NMR Spectroscopy of Inclusion Complex of Sodium Diclofenac with β-Cyclodextrin in Aqueous Solution

S. ASTILEAN,[1] CORINA IONESCU,[2] GH. CRISTEA,[1] S. I. FARCAS,[3] I. BRATU,[3] R. VITOC[1]

[1] Babes-Bolyai University, Department of Physics, 3400 Cluj-Napoca, Romania

[2] University of Medicine and Pharmacy, Department of Pharmacy, Biochemistry and Clinical Lab., 3400 Cluj-Napoca, Romania

[3] Institute of Isotopic and Molecular Technology, 3400 Cluj-Napoca 5, Romania

Received 16 October 1996; revised 21 October 1996; accepted 25 November 1996

ABSTRACT: The interaction between diclofenac (sodium salt of 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid) and β-cyclodextrin in aqueous solution has been investigated by ^{1}H-NMR spectroscopic technique. The technique is based on the shielding of the β-cyclodextrin and drug protons. The spectra showed upfield shifts of the β-cyclodextrin protons in the presence of diclofenac, and the diclofenac protons also shifted upfield in the presence of β-cyclodextrin. The changes in chemical shifts of suitable guest–host protons are consistent with the formation of an inclusion complex diclofenac/β-cyclodextrin. © 1997 John Wiley & Sons, Inc. Biospect **3:** 233–239, 1997

Keywords: cyclodextrins; diclofenac; inclusion complexes; chemical shifts; ^{1}H-NMR spectroscopy

INTRODUCTION

In recent years, the "microencapsulation" of drug molecules in the cavities of cyclodextrins (CD) has been extensively used in the pharmaceutical industry to produce more stable drug preparations with improved bioavailability.[1–5] Investigation of the driving forces of complexation and the structure of inclusion complexes appears to be of fundamental importance for understanding the biopharmaceutical functions of drug molecule.

CDs are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD), and eight (γ-CD) glucopyranose units that can be represented as a truncated cone structure. The molecular structure of β-CD and its schematic representation are presented in Figure 1.

The secondary hydroxyl groups are located on the wider rim of the torus, on the C2 and C3 atoms, whereas the primary hydroxyl groups are positioned on the opposite rim, on C6 atoms (the narrower rim). The C3-hydroxyl hydrogen is hydrogen bonded to the C2-hydroxyl oxygen of an adjacent glucopyranose ring, and this intramolecular hydrogen bonding accounts, in part, for the conical shape of cyclodextrins. The CH groups carrying the protons H-1, H-2, and H-4 are located on the exterior surface of the torus; consequently, the external face of CDs is hydrophilic. The interior of the torus, lined by two rings of CH groups (H-3 and H-5) and by glucosidic oxygen (O4), offers an environment of a much lower polarity than is present in water, so it can be considered as a hydrophobic cavity. The inner cavity diameters of α-, β-, and γ-CDs are about 5.7, 7.8, and 9.5 Å, respectively, and the depth of the cavity is 7.8 Å.[1]

Presented in part at the European Conference of the Spectroscopy of Biomolecules, ECSBM'95, Villeneuve d'Ascq, 3–8 September 1995, France.

Correspondence to: S. Astilean.

 CCC 1075-4261/97/030233-07

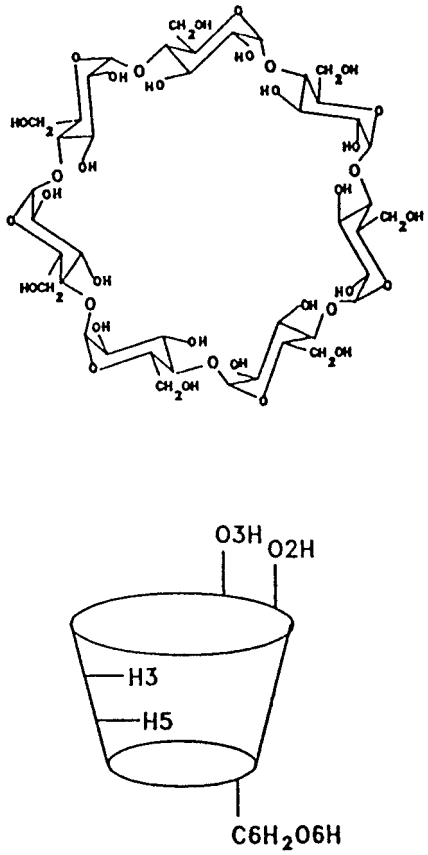

Figure 1. Molecular structure and schematic representation of β-CD.

