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Complexation of Manidipine with Cyclodextrins and their Derivatives

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Abstract. Manidipine (MDP, (±)-2-[-(diphenylmethyl)-1-piperazinyl]ethylmethyl-1,4-dihydro-2,6-dimethyl-4(m-nitrophenyl)-3,5-pyridinedicarboxylate methyl-ester) is a poorly soluble (<1 μg/mL) long acting antihypertensive drug. Salt forming with citric or tartaric acid results in a 400 to 600 fold solubility enhancement, respectively, which can be further increased by an order of magnitude with cyclodextrins. Dimethyl-βCD alone results in a more than 8000 fold solubility enhancement. Besides the strongly enhanced solubility ^{1}HNMR spectroscopy also proves the inclusion-type interaction between Manidipine and cyclodextrins. From the attained 5-8 mg/mL solubility of the drug in water an improved bioavailability and pharmacokinetics is expected.

Key words: manidipine, cyclodextrin, multicomponent complex, citric acid, tartaric acid, hydroxy acids.

1. Introduction

Manidipine (MDP), (±)-2-[-(diphenylmethyl)-1-piperazinyl]ethylmethyl-1,4-dihydro-2,6-dimethyl-4(m-nitrophenyl)-3,5-pyridinedicarboxylate methyl ester (see Figure 1) is a dihydropyridine-type long acting antihypertensive calcium antagonist vasodilator drug [1]. Its extreme lipophilicity results in a number of undesirable physicochemical and biopharmaceutical properties such as a very poor aqueous solubility and a strong tendency to get adsorbed on glass and plastic surfaces. Its dihydrochloride salt (MDP.2HCl) is actually marketed as once-a-day 20 mg tablets for the treatment of different kinds of hypertension [2]. Like other dihydropyridine derivatives, MDP exhibits high clearance and first pass metabolism [3] and hence a low systemic bioavailability. After administration of rising doses (5 to 20 mg) to healthy volunteers, a non linear increase of plasma levels was observed. Since it is unlikely that the fraction of the dose absorbed from the gastro-intestinal tract does not increase with the dose, this effect is probably due to a concentration dependent first-pass hepatic elimination of the drug. Although the aqueous solubility of the

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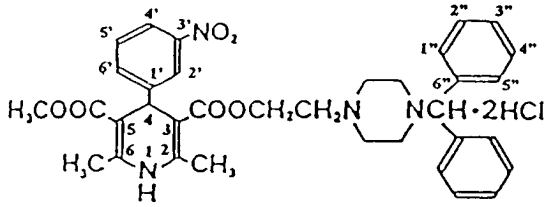

$C_{35}H_{38}N_4O_6.2HCl$

Mw: 683.63

Figure 1. Chemical structure of MDP.2HCl.

dihydrochloride salt is considerably higher than that of the corresponding base (0.38 mg/mL vs. less than 1 μg/mL), according to experiences with other similar drugs a further enhancement is necessary to attain a significant improvement in its absorption which in turn would lead to an increase in the MDP portal levels saturating partially the liver metabolism with a consequent improvement of the drug bioavailability.

Naturally occuring cyclodextrins (CDs) and 2-hydroxypropyl derivatives (HPβCD) are complexing agents which have been recently used to improve the aqueous solubility of other dihydropyridine derivatives [4]. In the present paper, the phase solubility diagrams in the presence of β- and γCD, DIMEB and RAMEB were obtained in order to test their efficacy in increasing the solubility of MDP base. Furthermore, as we have observed that the simultaneous salt formation and complexation in some cases dramatically increases the aqueous solubility of basic drugs [5], the phase-solubility studies were also extended to the MDP.2HCl : βCD system and to the MDP : βCD system in the presence of different organic hydroxy acids. Finally, ^{1}H NMR spectroscopy was also used to prove the interaction of MDP.2HCl with βCD.

