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Report

Effect of Cyclodextrin Derivatives on Indomethacin Stability in Aqueous Solution

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The effect of various cyclodextrins (CD) and cyclodextrin derivatives on indomethacin stability in phosphate buffer, pH 7.4, was investigated. The influence of CD-ring size, type of substituent, degree of substitution, substitution pattern, and influence of CD concentration were monitored. The indomethacin complex in solution was studied by ^{1}H-NMR spectroscopy to develop a molecular inclusion model. The most favorable ring size for the stabilization of indomethacin was the β-CD. The β-CD derivatives inhibited the hydrolysis of indomethacin more effectively than the parent CD. Among the studied CD derivatives, those with lipophilic substituents, such as ethyl or methyl, were superior to those with hydrophilic substitutents. The more hydroxyl groups of the glucose moiety are substituted, the better is the stabilizing effect. Further, the *p*-chlorobenzoic part of the indomethacin molecule is included in the CD channel.

KEY WORDS: cyclodextrines; cyclodextrin derivatives; indomethacin; stability; ^{1}H-NMR.

INTRODUCTION

Indomethacin is a nonsteroidal antiinflammatory drug which inhibits prostaglandin biosynthesis by inhibition of cyclooxygenase and therefore has analgesic, antipyretic, and antiphlogistic actions (1). It is a poorly soluble drug with a saturation solubility of 0.4 mg/ml in water (2). In aqueous solutions, it undergoes pH-dependent hydrolysis to 5-methoxy-2-methyl-indol-3-acetic acid and *p*-chlorobenzoic acid. The former can be further broken down to 5-methoxy-2,3-dimethylindol (3). Attempts have been made to stabilize indomethacin solutions by using cosolvents that reduce the dielectric constant of the mixture or by adding ionic or nonionic tensides which incorporate the indomethacin into micelles (4–6).

Hamada *et al.* investigated the interaction of indomethacin with α- and β-cyclodextrin (CD) and found that β-CD stabilized aqueous solutions, whereas α-CD tended to produce the opposite effect (7). Müller and Brauns found that indomethacin can be stabilized with hydroxyethyl-β-CD, but the solutions investigated were not physically stable during the investigated period of 6 months, due to microbial contamination (8).

Cyclodextrins have the remarkable property of forming molecular inclusion complexes with many drugs. These complexes have different properties than the free drugs. This is used in the field of pharmaceutics to improve aqueous solubility, chemical stability, and bioavailability of drug molecules (9,10). Besides the unsubstituted CD with 6, 7, and 8 glucose molecules (α-, β-, and γ-CD), other cyclodextrin derivatives are also used (11). In addition to increased solubility, the latter have the advantage of altering the phase solubility behavior in favor of solution isotherms of the A type (12). The alkylated and hydroxyalkylated cyclodextrin derivatives therefore appear to be more suitable for the production of solutions of poorly soluble drugs than nonsubstituted parent CD.

The aim of the study reported here was to investigate the effects of different CD on the stability of aqueous indomethacin solutions. The effects of the CD ring size, type of substituent, degree and pattern of substitution, and CD concentration and temperature were investigated.

NMR investigations were carried out to study the observed guest–cyclodextrin molecule interactions. An attempt was made, with the aid of NMR data, to develop a molecular model to define the structures of the molecule that are involved in the inclusion, and to calculate interactions energies.

MATERIALS AND METHODS

Materials

CD derivatives were kindly donated by Kalle AG (Wiesbaden, FRG), Wacker Chemie GmbH (Munich, FRG), and Chinoin (Budapest, Hungary) and were used as received. Except for the methyl derivatives, which were substituted mainly in positions 2 and 6 of the glucose molecule, all other derivatives were mixtures of randomly alkylated compounds. The determination of the molecular weight was cal-

