Journal of Inclusion Phenomena and Macrocyclic Chemistry **37:** 383–394, 2000.

Fluorometric and NMR Studies of the Naproxen–Cyclodextrin Inclusion Complexes in Aqueous Solutions

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(Received: 21 April 1999; in final form: 23 August 1999)

Abstract. Inclusion complexation processes involving four cyclodextrins and naproxen have been studied for the protonated and unprotonated forms of the guest molecule. The association constants have been evaluated from changes in the fluorescence intensity of naproxen following addition of a cyclodextrin to an aqueous naproxen solution. ^{1}H NMR NOESY and ROESY spectra have shown that two orientations of the guest molecule relative to β-cyclodextrin are possible.

Key words: naproxen, cyclodextrins, complexation, association constants, fluorescence, NMR

1. Introduction

Cyclodextrins (CDs) form host-guest complexes with a number of molecules. The fluorescence quantum yield is a physicochemical property of guest molecules that can be affected by complex formation. Changes in fluorescence intensity have been used for the determination of the association constants of complexes [1–7].

Naproxen, (+)-6-methoxy-α-methyl-2-naphthalene acetic acid (Scheme 1), and its sodium salt, is a non-steroidal anti-inflammatory drug. The solubility of the drug in water can be substantially enhanced upon complexation with beta-cyclodextrin (β-CD) [8–13].

Naproxen is a weak acid (pK_a = 4.2 [14]); in aqueous unbuffered solutions with neither acid nor base added, it exists as a mixture of dissociated and undissociated (anionic) forms and the pH of the solution is about 4.2 (at a concentration of 5×10^{-5} M). After addition of a CD, there exist four species in the solution, *viz*., the compound itself, dissociated or not, and two different complexes in either form. In order to deal with a simpler system, we have determined the association constants and carried out NMR measurements on solutions of naproxen acid in 0.1 M HCl (pH = 1) or in solutions of the naproxen sodium salt in diluted NaOH (1.25×10^{-4} M, pH $\approx$9). In the two solutions, naproxen was assumed to exist in the acidic, and in the anionic form, respectively.

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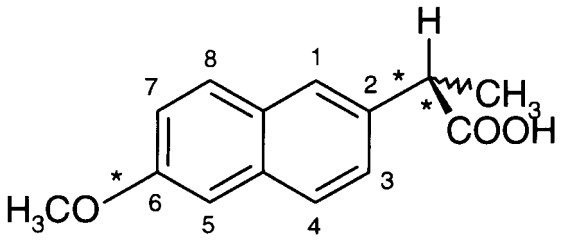

Scheme 1. Naproxen acid. Asterisks mark the bonds which served as rotation axes in searching for the most stable conformer.

The association constants of naproxen – CD complexes were measured by the fluorometric method, because naproxen is a strongly fluorescing compound and in aqueous solution its fluorescence intensity appeared to have been enhanced upon addition of cyclodextrins. In this way two conditions necessary for the determination of the association constant by the fluorometric method are fulfilled. The association constants of naproxen – CD complexes have been previously measured by phase solubility [10–12] in water, and by ^{1}H NMR in alkaline medium [15]; comparative determination of the constants for the acidic and anionic form of naproxen was made for β-CD [13, 16] and for 2-hydroxypropyl-β-CD (HP-β-CD) [17]. We have made such comparison for β-CD, HP-β-CD, methyl-β-CD (Me-β-CD), and gamma-CD (γ-CD), in order to compare the results obtained by different methods.

It is generally accepted that if association constants of complexes of a carboxylic acid and its anion with a cyclodextrin are compared, the association constant of the acid is greater than that of the anion. According to Bettinetti *et al.* [10], the affinity of the CD cavity for the neutral form of a given substrate is preferred to that for the ionized form. Such a regularity has indeed been observed for complexes of α-CD with benzoic acid [18, 19], benzoic acid derivatives [20–22], adamantanecarboxylic acid [23], and a series of alicyclic carboxylic acids [24], for β-CD complexed with 4-tert-butylbenzoic and nonanoic acids [25], and a series of alicyclic carboxylic acids [24], and for β-OH-CD complexed with indomethacin and naproxen [17]. Nevertheless, for complexes of some peracids with α-CD [25] and β-CD [26], and for a complex of *p*-nitrobenzoic acid with β-CD [27], the association constants of the unprotonated form were greater, as in the case of the *p*-nitrophenol complex with α-CD [28] and β-CD [29]. The corresponding values of the association constants were 220 and 1470 M^{-1} for complexes of the acidic and the anionic forms of *p*-nitrobenzoic acid [27]. On the other hand, population characteristics of cyclodextrin complex stabilities reported by Connors, revealed a twofold greater stability of ionic (mainly anionic) complexes with β-CD compared with uncharged complexes [30]. It seemed interesting to compare the association constants of the neutral and ionic naproxen complexes, for which the phase solubility method indicated larger stability of the uncharged form by another (fluorometric) method.