2. Experimental

2.1. MATERIALS

Manidipine dihydrochloride (MDP.2HCl) was licensed from Takeda Ltd (Osaka, Japan). β-Cyclodextrin (βCD), γ-cyclodextrin (γCD) and randomly methylated-β-cyclodextrin of D.S. 1.8 (RAMEB) were obtained from Wacker Chemie (Munich, Germany), heptakis-(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) from Cyclolab (Budapest, Hungary).

All other chemicals used were of analytical grade.

2.2. EQUIPMENT AND EXPERIMENTAL CONDITIONS

UV spectrophotometry. The quantitative determination of dissolved MDP was carried out on a Hewlett-Packard 8452 diode-array spectrophotometer at the absorption maximum of the drug (λ = 352–357 nm).

The stock solutions were prepared by dissolving 20-100 mg of MDP.2HCl in 10 mL of ethanol. The working standard solutions for the calibration curve were obtained after dilution of the stock solutions with 0.2 N HCl in 50% ethanol (final concentration range: 0.02–0.1 mg/mL).

^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra were recorded in D_2O at pD= 2.8 at 200.13 MHz on a Bruker ACF 200 spectrometer. The assignment of the protons of MDP.2HCl was previously performed in $D_2O : CD_3CN$ 1 : 1 by 2D ^{1}H, ^{1}H correlation (COSY) and steady state nuclear Overhauser effect difference (NOEDIFF) experiments. All chemical shifts were determined relative to external sodium 3-trimethyl-silyl-propionate (TSP) at 0 ppm (accurate to = 0.001 ppm). The association constant K_{ass} was evaluated according to the following equation [6], by setting the concentration of MDP.2HCl at 0.5 mM and varying that of βCD between 1 and 10 mM:

$$\frac{\Delta}{\Delta_{oo}} = \frac{1}{2}\left(\frac{H_o}{G_o} - \frac{1}{K_{ass}[G_o]} + 1\right) - \left[\frac{1}{4}\left(\frac{[H_o]}{[G_o]} + \frac{1}{K_{ass}[G_o]} + 1\right)^2 - \frac{[H_o]}{[G_o]}\right]^{1/2}$$

where K_{ass} is the association constant; H_o and G_o are the total concentrations of host and guest; Δ is the chemical shift difference (for a given proton) between the free guest and the complex; Δ_{oo} is the limiting value of this change for infinite host concentration. The program SIGMAPLOT^R [7] was used to carry out the non-linear regression analysis of the experimental data. The non-linear curve fitting has a major advantage over the linear treatment since no boundary condition with respect to the ratio of the concentrations of the two binding partners has to be respected during the titration. [6, 8]

2.3. PREPARATION OF MANIDIPINE BASE

1.36 g (1.99 mM) of MDP.2HCl was dissolved in 50 mL of methanol. An equivalent amount of 1N sodium hydroxide solution was added to the clear yellow solution. Then MDP was precipitated by diluting the solution with about 300 mL of water. The precipitate was isolated by filtration after thorough washing with water and dried at room temperature. The MDP content of the product was 98.9 $\pm$ 0.1%.

2.4. PREPARATION OF THE MDP.2HCl : βCD COMPLEX OF 1 : 5 MOLAR RATIO BY FREEZE-DRYING

6.84 g (100 mM) of MDP.2HCl and 65.73 g (500 mM) of βCD (water content 13.7%) were added to 2000 mL of distilled water, then ultrasonicated for 2 min. The slightly yellow suspension was stirred for 30 min at 40 °C under light protected conditions. The slightly opalescent, yellow solution obtained was frozen and lyophilized. The yield was 66.1 g (MDP.2HCl content: 9.8 $\pm$ 0.1%; loss on drying: 6.7 $\pm$ 0.11%).

2.5. SOLUBILITY MEASUREMENTS

The solubility isotherms were recorded according to Higuchi et al. [9]. An excess amount (25–60 mg) of MDP base or MDP.2HCl was weighed into scaled test tubes. γCD, RAMEB and DIMEB were added as aqueous solutions while βCD was added as a suspension due to its low aqueous solubility.