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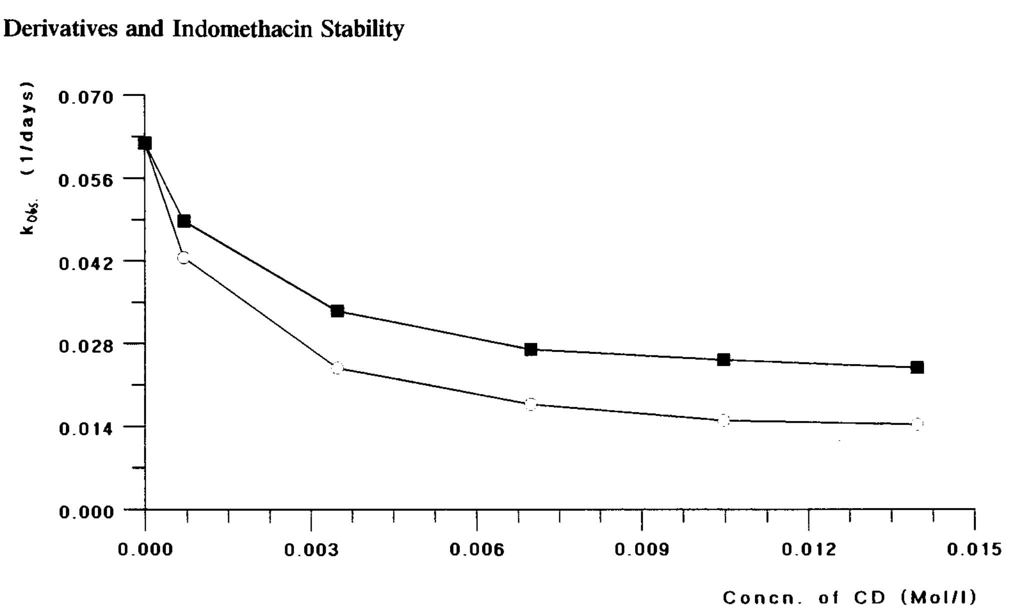


Fig. 1. Observed rate constants for the degradation of indomethacin as a function of CD concentration in phosphate buffer, pH 7.4, at 41°C. (○) Indomethacin + hydroxypropyl-β-CD MS 0.39; (■) indomethacin + β-CD.

culated via the molar degree of substitution. α-CD was purchased from Lehmann and Voss (Hamburg, FRG), β-CD from Chinoin (Budapest, Hungary), and γ-CD from Nihon Shokukin Kako Co. Ltd. (Tokyo). Indomethacin (Helm AG, Hamburg, FRG) was used in pharmaceutical-grade DAB 9 without further purification. All other reagents and solvents were of analytical grade, and double-distilled water was used throughout the study.

HPLC Method

The assay for indomethacin preparations was carried out with a stability-indicating HPLC method. The chromatograph consisted of a Gynkotek constant-flow pump 300B, a variable-wavelength detector Gynkotek SP-4 (Gynkotek, Munich, FRG), and a Shimadzu integrator C-R18 (Shimadzu, Kyoto, Japan). Injections were made with a Kontron autosampler MSI 660 (Kontron, Zurich, Switzerland) equipped with a 100-μl Rheodyne injection value (Rheodyne, Cotati, USA).

The chromatograph was operated at a flow of 2.0 ml/min and the eluent was monitored spectrophotometrically at 265 nm. A column (5-mm i.d. × 25 cm) containing 5-μm Shandon ODS Hypersil RP 18 (Shandon, Runcorn, UK) was used for separation. The mobile phase consisted of acetonitrile/tetrahydrofuran/acetic acid/water (50:5:0.2:44.8). The chromatographic analyses were carried out at 20°C. Peak areas of the intact indomethacin were measured and compared with areas of known amounts of external standards.

Preparation of Solutions

To investigate the stability of various cyclodextrins and the effect of temperature, 0.5 mg/ml indomethacin (1.4×10^{-3} *M*) dissolved in phosphate buffer, pH 7.4, Eur. P. was mixed with 10 times excess of the respective CD (1.4×10^{-2} *M*).

In the investigations of different CD concentrations, the indomethacin solutions were prepared in the same buffer to give 0.25 mg/ml (7×10^{-4} *M*). β-CD or hydroxypropyl-β-CD (MS 0.39) was added to these solutions at concentrations of 7×10^{-4} to 1.4×10^{-2} *M*.