The above characteristics of CDs allow various types of drugs to be encased in the cavity, forming noncovalent inclusion complexes either in the solid phase or in aqueous solutions, leading to widespread applications in the pharmaceutical field, analytical chemistry, chemical synthesis, and catalysis.[1–12]

Sodium diclofenac (DCF) is the salt of 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid. DCF is a potent nonsteroidal anti-inflammatory drug (NSAID) from the group of the arylalcanoic acid derivatives with large therapeutic applicability in the symptomatic standard treatment of rheumatic affections.[13–15] The structural elements of the DCF molecule are indicated in Figure 2. They include a phenylacetic group, a secondary amino group, and a dichlorophenyl ring (the two ortho positions are occupied by chlorine atoms). The chlorine atoms cause maximal twisting of phenyl rings. The X-ray analysis of the molecular structure showed the value of the torsion angle between the two aromatic rings of 58–69° and the existence of an intramolecular hydrogen bond between the carboxyl oxygen and the amino hydrogen.[13,14]

The anti-inflammatory activity of DCF and most of its other pharmacological effects are thought to be related with the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory process. DCF is a potent inhibitor of cyclooxygenase, thereby decreasing the synthesis of prostaglandin, prostacyclin, and thromoxane products. The high activity of DCF as an inhibitor of cyclooxygenase at the enzyme level was explained by lipophilicity and steric properties that governs its fit to the receptor. Like other NSAID, DCF is highly enzyme and protein bonded. From this point of view, the complexation of DCF with CDs can play the role of model for the "receptor site" of cyclooxygenase, the complexation mimicking the substrate-specific interactions, and lightening the arrangement of the aromatic ring within the receptor cavity. Moreover, it is also possible that in some therapeutic formulae the association of DCF with CDs enables the optimization of physicochemical and pharmaceutical properties of DCF, with the purpose of obtaining more stable orally administrable pharmaceutical preparations, with improved therapeutic effects. Consequently, the

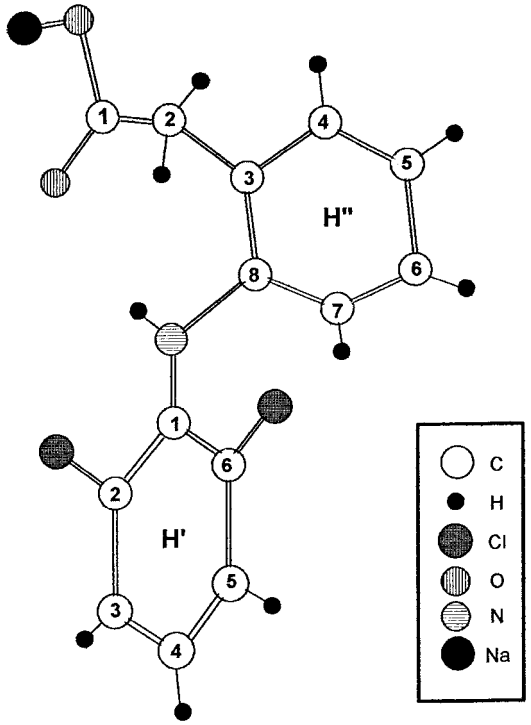

Figure 2. Molecular structure of sodium diclofenac (DCF).

understanding of the complexation between DCF and CDs appears to be of great importance.

The purpose of the present work is to examine the interaction of DCF with β-CD in D_2O solution via ^{1}H-NMR spectroscopy. The NMR method for investigation of host–guest compounds relies on the analysis of chemical shift variations of protons directly involved in the interaction. For example, in the case of aromatic guests, H3 and H5 of glucose units, namely the protons inside the cavity, undergo high-field shifts due to anisotropic shielding by the aromatic ring of the guest, whereas the protons of the guest that interact with the nonpolar cavity of CDs experince low-field shifts.[4,6–11]

It is expected that the monitoring of chemical shift variations will allow us to prove the complexation between DCF and β-CD and to discriminate between the possible ways of complexation.