The solubility isotherms of the MDP base:βCD system were also obtained in the presence of 20 μmol/mL of citric or L(+) tartaric acid. The samples were stirred at 25 $\pm$ 2 °C for 24 hours, then filtered. The pH of the filtered solutions of the base/hydroxyacid/CD combinations was 3.5, of the hydrochloride/CD combination 2.5.

The dissolved MDP concentration was measured by UV spectrophotometry after an appropriate dilution with 0.2 N HCl in 50% of ethanol.

The association constants in water and pH 1.4 HCl were calculated according to the equation:

$$K_{ass} = \frac{\text{tg } \alpha}{S_0(1 - \text{tg } \alpha)}$$

where S_o corresponds to the aqueous solubility of MDP.2HCl; tg α is the slope of the initial straight section of the curve.

3. Results and Discussion

3.1. SOLUBILITY STUDIES

The aqueous solubility of RAMEB and DIMEB is higher than that of naturally occurring CDs, so, in concentrated solution, a higher dissolved MDP concentration could be achieved. Figure 2 shows the phase solubility diagrams of MDP base in the presence of βCD, γCD, RAMEB and DIMEB. It is interesing that these CDs give different types of solubility diagrams [9]. With βCD, a typical B_S curve was obtained: a linear increase of MDP solubility was observed with increasing βCD concentration up to about 0.58 mg/mL at 34 mg/mL of βCD concentration, then the complex started to precipitate.

The solubility enhancement factor was 580 fold. In the presence of γCD, a typical A_L curve was obtained with a very low slope, indicating that it is practically ineffective in solubilizing MDP. Both DIMEB and RAMEB gave an A_P type. DIMEB resulted in a more than 8000 fold solubility enhancement (8 mg/mL of MDP in 15% solution).

Because simultaneous salt formation and complexation, in some cases, dramatically increases the aqueous solubility of basic drugs [5, 10–12], the effect of different counter ions was also investigated. Figure 3 shows the B_S type phase solubility diagrams of the MDP base: βCD system in the presence of citric and tartaric acid. In the presence of hydroxy acids, the solubility of plain MDP base significantly increases. Upon addition of 20 μmol/mL of citric or tartaric acid,