In order to gain some information about intermolecular interactions accompanying complexation, we decided to study complexes of each naproxen form with β-CD by means of NMR spectroscopy. The ^{13}C NMR study [10] of naproxen complexes with α-, β-, and γ-CD in aqueous unbuffered solutions carried out by the method of continuous variation of concentration has shown the 1 : 1 complex to be preferrred in the case of β-CD. With β-CD used in double molar excess, the low frequency shift of the carboxylate carbon atom of 1 ppm was observed, whereas both methyl groups showed a negligible high frequency shift of ca. 0.2–0.5 ppm under the same experimental conditions. A low frequency shift three times as large ($\sim$3 ppm) was observed for the carboxylate group interaction with the interior of the cavity [18], hence the results reported have been interpreted to be due to a rather strong hydrogen bonding of the carboxylate with the hydroxyls of the rim. While other aromatic resonances show a moderate low frequency shift (less than 1 ppm), the naproxen molecule has been inferred to be preferentially axially fitted into the cavity with the carboxylate group entering the cavity on the wider diameter rim side.

In the paper [31] devoted to the investigation of complexes of substituted cyclohexanecarboxylic acids and phenylalkanoic acids with α-, β-, and γ-CDs the NMR CIS and ROESY results indicated the presence of isomeric complexes with the COO^- functional group partly in and partly out of the cavity.

We expected that ^{1}H NMR investigation would give complementary information about the system, especially the two-dimensional (2D) ROESY and NOESY techniques which immediately show any particular interaction as a corresponding cross peak and are established as a far more sensitive tool for studying intermolecular interactions and host-guest complexes [32].

2. Experimental

2.1. MATERIALS

β-CD and HP-β-CD were purchased from Sigma Chemical Co. (St. Louis, Missouri). γ-CD was from Merck, Darmstadt, Germany. Me-β-CD (12.7 methyl groups per molecule) was from Amaizo, Hammond, Indiana. The water content in the CDs was determined by drying at 105°C to constant weight. Solutions of naproxen and CDs were prepared freshly each day. The NMR samples were prepared with deuterated water (99.9%D) purchased from the Radioisotope Centre Polatom, Świerk, Poland.

2.2. FLUORESCENCE MEASUREMENTS

Fluorescence spectra were measured with a Shimadzu RF-5000 spectrofluorometer equipped with a thermostatically controlled cell compartment. Spectra were measured at 35°C. The excitation wavelength was 329.6 nm; at this wavelength, the molar absorption coefficients of naproxen acid and naproxen anion were about

1520 M^{-1}, and 1600 M^{-1}, respectively. The maximum value at 356.4 nm was taken as a measure of fluorescence intensity.

For fluorescence titration, a naproxen solution (about 5×10^{-5} M), 2 mL, in 0.1 M HCl or in water with a very small amount of 0.025 M NaOH added to adjust pH to 8–9, was titrated in a fluorescence cell equipped with a Teflon stopper and a magnetic stirrer by adding successive amounts of a CD dissolved in an identical naproxen solution at a concentration of about 10 mM. The portions added were in the range 10–100 μL. The final concentrations of CDs ranged from 0 to $\sim$2.7 mM. After each titration, the fluorescence spectrum as well as the intensity at the maximum wavelength was recorded as a function of ligand concentration.

2.3. NMR EXPERIMENTS

The ^{1}H NMR spectra were run on a VARIAN INOVA 500 MHz instrument by using routine software for COSY, NOESY, and ROESY types of experiments. Sodium 3-trimethylsilyltetradeuteropropionate, d_4-TSPA, was used as an internal reference. The equimolar concentrations of both components were 10 mM or 1 mM for naproxen anion. In the case of naproxen acid, due to its very low solubility, only 0.1 mM concentration was obtained when fourfold excess of β-CD was used. The COSY type spectra were acquired by using spectral widths of 4600 Hz in both dimensions, acquisition time 0.223 s, 4 transients per 256 increments, and 2048 data points in the F2 domain. For the NOESY type spectra, the corresponding parameters were: 4600 Hz, acquisition time 0.223 s, 384 transients per 160 increments, mixing time 300 ms, and 2048 data points in the F2 domain. The 2D ROESY measurements were acquired under the experimental conditions: 4 kHz spin lock field in cw mode centered at water resonances and 300 ms mixing time duration.