EXPERIMENTAL

Materials

β-CD (99.9%) was kindly supplied by Merck; DCF (99.4%) by "Terapia" Cluj-Napoca, Romania; and D_2O (99.88%) by Institute for Cryogenics and Isotopic Separations, Rm-Vâlcea, Romania.

Sample Preparation

The compounds were prepared by slow evaporation of an aqueous solution obtained by dissolving the sodium salt (DCF) and cyclodextrin (β-CD) in distilled water at 343 K. A white crystalline material which precipitated was collected by filtration, washed with diethyl ether, and dried at 308 K. Several DCF/β-CD compounds were also prepared with molar ratios of 0.5, 1.0, 1.5, 2.0, and 2.5.

NMR Measurements

All ^{1}H-NMR spectra were recorded in D_2O solutions with sodium 2,3-dimethyl-2-silapentane-5-sulfonate as the standard. The D_2O solutions of free DCF, free β-CD, and DCF/β-CD compounds were equilibrated in the probe for approximately 5 min before each run. High-resolution NMR spectra were obtained at room temperature using a Gemini-300 Varian spectrometer operating at 300 MHz.

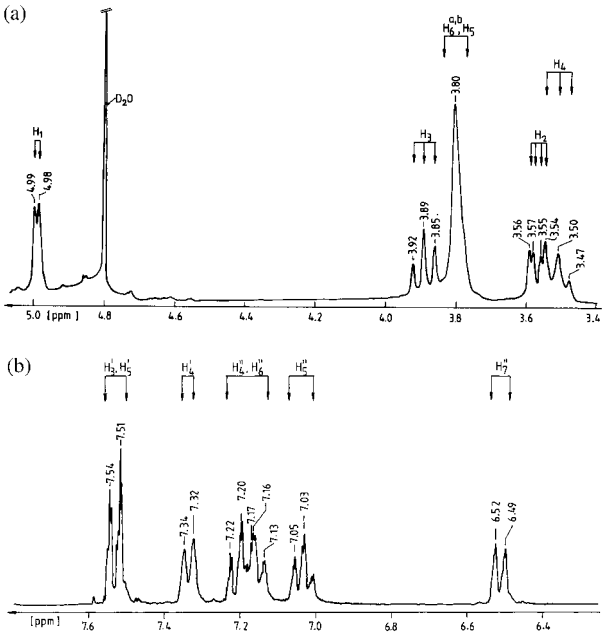

Figure 3. (a) ^{1}H-NMR spectrum of β-CD in D_2O. (b) ^{1}H-NMR spectrum of DCF in D_2O. (The $C^{(2)}H$-2 resonances are not shown.)

RESULTS AND DISCUSSION

^{1}H-NMR spectroscopy has been used previously to examine the mode of interaction of β-CD with a variety of aromatic substrates, such as substituted benzoic acids or substituted phenols, with some NSAIDs such as indomethacin, flufenamic acid, mefenamic acid, meclofenamic acids, and other drug molecules.[4,8–11] It was recognized that the upfield shifts of the CD proton resonances and the downfield shifts of guest proton resonances can be related to guest–host complex formation.

^{1}H-NMR Study of the DCF/β-CD Inclusion Complex

^{1}H-NMR peak assignments (see proton numbering in structure above) were achieved according to previous work.[9,10,16] The ^{1}H-NMR spectra of pure compounds in D_2O solution are shown in Figure 3(a,b), respectively.

For β-CD: H-1 (7H, 4.99 ppm, 4.98 ppm, doublet); H-3 (7H, 3.92 ppm, 3.89 ppm, 3.85 ppm, triplet); H-2 (7H, 3.58 ppm and 3.57 ppm, 3.55 ppm and 3.54 ppm, two doublets); H-4 (7H, 3.54 ppm, 3.50 ppm, 3.47 ppm, triplet); H-5,6 (21H, overlapped multiplets in the 3.90–3.70 ppm region). At this stage we note that the expected