All these solutions were filtered through a 0.22-μm membrane filter into presterilized 2-ml glass ampoules under aseptic conditions. The ampoules were stored at 41°C in the dark. The solutions for the Arrhenius plot were stressed from 31 to 71°C. The pH of the sample solutions was ascertained to be identical before and after the reaction (pH meter pMX 2000, WTW, Weinheim, FRG).

NMR Studies

^{1}H-NMR measurements were performed using a 360-MHz NMR spectrometer AM 360L (Karlsruhe, FRG). The measurements were performed in 0.03 m*M* phosphate buffer, pH 7.4, at room temperature with t-butanol as internal standard because no interactions with CD have been observed (13). The ^{1}H-proton chemical shifts of the various spin systems of indomethacin were determined in the absence and in the presence of increasing concentrations of hydroxypropyl-β-CD MS 0.39 and hydroxypropyl-y-CD MS 0.46. (CD concentrations were 2.35×10^{-4} to 2.54×10^{-3} *M*; the indomethacin concentrations were 9.4×10^{-4} to 8.4×10^{-4} *M*).

Table I. Rate and Stability Constants of Indomethacin–CD Complexed at 41°C in Phosphate Buffer, pH 7.4

	k_1 (d^{-1})	k_2 (d^{-1})	K_A (mol^{-1})	k_1/k_2
Indomethacin	0.0618	—	—	—
Indomethacin + β-CD	—	0.0194	619	3.2
Indomethacin + HP-β-CD MS 0.39	—	0.0096	774	6.5

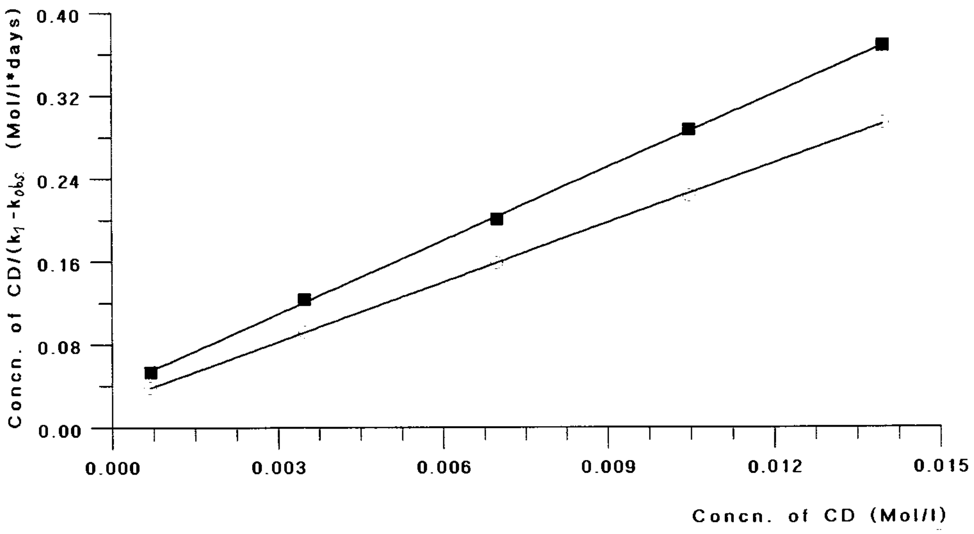


Fig. 2. Determination of k_1 and k_2 for indomethacin–CD complexes by plotting kinetic data (Fig. 1) according to Eq. (1). (○) Indomethacin + hydroxypropyl-β-CD MS 0.39; (■) indomethacin + β-CD.

Computer Modeling

The X-ray coordinates of the β-CD–benzocaine complex were used for modeling of the inclusion complexes (14). Bond lengths and angles of the compound studied were optimized using the MMX program. Using these start conformations, a conformational search was performed using CAMSEQ, and from these results the most frequent populations of minimal energy were selected by the FAMILY program (15) and docked into the CD. Docking and calculating of binding energies were performed by the CHEMP-CCS program (16).