3. Calculations

The structures of the two forms of naproxen were first optimized by using the semiempirical AM1 method. During this optimization the most stable conformers were found with respect to rotation of the functional groups of the molecule around three single bonds (marked with *, Scheme 1) every 30°. Finally, the structures of the most stable conformers were used as the starting structures for *ab initio* calculations at the HF/3-21G* level with the use of the Gaussian 94 program [33]. Details of the conformers energies and structures will be described in a forthcoming paper.

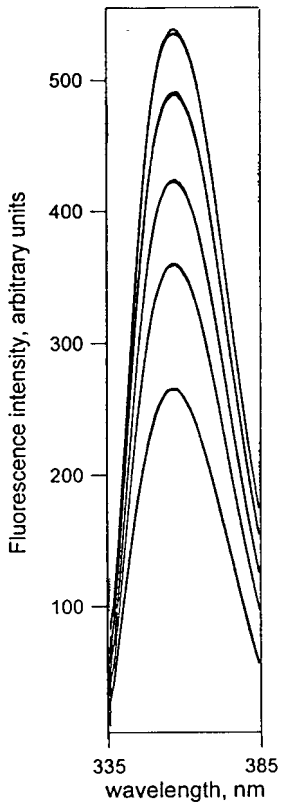

Figure 1. Enhancement of the fluorescence intensity of naproxen acid (pH 1) by the addition of methyl-β-cyclodextrin. Concentrations of Me-β-CD from top to bottom: 0.14, 0.053, 0.019, 0.0076, and 0 mM.

4. Results and Discussion

4.1. ASSOCIATION CONSTANTS

Addition of each of the four CDs to naproxen at pH values 1 and 9 resulted in an increase in fluorescence intensity. The fluorescence spectra of naproxen acid containing varying concentrations of Me-β-CD and γ-CD are shown in Figures 1 and 2, respectively. The influence of Me-β-CD on the fluorescence intensity was much greater owing to the greater association constant with that ligand (concentrations of γ-CD in Figure 2 were much greater than that of Me-β-CD in Figure 1).

Evaluation of the association constant of a complex between two interacting species must be preceded by the determination of complex stoichiometry. In order to test the stoichiometry by the fluorometric method, Benesi–Hildebrand (double reciprocal) plots were used, namely $1/\Delta F$ *vs* 1/[CD], where ΔF is the increment of fluorescence intensity upon addition of a cyclodextrin at a concentration [CD]. The plots were linear with typical regression coefficients exceeding 0.999. The linearity

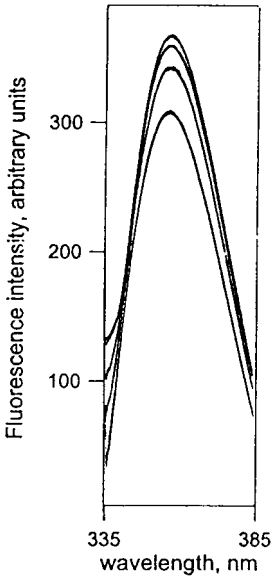

Figure 2. Enhancement of the fluorescence intensity of naproxen anion by addition of γ-CD. Concentration of γ-CD from top to bottom: 7.9, 5.8, 3.2, and 0 mM.

of the Benesi–Hildebrand plots is an indication of only one complex present in the solution, of a host-guest composition of 1 : 1. Similar results were obtained with the Scatchard and Scott plots [34]. These results are in accordance with those obtained by using ^{13}C NMR measurements [10]. For γ-CD, the plots could not be constructed because of the very small changes in fluorescence intensity upon the addition of these CDs, accompanying weak host-guest interactions and low association constants. With γ-CD, the association constant values are burdened with large errors and are estimated for a tentative stoichiometry of 1 : 1.

The linear transformations of binding isotherms used in the graphical methods applied for the determination of association constants do not properly weight the data [34]. For example, the Benesi–Hildebrand (double reciprocal) plots tend to place more emphasis on lower rather than higher concentration values. As a result, the value of the slope depends strongly on the ordinate value corresponding to the point at the lowest cyclodextrin concentration [5]. Therefore, the association constants were calculated by using a nonlinear least-squares regression analysis.