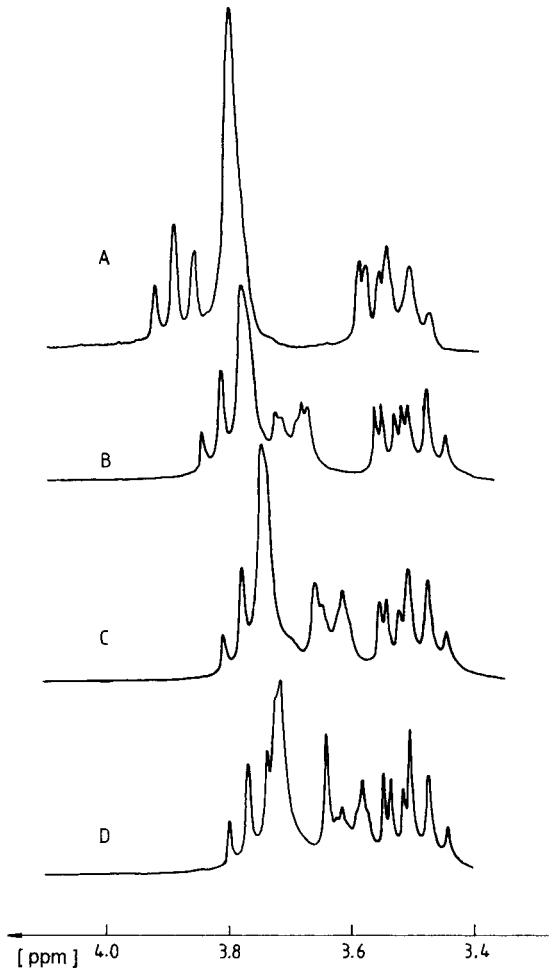

Figure 4. ^{1}H-NMR spectra of DCF–β-CD complex in D_2O at various molar ratios (DCF : β-CD): (A) 0, (B) 0.5, (C) 1, and (D) 1.5.

H-5 signal could not be clearly observed in the spectrum of pure β-CD because of the overlapping with the H-6 signal (H_a-6 and H_b-6 protons), all resonances appearing as a strong and unresolved broad peak [Fig. 3(a)]. However, a sharp signal progressively shifting to a higher field, assigned to H-5, becomes apparent in the presence of increasing amounts of DCF (Fig. 4). The same behavior has also been described for phenothiazine, tolbutamide, chloropropamide, and salbutamol complexes with β-CD.[7,8]

For DCF, the protons exhibit a quasi-well-resolved structure of resonances [Fig. 3(b)]. The presence of carboxylic group in the structure of DCF deshields the neighbor protons attracting electron density; the more affected are $C^{(2)}$ H-2 protons, which resonate at 3.74 ppm. The phenylacetate ring protons (H″-4 at 7.22–7.20 ppm, H″-6 at 7.17–7.13 ppm, H″-5 at 7.05–7.03 ppm, and H″-7 at 6.52–6.49 ppm) are affected by the proximity of two groups, the carboxylic group and 2,6-diclorophenyl ring, respectively. The signals of 2,6-diclorophenyl ring protons appear further down field (H′-3 at 7.54 ppm, H′-5 at 7.51 ppm, and H′-4 at 7.34–7.32 ppm).

In a first step, the formation of the complex is studied by comparing the ^{1}H-NMR spectra of β-CD in the absence and, respectively, in the presence of DCF at various concentrations. The spectra are shown in Figure 4 and the chemical shift variations of β-CD protons are presented in Table I. The chemical shift variations of individual resonances of β-CD protons as a function of molar ratio (DCF : β-CD) are plotted in Figure 5.

At this point, it is important to recall that the β-CD molecule adopts the conformation of a torus where H-3 and H-5 protons are located inside the cavity, whereas H-2 and H-4 are outside the torus and in contact with the aqueous medium. The H-6 protons of the primary alcohol group are on the narrow side and H-1 is in the glycosidic bond plane of β-CD. By comparison of the spectra of co-precipitated compounds with the spectrum of pure β-CD in D_2O solution, several chemical shift variations appear. All β-CD protons experience a shielding effect, their peaks moving to higher fields from their initial position, which clearly suggests that DCF interacts with β-CD protons, located within or outside of the torus. The most significant changes are experienced by H-5, H-3, and H-6 protons, indicating the existence of an important shielding effect in the cavity. On the other hand, the fact that $\Delta\delta$ H-3 $>$ $\Delta\delta$ H-6 at all concentrations proves an increased shielding in the cavity strictly in this way, (i.e., from secondary hydroxyl side to primary H-6 protons). As discussed in the literature,[4,7–11,16] these results suggest a more or less complete inclusion of the DCF moiety in the cavity of β-CD cavity through the larger rim of the torus.