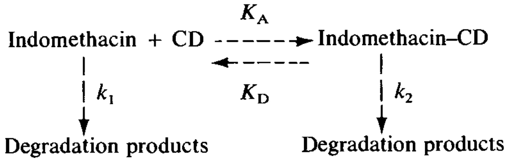
RESULTS AND DISCUSSION

Effects of Concentration

Figure 1 shows the influence of β-CD and hydroxypropyl-β-CD MS 0.39 concentrations on the degradation of indomethacin. The pseudo first-order degradation rates decreased hyperbolically with increasing CD concentration, showing characteristic saturation kinetics. Figure 1 shows that hydroxypropyl-β-CD (HP-β-CD) is a better stabilizing agent than the parent β-CD at identical CD concentrations. The dependence of the observed rate constants k_{obs} of the hydrolytic reaction on the CD concentration was quantitatively treated by Eq. (1) (17).

$$\frac{[CD_t]}{(k_1 - k_{obs})} = \frac{[CD_t]}{(k_1 - k_2)} + \frac{1}{K_A \times (k_1 - k_2)} \qquad (1)$$

Following Scheme I on the basis of a 1:1 complexation, where K_A is the stability constant of the complex, k_1 is the pseudo first-order rate constant of uncomplexed indomethacin, k_2 the pseudo first-order rate constant of the inclusion complex, and CD_t the total concentration of CD, Lineweaver–Burk plots are obtained by plotting $[CD_t]/(k_1 - k_{obs})$ vs the CD concentration. The plots from Eq. (1), based on the data for Fig. 1, gave a straight line with a correlation coefficient >0.999 (Fig. 2).



Scheme I

Both β- and hydroxypropyl-β-CD decelerate the hydrolysis of indomethacin. β-CD decelerates the degradation rate by a factor of 3, and HP-β-CD by a factor of 6.

Apparently, the *p*-chlorobenzoic acid moiety of indomethacin is sensitive to hydrolysis and is better protected from the attack of the hydroxyl ions by the hydroxypropyl groups of the β-CD ring than by enclosure in the parent β-CD.

Effect of Temperature

The effect of temperature on the hydrolysis rate of indomethacin was investigated to gain further insight into the decomposition mechanism. Figure 3 shows the Arrhenius plots of the degradation rates in the absence and presence of β-CD and HP-β-CD MS 0.39 over the temperature range of 31 to 71°C. The activation energy, the frequency factor, and the value of 10% degenerated indomethacin ($t_{90\%}$) at 20°C were calculated using the Arrhenius equation and are summarized in Table II.

Effect of Ring Size

Figure 4 shows the degradation of indomethacin in the absence and presence of α-, β-, and γ-CD. Both β- and γ-CD inhibit the hydrolysis, while the hydrolysis of indomethacin was accelerated with α-CD. The 50% degradation value at 40°C is 10.7 days without any CD, 28.3 and 16.5 days with β- and γ-CD, and 6.6 days for α-CD, respectively. The most