The results of measurements of the association constants for naproxen acid and naproxen anion are given in Table I together with the values previously measured for β-CD. The results of measurements in water, where the acidic and the anionic forms of naproxen coexist together, are added for comparison with the previous data of Bettinetti [10] and Melani [12]. All previously reported values are measured by the phase solubility method (only those earlier data are quoted that were obtained at 35 or 37°C; values obtained at 25°C or at room temperature are omitted in the table). The agreement of the results for naproxen in water measured by the

Table I. Association constants of naproxen with four cyclodextrins at 35°C, measured fluorometrically, with the data taken from literature, obtained using phase solubility. Reproducibility of the results was 3–17% R.S.D. for first three CDs and 33% R.S.D. for γ-CD

Cyclodextrin	Naproxen acid	Naproxen anion	Naproxen in H_2O
β-CD	1950 (1070[13]) (975[16])	620 (26[16])	1335 (1388[10]) (1388[12])
β-OH-CD	2600	540	1880 (1973[10]) (1726[12])
Me-β-CD	6100	700	4100 (5855[10])
γ-CD	60	60	(79[12])

two methods is acceptable, taking into consideration that, in unbuffered solutions, a slight difference in pH would change the proportion of the two naproxen forms. For naproxen in the acidic and the basic environments with β-CD, our values are higher than those measured by the phase solubility method [13, 16]. For β-OH-CD, values measured at 25°C were 1670 and 331 M^{-1} for acid and anion, respectively [17]. They would be still lower at 35°C, so our fluorometric values are also larger than those measured by phase solubility with this ligand. The differences can be due to a systematic error of one or both of the methods, but there exists another possibility: if the complexation equilibrium is achieved sufficiently quickly, monitoring of the fluorescence intensity provides us with the opportunity to study complexation behavior in the substrate excited state [3]. The difference between the association constants determined by the two methods can be due to the different complexation degrees in the ground and excited states.

Striking are the low association constants of both naproxen forms with γ-CD, shown previously by Melani using the phase solubility method [12]. For another compound with the naphthalene ring, 1-aniline-8-naphthalenesulfonate, the reported association constants for complexes with β- and γ-CD were respectively 64 and 1260 M^{-1} [35]. On this basis the binding was concluded to be more effective if there is enough room for high mobility of the lipophilic guest moieties in the cavity. Our results do not confirm this inference; they agree with the results of Eftink *et al*. which suggest that for complexes of alicyclic carboxylic acids and carboxylates with α- and β-CD [24], the larger values of the association constants with the latter CD may well reflect a deeper penetration of the guest into the β-CD cavity and enhanced van der Waals interactions. The same conclusion was arrived at by Ber-