Only a small upfield shift is observed for the signals of H-1 equatorial protons and H-2 and H-4 axial protons, respectively. This weak upfield shift of external protons suggests that the DCF molecules might also interact with the exterior surface of the torus via the moiety suspended on the rim of the torus. On the other hand, the complex formation by a partial inclusion of DCF into the β-CD cavity may also be consistent with a mechanism of association, where the DCF molecule is linked to the external part of the β-CD torus. Furthermore, a quite different concentra-

Table I. Changes in Chemical Shifts of β-CD Protons in the Presence of Increasing Concentrations of DCF

Molar Ratio DCF: β-CD	H-1	H-2	H-3	H-4	H-5	$H_{a,b}$-6
0.5	−0.02	−0.03	−0.08	−0.03	−0.13	−0.03
1.0	−0.04	−0.05	−0.12	−0.04	−0.20	−0.06
1.5	−0.05	−0.06	−0.14	−0.05	−0.24	−0.10
2.0	−0.04	−0.05	−0.11	−0.04	−0.22	−0.05
2.5	−0.04	−0.05	−0.12	−0.03	−0.24	−0.08

Chemical shifts in ppm are calculated from the relation $\Delta\delta = \delta_{DCF/\beta\text{-CD}} - \delta_{\beta\text{-CD free}}$.

tion dependence of chemical shifts was observed for each of the resonances (i.e., the inside protons respond more to the increasing concentration than the outer protons; (Fig. 5). The first behavior of the resonances agrees with an inclusion process, and the latter is not exclusive of an external association between the two molecules.

In a second step, to confirm the complexation of DCF with β-CD, the chemical shift variations of DCF protons were also investigated. The chemical shifts of different DCF protons are presented in Table II.

Chemical shift variations of all protons of DCF in the presence of β-CD are also clear evidence for the host–guest interaction and formation of the complex. All protons of DCF experienced an unusual shielding effect, the peaks moving to higher fields from their initial position.[10] Although not frequent, this kind of shielding can be related to the protruding of a moiety of DCF molecule into the β-CD cavity, so that the magnetic influence of one aromatic ring on the protons of the other ring can be reduced relative to the status when the diclofenac molecule is in its free form in solution. The guest molecule DCF has a nonplanar configuration, and the "hidden" diamagnetism of aromatic moiety could be compensated or overwhelmed by the diamagnetic proximity of host cavity.

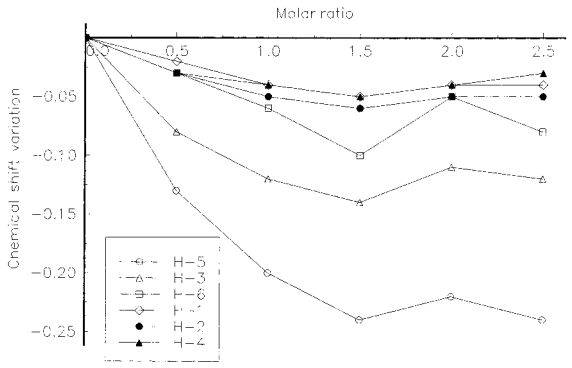

Figure 5. Chemical shift variations of β-CD protons as a function of molar ratio (DCF : β-CD).

The upfield shifts of phenylacetate ring protons (H″-7, -6, -5, -4) are greater than the upfield shifts of dichlorophenyl ring protons (H′-3, -4, -5), suggesting that the phenylacetate ring is more strongly influenced by the presence of β-CD. The size of the DCF molecule is compatible with its inclusion into the β-CD cavity. The diameter of the dichlorophenyl ring of DCF is very close to the maximum diameter of the host cavity (7.8 Å), and, consequently, it is more plausible to admit that the phenylacetate ring is accepted inside the β-CD cavity.

Molar Ratio Dependence of DCF:β-CD Inclusion Complex

The chemical shift variations of proton resonances as a function of the molar ratio DCF : β-CD are generally used to determine the stoichiometry of the inclusion complex, the stoichiometry corresponding to the steady state in the curve.[11]

To determine the possible stoichiometry, we examined only the chemical shift variations of internal protons of the β-CD molecule, which correlate with an inclusion process. As shown in Figure 5, the variation of chemical shift as function of molar ratio DCF:β-CD is quantitatively very different from one proton to another. However, each curve presents a plateau value above the molar ratio 1 : 1, so that the probable stoichiometric ratio of the inclusion complex is 1 : 1. In particular, a mechanism of association more complex than a single inclusion process should be also examined.