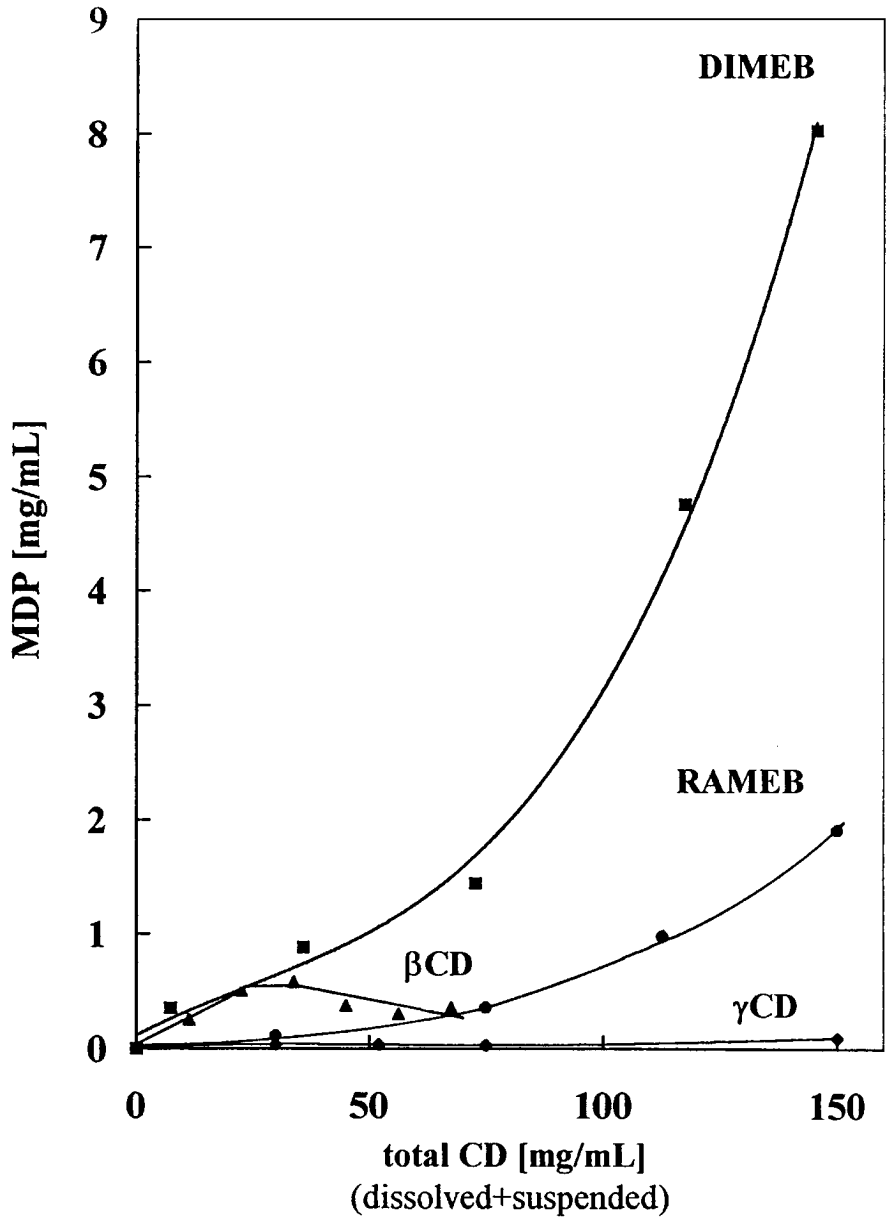

Figure 2. Solubility isotherms of Manidipine base with βCD, γCD, DIMEB and RAMEB.

0.48 or 0.64 mg/mL dissolved MDP concentrations were achieved corresponding to enhancement factors of 480 and 640, respectively. The simultaneous presence of βCD results in a synergetic effect; with tartaric acid, about 8.7 mg/mL MDP concentration can be reached at 45 mg/mL βCD concentration (approx. 1 : 1.4 : 2.7