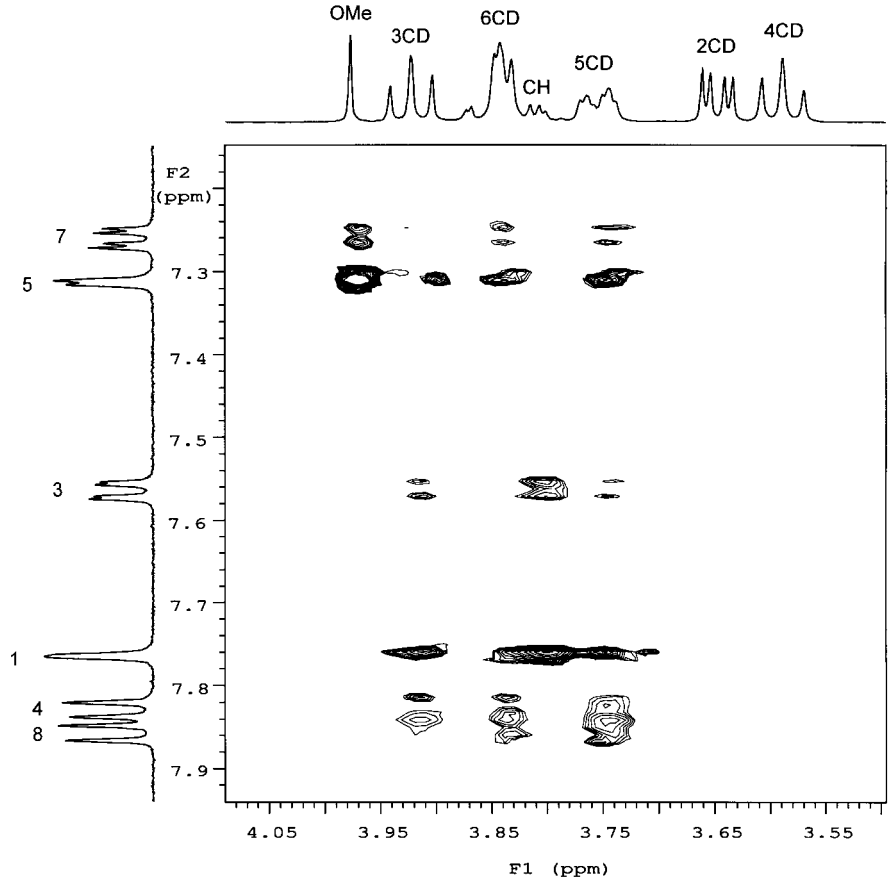

Figure 3. 2D ROESY spectrum of naproxen anion at concentration 1 mM with β-cyclodextrin.

geron *et al*. who stated that in view of the dependence of London dispersion forces on the distance between the interacting species (r^{-6}), any substance which fits into cyclohexaamylose is likely to bind more weakly in the cycloheptaamylose cavity owing to the greater diameter of the heptamer's cavity and the greater distance between the host and the guest molecules [36]. The same reasoning can perhaps be used to explain the low association constants of the naproxen complexes with γ-CD as compared with the association constants of the complexes with β-CD and its derivatives.

Our attempts to measure the association constants of naproxen with α-CD failed, because the influence of α-CD on the fluorescence intensity was too small to be measurable.

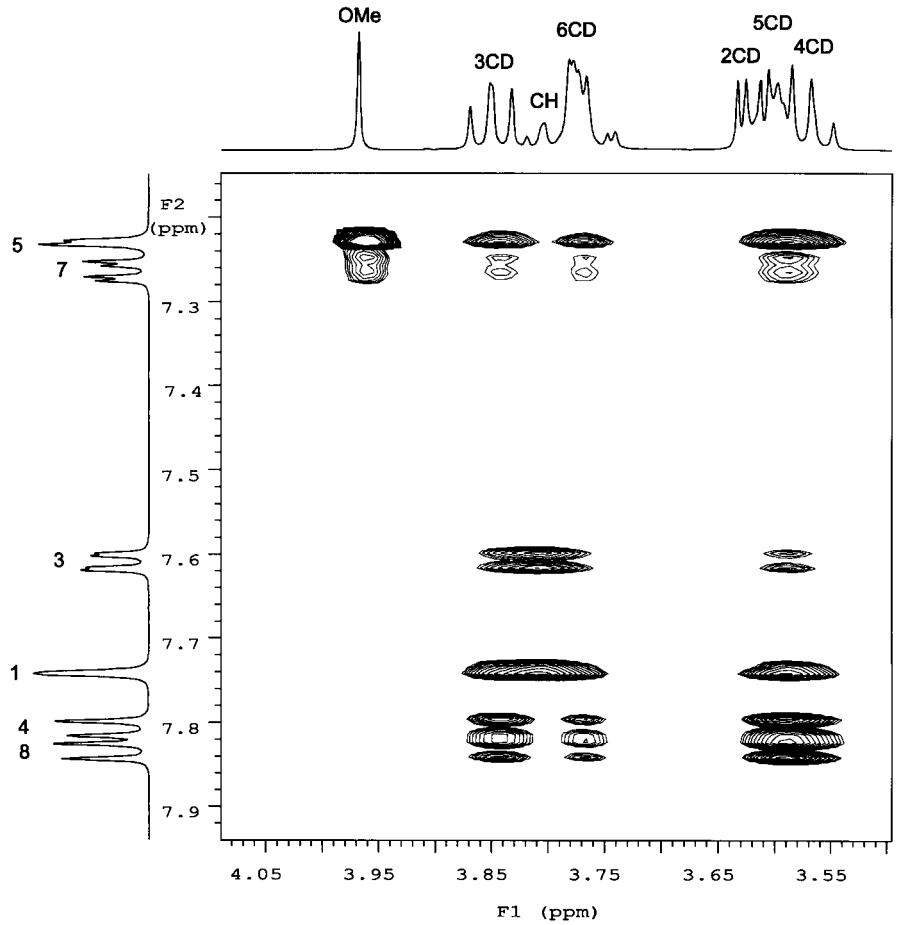

Figure 4. 2D ROESY spectrum of naproxen anion at concentration 10 mM with β-cyclodextrin.