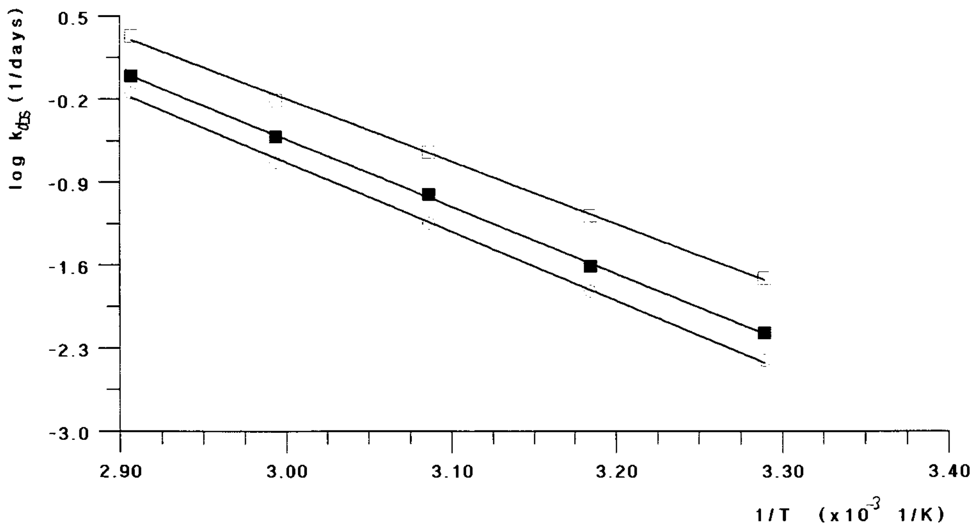


Fig. 3. Arrhenius plots of the degradation of indomethacin in the absence and in the presence of CDs in phosphate buffer pH 7.4. (□) Indomethacin alone; (○) indomethacin + HP-β-CD MS 0.39; (■) indomethacin + β-CD.

favorable ring size for the formation of the inclusion complex and for the stabilization of indomethacin is therefore the CD with seven glucose units.

Effect of Type of Substituent

The influence of various CD derivatives with almost identical degrees of substitution on indomethacin degradation have been studied. Table III shows that the most favorable substituent for improving the chemical stability of indomethacin is the ethyl group. It is evident, however, that all CD derivatives of either γ- or β-CD derivatives have a greater influence on hydrolysis than the parent CD. There are only minor differences in the stabilizing effect of the more hydrophilic substituents such as hydroxypropyl, hydroxyethyl, and hydroxybutyl of the β- and γ-CD derivatives. The trimethylammoniumhydroxypropyl-β-CD derivative has less effect on indomethacin degradation than the parent β-CD because of its ionic state. The more lipohilic substituents, such as methyl or ethyl, are the most effective stabilizing agents among the CD derivatives studied. It appears that they can better protect the hydrolytically sensitive *p*-chlorobenzoic acid part of the indomethacin molecule from hydrogenium or oxygenium attack.

Table II. Activation Parameters and $t_{90\%}$ Value at 21°C for the Degradation of Indomethacin in the Absence and in the Presence of CDs in Phosphate Buffer, pH 7.4

	E_A (kJ/mol)	A (days^{-1})	$t_{90\%}$ (days)
		($\times 10^{15}$)	
Indomethacin alone	97.6	1.2	19
Indomethacin + β-CD	105.9	11.2	61
Indomethacin + HP-β-CD	107.3	11.9	103

Effect of Degree of Substitution

Table IV shows the influence of the degree of substitution of hydroxyethyl- and hydroxypropyl-β-CD on indomethacin degradation. The data indicate that a higher degree of substitution of the CD gives a better stabilizing effect on the indomethacin degradation. The more the hydroxyl groups of the glucose molecules are substituted, the better is the deshielding effect of the CD for the indomethacin molecule because of the longer interior of the CD cavity.

Effect of Substitution Pattern

Table V compares the effect of dimethyl-β-CD (DIMEB), the methyl derivative stubstituted mainly in positions 2 and 6, with a randomly alkylated methyl compound. Both are of an almost identical degree of substitution (MS 2.0 and MS 1.79). It can be concluded, with a half-life of 117.5 days at 41°C compared with the half-life of the randomly substituted CD of 77.0 days, that DIMEB is the more powerful tool for stabilizing indomethacin solutions with CD. The marked influence of the substitution pattern was also observed in the complexing ability of the CD derivatives (8). Obviously the distribution of the substituents in the CD molecule is very important. Pitha *et al.* (18) suggest three principal structural types of hydroxylalkyl-CD. The substituents may be distributed equally between the glucoses of CD, clustered on one or two, glucose molecules, or form an oligomeric chain (18). With an optimum protection of the indomethacin molecule in the CD interior space, the even distribution of the substituents on the CD ring is probably advantageous. During synthesis of the randomly alkylated methyl-β-CD, clusters have apparently formed which are disadvantageous for the stabilization of the indomethacin, as a facilitated attack of H^+ and OH^- ions can take place at the free unsubstituted sites of the CD molecule, as with the parent CD.