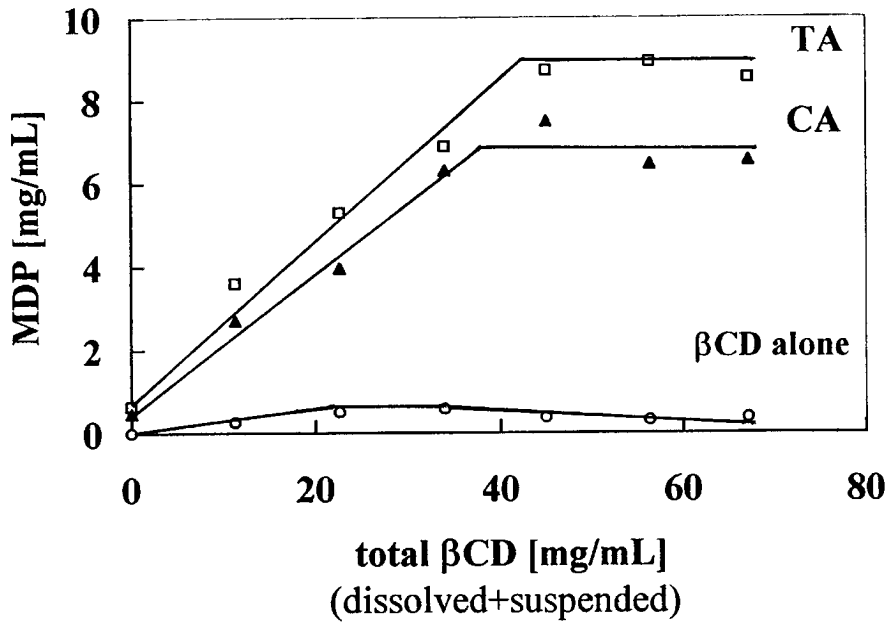

Figure 3. Solubility isotherms of Manidipine base in βCD system in the absence or presence of 20 μmol/mL hydroxy acids (TA = L(+) tartaric acid, CA = citric acid).

molar ratio), while, with citric acid, 7.5 mg/mL MDP can be dissolved at the same βCD concentration (approx. 1 : 1.6 : 3.2 molar ratio). These values correspond to 7500- and 8700-fold solubility enhancement factors, respectively. The results are summarized in Table I. In both cases, the solubility of βCD also increases by about 3-fold. Also the aqueous solubility of MDP.2HCl (0.38 mg/mL) increases with βCD (Figure 4): about 4.8 mg/mL (4.3 mg/mL MDP base) can be dissolved at 50 mg/mL βCD concentration. A similar solubility curve was obtained in HCl solution, pH 1.4. Assuming a 1 : 1 stoichiometry, the association constants of the MDP.2HCl : βCD complex, calculated from the initial straight line of the solubility curves, were 4.2×10^2 mol^{-1}L in water (pH 2.8) and 3.5×10^2 mol^{-1}L at pH 1.4. The βCD – present in the system – may be free, dissolved βCD, non-dissolved, dissolved complex and solid complex. No attempts have been made to determine the ratio of these components. The effect of hydroxy acids on the solubility enhancement of βCD has been studied, and has been published in preliminary form [13]. The detailed studies will be published soon elsewhere.

3.2. NMR STUDIES

The interaction of MDP.2HCl with βCD was investigated also by means of ^{1}H-NMR spectroscopy. Partial 200 MHz ^{1}H-NMR spectra of the plain compounds and a mixture of MDP.2HCl : βCD in 1:2 molar ratio are displayed in Figure 5. The

Table I. Solubility of MDP base in different media (determined by UV-photometry)

Medium	Solubility of MDP base (mg/mL)	Solubility enhancement factor
Water	∼0.001	1
20 μmol/mL HCl	0.33	330
20 μmol/mL TA	0.64	640
20 μmol/mL CA	0.48	480
30 μmol/mL βCD	0.58	580
40 μmol/mL βCD	0.37	370
40 μmol/mL βCD in 20 μmol/mL TA	8.73	8730
40 μmol/mL βCD in 20 μmol/mL CA	7.50	7500

TA= L(+) tartaric acid, CA= citric acid.