Structure of Inclusion Complex

A recently published paper reported the first synthesis and the X-ray crystal structure of a DCF :

Table II. Changes in Chemical Shifts of Some DCF Protons in the Presence of Different Concentrations of β-CD

Molar Ratio DCF: β-CD	H′-3, -5	H′-4	H″-4, -6	H″-5	H″-7	$C^{(2)}$ H-2
0.5	−0.05	−0.10	−0.11	−0.12	−0.15	−0.02
1.0	−0.08	−0.10	−0.12	−0.15	−0.15	−0.10
1.5	−0.10	−0.11	0.14	−0.15	−0.15	−0.12
2.0	−0.06	−0.10	0.14	−0.14	−0.15	−0.10
2.5	−0.06	−0.10	0.14	−0.16	−0.15	−0.10

Chemical shifts in ppm are calculated from the relation $\Delta\delta = \delta_{DCF/\beta\text{-CD}} - \delta_{DCF\ free}$.

β-CD inclusion complex.[17] X-ray data indicate that the complex exists in the solid state, and the binding complexation force has been assumed to be hydrogen bonding and hydrophobic interaction. The solid-state structure of the inclusion complex has been resolved. The phenylacetate ring of the guest molecule is fully inserted in the β-CD cavity; one carboxylate oxygen atom is hydrogen bonded to a primary hydroxyl group of the host molecule, whereas one other carboxylate oxygen atom is intramolecular hydrogen bonded. The dichlorophenyl residue rests on the secondary face of the host and the $C^{(2)}$ H-2 hydrogens are situated at the periphery of the β-CD molecule, nearly on its primary face.[15,17]

The present ^{1}H-NMR study suggests that the complex structure observed in the solid state can also be conceived in D_2O solution. It is expected that the conformation of two molecules is distorted in the solution complex. The mobility of the β-CD macrocyclic rings is quite limited upon the accommodation of the phenylacetate ring into the cavity. Consequently, the "microencapsulation" can induce some conformational changes, particularly in the angle between glucose units of the host and also in the angle between two phenyl rings of the guest.[17,18]

At this stage, the ^{1}H-NMR results are not enough to completely elucidate the geometry and the nature of intermolecular binding. Complementing the present ^{1}H-NMR results, other information concerning the complex formation, both in solution and in the solid state, was obtained by Fourier transform IR spectroscopy, Raman spectroscopy, and X-ray analysis.[19,20] In view of our findings, the complexation is consistent with the insertion of phenylacetate ring and carboxylic group of DCF in the cavity of β-CD and with hydrogen binding of one carboxylate oxygen of DCF to a primary hydroxyl group of the host, directly or via a water molecule. We hope that these experiments will help to improve the understanding of DCF–β-CD interactions, although a complete characterization of the geometry of the complex in aqueous solution remains to be accomplished.

CONCLUSIONS

We present here some results of an ^{1}H-NMR study of the interaction between DCF and β-CD molecules in D_2O solution. On the basis of experimental data, the hypothesis of complexation can be formulated. The chemical shift variations of H-3 and H-5 resonances in β-CD and H″/H′ in DCF both suggest an inclusion reaction. The solution conformation of the inclusion complex is characterized by the phenylacetate ring of DCF deeply inserted into the lipophilic cavity of the host, the entry occurring through the larger rim of β-CD truncated cone. Intermolecular interactions such as the hydrophobic interaction, hydrogen bonding, and other physical forces are involved in the inclusion complexation process.

REFERENCES

1. J. Szejtli, *Cyclodextrins and their Inclusion Complexes,* Akademiai Kiado, Budapest, 1982.
2. S. P. Jones, D. J. W. Grant, J. Hadraft, and G. P. Par, "Cyclodextrins in pharmaceutical sciences. I. Preparation, structure and properties of cyclodextrins and cyclodextrin inclusion compounds," *Acta Pharm. Tech.,* **23,** 213–223 (1983).
3. K. Uekama, "Pharmaceutical application of cyclodextrin complexation" *Yagugaku Zasshi,* **101,** 857–873 (1981).
4. C. Fronza, A. Mele, E. Redenti, and P. Ventura, "^{1}H NMR and molecular modeling study on the inclusion complex β-cyclodextrin-indomethacin," *J. Org. Chem.,* **61,** 909–914 (1996).