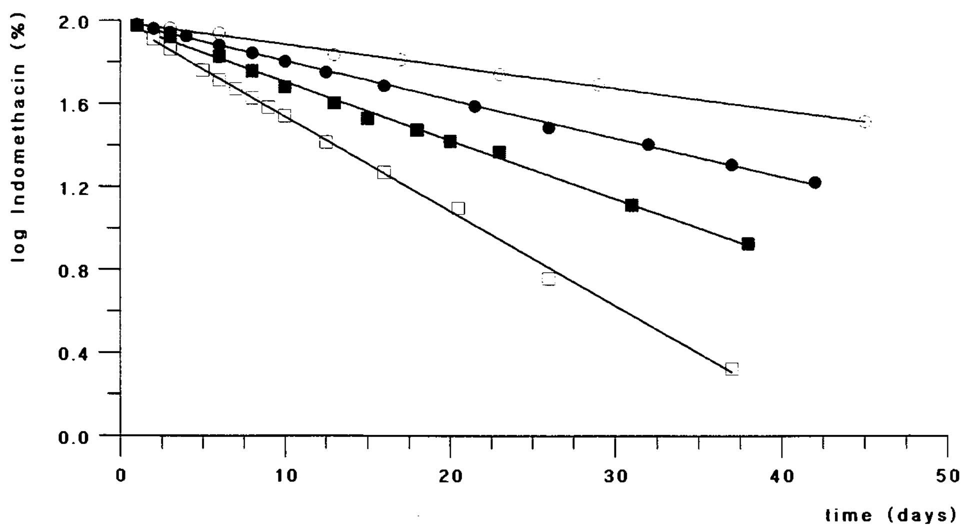


Fig. 4. Degradation of indomethacin in the presence of CDs with various ring sizes in phosphate buffer pH 7.4 at 41°C. (■) Indomethacin alone; (○) indomethacin + β-CD; (□) indomethacin + α-CD; (●) indomethacin + γ-CD.

As can be seen from Table V, the formation of mixed ethers has no synergistic effect for the stabilization of aqueous indomethacin solutions, Thus, for example, the $t_{50\%}$ value for methylhydroxyethyl-β-CD DS 1.51 MS 0.22 is 66.6 days. For a DC derivative that is substituted only with methyl groups, the $t_{50\%}$ at a substitution rate of 1.79 is 77.0 days, and for a CD derivative that has been substituted only with hydroxyethyl groups, it is 33.5 days at an MS of 0.50.

NMR Studies

^{1}H-NMR technique was employed to examine the inclusion mode in aqueous solution. Figures 5 and 6 show the change in chemical shift of different indomethacin protons as a function of increasing concentrations of hydroxypropyl-β-CD MS 0.39 and hydroxypropyl-γ-CD MS 0.46 at constant drug concentrations. The ^{1}H-NMR signals of the different drug protons indicate large differences in the change of chemical shift on complex formation. Especially, protons c and b are clearly shifted downward as a function of the complex concentrations (accuracy, 0.001 ppm). This can be explained by the fact that, during complex formation, the *p*-chlorobenzoic acid part is taken into the β-CD ring and that, as a result, the substituted indol moiety, which is spatially too large for inclusion, butts onto the margin of the CD ring. This produces an interference in the magnetic field of the involved protons and provides an explanation for the especially marked shifts in protons b, c, and j.

Figure 5 also shows that the shifts, i.e., the interactions of indomethacin with the interior space of the CD, are more marked with hydroxypropyl-β-CD than with the one-glucose unit-larger hydroxypropyl-γ-CD. Apparently, the indomethacin molecule fits into the spatial dimensions considerably better in the β-CD ring, while the γ-CD derivative is too large for it. Indomethacin fits tightly into β-CD channels and more loosely into the larger interior space of γ-CD channels. These investigations explain the findings made during the stability tests that the β-CD derivatives stabilize the indomethacin considerably better than the corresponding γ-CD derivatives. Assignment of the individual CD protons is un-