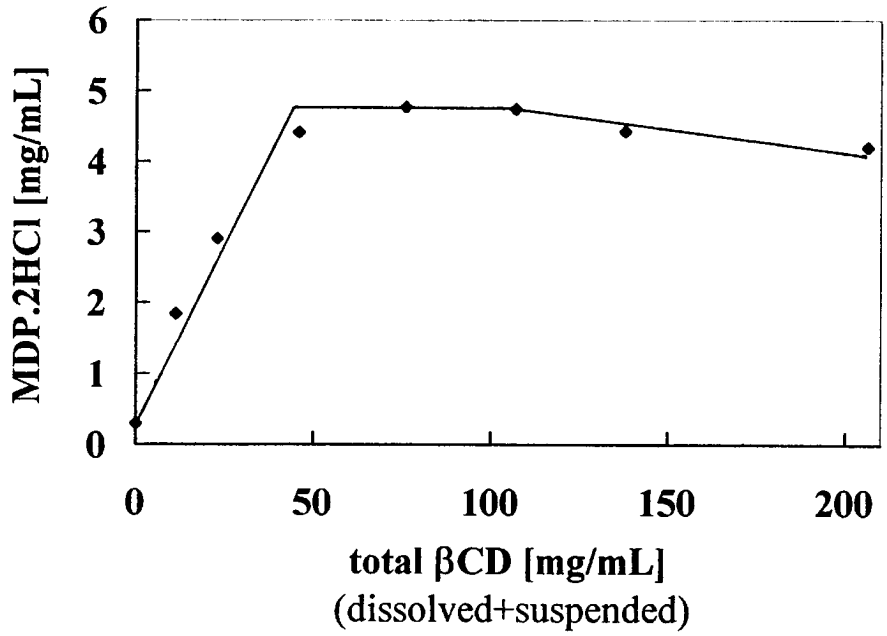

Figure 4. Solubility isotherm of MDP.2HCl with βCD.

H-3 and H-5 protons, which are located inside the cavity of βCD, shift much more than the other protons on the outer surface on addition of MDP.2HCl. This implies that the guest molecule is inserted into the cavity [14–16]. Also the protons of MDP.2HCl experience chemical shift changes upon addition of βCD. In particular the H-6' proton of the m-nitro-phenyl ring and of the ortho protons H-1$''$ of the geminal diphenyl group shift significantly upfield, while the H-4$'$ proton of the m-nitro-phenyl ring shift downfield. The directions of displacement on the MMR

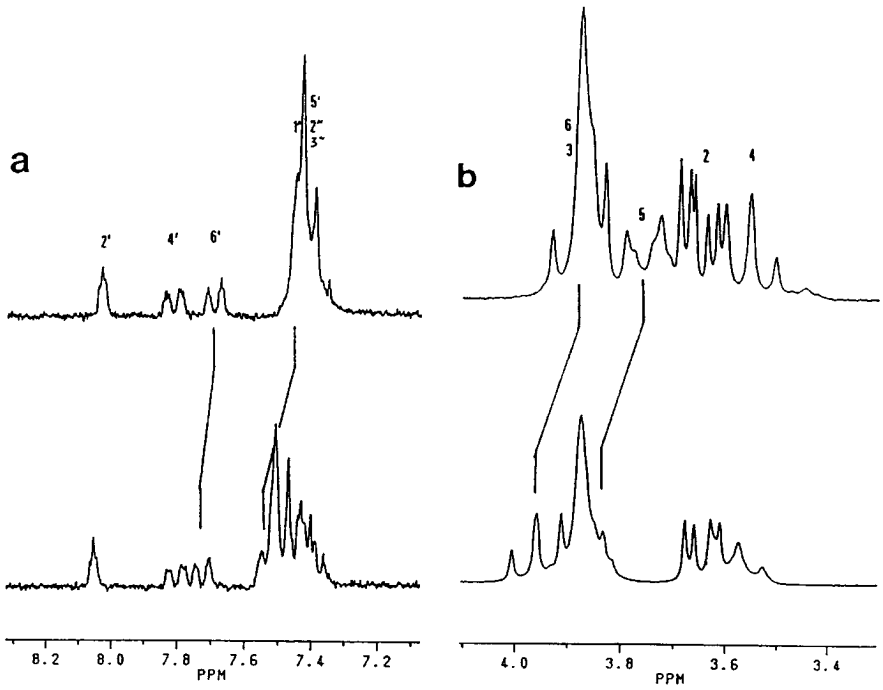

Figure 5. Partial 200 ^{1}H-NMR spectra of (a) aromatic region of the MDP.2HCl : βCD 1 : 2 mixture (upper trace) in comparison to MDP.2HCl (lower trace); (b) aliphatic region of the MDP.2HCl : βCD 1 : 2 mixture (upper trace) in comparison to βCD (lower trace).

