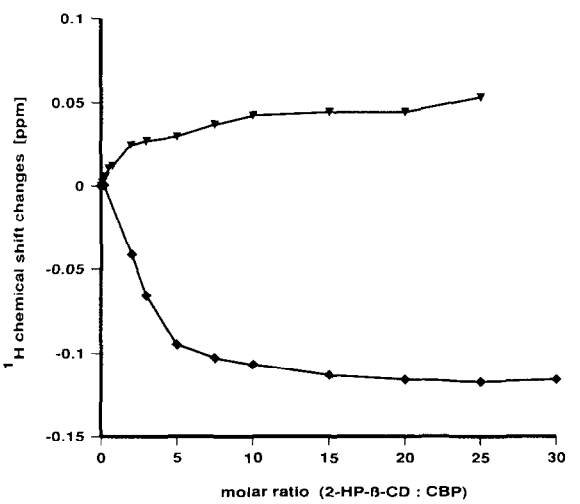


Fig. 8. Binding curve of (−)-2-(4-chlorobenzyl)pyrrolidine with 2-HP-β-CD: aromatic protons (▼); aliphatic benzyl-CH_2 protons (♦).

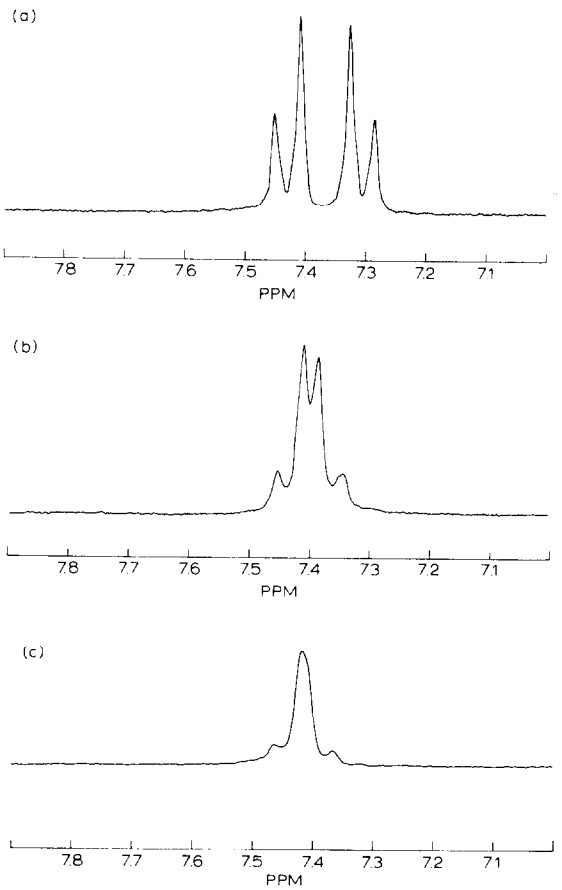


Fig. 9. Downfield part of the ^{1}H-NMR spectra of (−)-2-(4-chlorobenzyl)pyrrolidine (0.005 M in D_2O) with increasing molar ratios of 2-HP-β-CD : CBP: 0 (a), 5 (b), 15 (c).

with 2-HP-β-CD in D_2O in order to obtain binding curves. Upon inclusion of aromatic structures, the chemical shift of protons located inside the cyclodextrin cavity should be affected due to the magnetic anisotropic effect. However, the H-3 and H-5 protons of 2-HP-β-CD could not be assigned to resonance signals because 2-HP-β-CD consists of a multitude of isomers (Albers, 1991). On the other hand, cyclodextrin-induced ^{1}H-chemical shift changes of the guest proton resonances could be observed. In the ^{1}H-NMR spectrum of DPP in Fig. 5, the integral over 7.2–7.5 ppm displays a number of 10 aromatic proton signals. The observed multiplicity could be attributed to interdependence of the ring current effects of the geminal diphenyl group. The piperidine protons of DPP appeared as an ABCD spin system well known for morpholine. The ^{1}H-NMR spectrum of CBP is characterized by the typical, centrosymmetric AA'BB' spin system (Bovey, 1969) shown in Fig. 6 and separately in Fig. 9a, as well as strongly split lines for pyrrolidine and aliphatic benzyl-CH_2 protons, Plots of the change in chemical shifts of the guest molecule resonances vs the molar ratio of 2-HP-β-CD to guest are displayed in Figs 7 and 8 for the amines DPP and CBP, respectively. In the case of CBP, the resonances of pyrrolidine were not examined because, upon addition of 2-HP-β-CD, the multiplicity changed which resulted in ambiguous assignment of signals. The ^{1}H-chemical shift of the 4-chlorobenzyl-AA'BB' spin system relates to its

centre according to $(\nu_1 + \nu_2)/2$. Upon addition of 2-HP-β-CD, the aromatic proton resonances of DPP revealed an upfield shift, whereas those of CBP exhibited a downfield shift. The magnitudes of the maximal induced shifts of the aromatic signals of DPP and CBP were similar, 0.06 and 0.05 ppm, respectively. However, the directions of displacement on the NMR frequency scale were opposite, indicating the difficulty of interpretation in terms of shielding and deshielding effects. In Fig. 7, no appreciable differences in the chemical shift displacement of the aromatic protons of DPP occurred above a molar ratio of 2. The 2,6 protons in the *ortho* position of the piperidine nitrogen progressively shifted downfield, whereas the 3,5 protons in the *meta* position remained unaffected. In the spectra of DPP, considerable line broadening and decreased intensities were observed with increasing amounts of 2-HP-β-CD resulting in complete destruction of the characteristic ABCD spin system and a single signal for the aromatic protons above a molar ratio of 1. The latter effect can be attributed to the disturbance of the interdependent ring current effects as well as to an enhancement of the transverse relaxation process according to the dependence of the transverse relaxation time on the full-width at half-maximum of the resonance signals: $\Delta\nu = (\pi T_2)^{-1}$. To summarize, the NMR data for DPP suggest the formation of 1:2 complexes with inclusion of the phenyl moiety and partial inclusion of the piperidine moiety.