Table III. Influence of Cyclodextrins with Various Types of Substituents with Nearly Identical Degrees of Substitution on Indomethacin Degradation in Phosphate Buffer, pH 7.4; t = 41°C

CD	R	MS	k_{obs} $\times 10^{-2}$ (days^{-1})	$t_{50\%}$ (days)
—	—	—	6.50	10.7
β	—	—	2.45	28.3
β	$-CH_2-CH_3$	0.60	1.11	62.4
β	$-CH_3$	0.40	1.52	45.6
β	$-CH_2-CHOH-CH_3$	0.48	1.63	42.5
β	$-CH_2-CH_2-OH$	0.50	2.07	33.5
β	$-CH_2-CHOH-CH_2-CH_3$	0.58	1.80	38.5
β	$-CH_2-CHOH-CH_2$ $-N(CH_3)_3{}^+Cl^-$	0.51	3.59	19.3
γ	—	—	4.21	16.5
γ	$-CH_3$	0.40	3.14	22.1
γ	$-CH_2-CHOH-CH_3$	0.46	3.43	20.2
γ	$-CH_2-CH_2-OH$	0.50	3.91	17.7

Table IV. Influence of Hydroxyethyl- and Hydroxypropyl-β-CDs with Various Degrees of Substitution on Indomethacin Degradation in Phosphate Buffer, pH 7.4; t = 41°C

CD	R	MS	k_{obs} $\times 10^{-2}$ (days^{-1})	$t_{50\%}$ (days)
β	$-CH_2-CH_2-OH$	0.50	2.07	33.5
β	"	0.60	1.92	36.1
β	"	1.0	1.76	39.4
β	"	1.60	1.42	48.8
β	$-CH_2-CHOH-CH_3$	0.48	1.63	42.5
β	"	0.60	1.52	45.6
β	"	0.90	1.27	54.6
β	"	1.12	1.03	67.3

Table V. Influence of Cyclodextrins with Various Substitution Pattern on Indomethacin Degradation in Phosphate Buffer, pH 7.4; t = 41°C

CD	R	DS MS	k_{obs} $\times 10^{-2}$ (days^{-1})	$t_{50\%}$ (days)
β	$-CH_3$[a]	2.0	0.59	117.5
β	$-CH_3$	1.79	0.90	77.0
β	$-CH_3$	1.51	1.04	66.6
	$-CH_2-CH_2-OH$	0.22		
β	$-CH_2-CH_2-OH$	0.50	2.07	33.5
β	$-CH_3$	0.85	1.13	61.3
	$-CH_2-CHOH-CH_3$	0.43		
β	$-CH_3$	1.0	1.16	59.8
β	$-CH_2-CHOH-CH_3$	0.48	1.63	42.5

[a] Methyl-β-CD, predominantly substituted in positions 2 and 6 (DIMEB).

fortunately not possible because of the complex composition of the CD derivatives.

Computer Modeling

For modeling of the inclusion complex between indomethacin and β-CD, the selected low-energy conformations were docked into the CD. The distances between the CD atoms and those parts of the drugs which were influenced by complexation were monitored. The conformations that were in agreement with the NMR data showed no significant difference in calculated binding energy. Figure 7 shows the modeling of the indomethacin molecule docked into the polymer ring structure of β-CD. β-CD X-ray coordinates were selected for this modeling because CD derivatives are amorphous and thus no crystal data are available. A similar inclusion complex between indomethacin and HP-β-CD is assumed. Figure 7 demonstrates the inclusion of the p-chlorobenzoic part of the indomethacin molecule into the CD channel. The CD ring is cut for better illustration and shows the CD ring with the glucose molecules in the back. The indol part of the indomethacin proved to be too large for inclusion.