frequency scale were different, indicating the difficulty of interpretation in terms of shielding and deshielding effects. However, it seems likely that two types of isomeric complexes are present, one with the m-nitro-phenyl ring included, and the other with one of the aryl groups of the diphenylmethane moiety included. A 'bimodal' inclusion process has been already reported for other systems [17, 18] and it is consistent with that reported for similar dihydropyridine derivatives. [4] The proposed molecular model of the complex in solution could be refined by other NMR experiments (e.g. nuclear Overhauser effects). The association constant was determined by using the equation reported in the Experimental as a function of the molar ratio. The change in the chemical shift of different protons of MDP.2HCl was monitored and the H-4′ proton belonging to the m-nitro-phenyl ring experienced the largest shift upon increasing the βCD amount. The curve of the best fit is plotted in Figure 6. The value of the association constant obtained from this procedure turned out to be 3.4×10^2 M^{-1} L (pD 2.8), in close analogy with the results of the phase-solubility analysis.

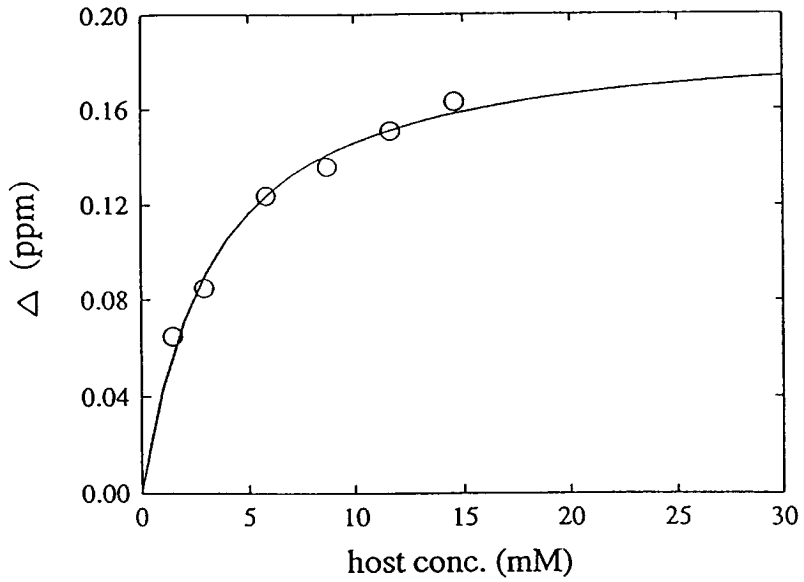

Figure 6. Non linear curve fitting of the chemical shift change of the H-4′ proton of MDP.2HCl (o) during titration with βCD.

3.3. COMPLEX PREPARATION

A MDP.2HCl : βCD complex (1 : 5 molar ratio) was prepared by freeze-drying according to the method described in Section 2.4. 200 mg of this complex can be dissolved in 4 mL of water resulting in a slightly opalescent solution of 4.8 mg/mL MDP.2HCl concentration. The pH of the solution was 2.5. The complex was used to prepare tablets (200 mg, MDP.2HCl content approx. 10%) for preliminary pharmacokinetics studies in animals.

4. Conclusions

The different solubility enhancing effects of the various hydroxyacids (counterions) can not be explained by pH-difference, because at identical pH rather different solubilities can be attained with the various acids.

βCD alone, without hydroxyacids, resulted only in an insignificant solubility enhancement (Figure 3), while in a bi-component system DIMEB resulted in the highest – 8000 fold – solubility enhancement. In the multicomponent system drug/hydroxyacid/CD the chemically non-modified βCD attained a similar performance. This is very important, because the oral administration of βCD – in reasonable doses – is devoid of any toxic side-effects, it is registered already in various Pharmacopoeia. Consequently the approval for human application of a MDP/βCD/hydroxyacid complex will not be refused by the competent health authorities due to lack of adequate toxicological documentation. Such documenta-

tion is presently not yet available for DIMEB, therefore despite its excellent drug solubilizing properties, its pharmaceutical application in oral formulations is not expected within the coming years.

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