In Fig. 7, the signal of the aliphatic protons of benzyl-CH_2 shows a strong upfield shift that can easily be explained by the inclusion of the 4-chlorobenzyl moiety by 2-HP-β-CD. In the free state, the aliphatic benzyl-CH_2 protons are deshielded owing to the proximity to the benzene ring. If this effect is altered, e.g. by complexation of the phenyl moiety, the aliphatic benzyl protons are shifted upfield. Furthermore, as a result of the deep insertion of the phenyl moiety into the cyclodextrin cavity, shielding effects of the cyclodextrin itself would affect the aliphatic benzyl-CH_2 protons. The displacement of aromatic protons of CBP, with respect to the centre $(\nu_1 + \nu_2)/2$ of the AA'BB' spin system is accompanied by a strong effect of 2-HP-β-CD on the spacings

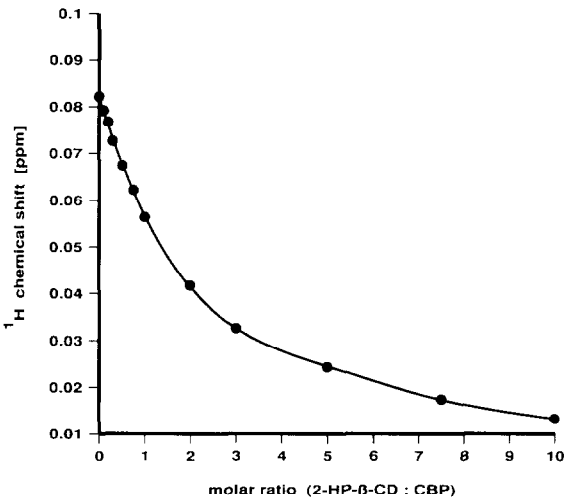


Fig. 10. Plot of the distance of the inner, intense lines ν_1 and ν_2 in the spin system AA'BB' of (−)-2-(4-chlorobenzyl)pyrrolidine vs the molar ratio of 2-HP-β-CD:CBP.

of the single lines from the centre without deviation of the spin system from strict centrosymmetry as shown in Fig. 9a–c. If ν_1 and ν_2 are the resonance frequencies of the inner lines in the AA'BB' spin system with $\nu_1 > \nu_2$, the spacing between ν_1 and ν_2 is given as $2[\nu_1-(\nu_1 + \nu_2)/2]$. In Fig. 10, the distance between the inner lines in the AA'BB' spin is plotted vs increasing molar ratio of 2-HP-β-CD:CBP in ppm. With increasing amounts of 2-HP-β-CD, the distance between ν_1 and ν_2 decreases and the curve declines asymptotically to zero. Depending on the resolution of the NMR spectrometer, this value was recorded at a molar ratio of 15 (Fig. 9c). Summarizing, the effect of 2-HP-β-CD on the ^{1}H chemical shifts of CBP clearly indicates the inclusion of the 4-chlorobenzyl moiety into the cyclodextrin cavity.

Conclusions

Partial structures of the DHPs that were presumed to interact with 2-HP-β-CD on the basis of phase solubility behaviour and stability studies have been used for ^{1}H-NMR investigations in order to elucidate the interaction mode with 2-HP-β-CD. In view of the structural requirements

of guest molecules, the structural conditions of DHP esters are different as compared with the respective amines, the interactions of the ester amine position with cyclodextrins being particularly inhibited. However, the conclusions concerning the interaction mode of the corresponding DHP esters with 2-HP-β-CD are valid bearing in mind that the same spin systems for the aromatic resonances discussed above occur in the ^{1}H-NMR spectra of the DHP esters, and considering the spatial exposure of the aromatic ester side chain. In this respect, it is concluded that the aromatic side chain of the DHP esters is mainly involved in the inclusion complexation of aromatic DHP esters with cyclodextrins. The application of 2-HP-β-CD for solubilization of drugs with low solubility does not necessarily lead to increased physical and chemical stability. The degree of solubilization of ionic active substances is decisively influenced by the pH. The course of the solubility/pH curve should therefore be established for new active substances. It is then necessary to check whether the pH range corresponding to high solubility of the complex can be reconciled with sufficient stability of the special active substance being investigated. When solubilizing new drug molecules with cyclodextrin derivatives, structure-interaction relationships determining the inclusion phenomena must be taken into consideration. In this respect, aromatic substitution of the potential guest molecule, especially with chlorobenzyl or linear monoaryl functions, leads to ideal structural requirements concerning inclusion complexation and therefore solubilization with 2-HP-β-CD.

The preferential use of 2-HP-β-CD as a parenteral drug carrier is mainly based on its high solubilizing capacity depending on structural requirements of the guest molecule together with the physiological tolerance of the excipient. The adsorption-diminishing effect of 2-HP-β-CD revealed in the present study represents further evidence in support of the argument for the usefulness of the CD derivative. This might become decisive in the case of highly biopotent and expensive drug molecules such as new peptide hormones that are expected to gain increasing importance in the near future.

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