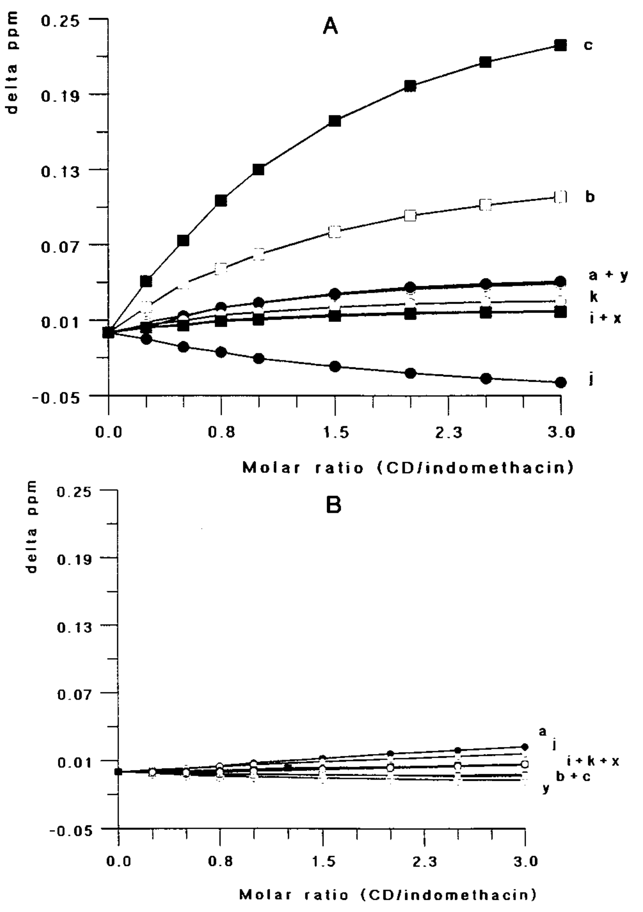


Fig. 5. (A) ^{1}H-chemical shift displacements of indomethacin protons upon the addition of HP-β-CD MS 0.39. (B) ^{1}H-chemical shift displacements of indomethacin protons upon the addition of HP-γ-CD MS 0.46.

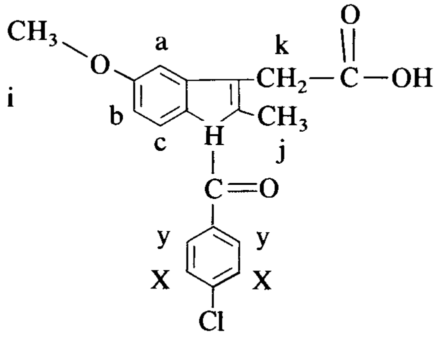


Fig. 6. Chemical structure of indomethacin.

CONCLUSION

An essential advantage of the CD derivatives compared with the parent CD in stabilizing drugs is their excellent water solubility. The low water solubility of β-CD [1.8%

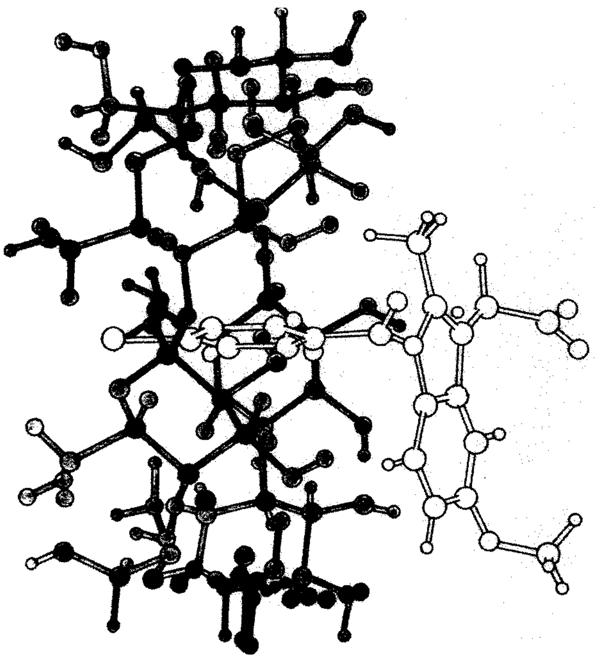


Fig. 7. Computer model of the inclusion complex between indomethacin and β-CD.

(m/v)] is often insufficient to stabilize drugs at therapeutic doses because the drug:CD ratio has an important influence on the stability effects that can be achieved.

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