5. A. Marini, V. Berbeni, G. Bruni, P. Mustarelli, F. Giordano and M. Villa, "Termoanalytical and spectroscopic characterization of β-cyclodextrin/ketoprofen inclusion complexes," *J. Inclusion Phenomena Mol. Recogn. Chem.,* **22,** 221–234 (1995).
6. A. L. Thakkar and P. V. Demarco, "Cyclohepta-amylose inclusion complexes of barbiturates: correlation between proton magnetic resonance and solubility studies," *J. Pharm. Sci.,* **60,** 652–653 (1971).
7. M. Otagiri, K. Uekama, and K. Ikeda, "Inclusion complexes of β-CD with tranquilizing drugs phenothiazines in aqueous solution," *Chem. Pharm. Bull.,* **23,** 188–195 (1975).
8. H. Ueda and T. Nagai, "NMR spectroscopy of inclusion compounds of tolbutamide and cloropropamide with β-CD in aqueous solution," *Chem. Pharm. Bull.,* **28,** 1415–1421 (1980).
9. M. V. Rekharsky, F. P. Schwartz, Y. B. Tewari, R. N. Goldberg, M. Tanaka, and Y. Yamashoji, "Thermodinamic and NMR study of the interactions of cyclodextrins with cyclohexane derivatives," *J. Phys. Chem.,* **98,** 4098–4103 (1994).
10. V. K. Smith, T. T. Ndou, and I. M. Warner, "Spectroscopic study of the interaction of catechin with α-, β-, γ-, cyclodextrins," *J. Phys. Chem.,* **98,** 8627–8631 (1994).
11. S. Crouzy, F. Fauvelle, J-C. Debouzy, M. Göschl, and Y. Chapron, "Investigation of the α-cyclodextrin-myo-inositol phosphate inclusion complex by NMR spectroscopy and molecular modeling," *Carbohydr. Res.,* **287,** 21–35 (1996).
12. E. Lamcharfi, G. Kunesch, C. Meyer, and B. Robert, "Investigation of cyclodextrin inclusion compounds using FT-IR and Raman spectroscopy," *Spectrochimica Acta (Part A),* **51,** 1861–1870 (1996).
13. A. R. Sallmann, "The hystory of diclofenac," *Am. J. Med.,* **80,** 29–33 (1986).
14. P. Moser, A. R. Sallman, and I. Wiesenberg, "Synthesis and quantitative structure-activity relationships of diclofenac analoques," *J. Med. Chem.,* **33,** 2358–2368 (1990).
15. E. C. Van Tonder, M. R. Caira, S. A. Botha, and A. P. Lötter, "Comparison between diclofenac and diclofenac sodium tetrahydrate," *Pharm. Res.,* **10,** S-163 (1993).
16. D. K. Demertzi, D. Mentzafos, and A. Terzis, "Metal complexes of the anti-inflammatory drug sodium [2-[2,6-dichlorophenyl)amino]phenyl]acetate (diclofenac sodium). Molecular and crystal structure of cadmium diclofenac," *Polyhedron,* **12,** 1361–1370 (1993).
17. M. R. Caira, V. J. Griffith, L. R. Nassimbeni, and Van B. Oudtshoorn, "Synthesis and X-ray crystal structure of β-cyclodextrins diclofenac sodium undecahydrate, a β-CD complex with a unique crystal packing arrangement," *J. Chem. Soc., Chem. Commun.,* 1061–1062 (1994).
18. H. Dodziuk and K. Nowinski, "Structure of cyclodextrins and their complex. Do cyclodextrins have a rigid truncated-cone structure?" *J. Molec. Structure (Theochem),* **304,** 61–68 (1994).
19. I. Bratu, C. Ionescu, S. Astilean, E. Indrea, J. P. Huvenne, and P. Legrand, "FT-IR and X-Ray spectroscopic investigations of Na diclofenac/cyclodextrins interactions," *Spectr. Acta (a), Biomolec. Spectr.,* to appear.
20. C. Ionescu, E. Curea, M. Bogdan, G. Cristea, S. Astilean, and R. Vitoc, "NMR study of the interaction of sodium diclofenac with cyclodextrins," in 6th European Conference on the Spectroscopy of Biological Molecules, Villeneuve d'Ascq, Lille, France, 3–8 Sept., 1995.