4.2. ^{1}H NMR EXPERIMENTS

The assignment of proton resonances of a neat naproxen sample in water and in the presence of β-CD was achieved by examining the scalar interactions by means of a COSY experiment. In order to determine the dipolar contacts for both intra- and intermolecular interactions, NOESY (anionic form) and ROESY (both forms) experiments were made. In our case 2D ROESY experiments were found to be the more sensitive ones.

The 2D ROESY spectra clearly provide evidence for interactions of naproxen with β-CD protons in terms of NOE enhancement of appropriate signals during the mixing time. Proton dipolar contacts correlation in the ROESY spectra are shown in Figures 3–5 for two anion concentrations and for the carboxylic form. The spectra show both intramolecular and intermolecular contacts. In all spectra

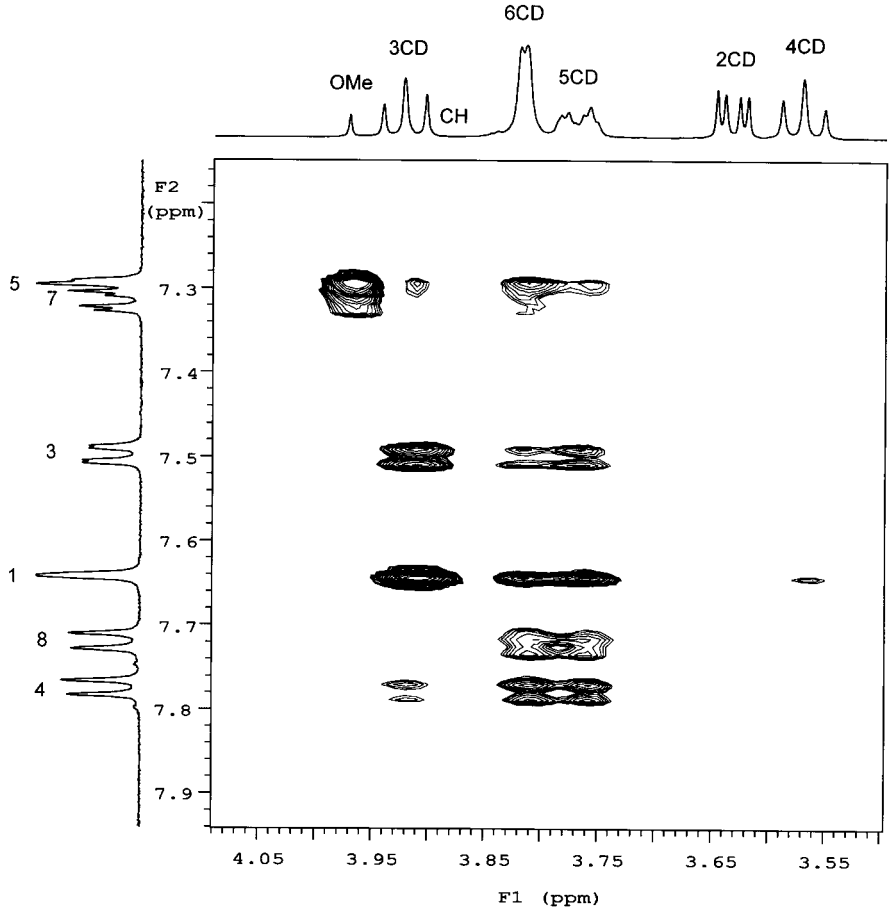

Figure 5. 2D ROESY spectrum of naproxen acid at concentration 0.1 mM with β-cyclodextrin.

reported, intense correlation peaks are observed between naproxen protons and H-3, H-5 and H-6 of β-CD and intermolecular contacts are clearly absent for protons H-2 and H-4 of β-CD which are found on the outer surface of the host molecule (in the case of cyclohexanecarboxylic acid, intermolecular interactions with the outer surface of α-CD were evident as cross-peaks to 2 and 4 protons of α-CD) [31]). This is a clear evidence that the inclusion phenomenon has indeed been observed.

In principle, two modes of inclusion are possible with the carboxylic group lying in the primary hydroxyl end (narrower rim) of the CD molecule or near the secondary hydroxyl end (wider rim). Both modes were found to occur in interactions of carboxylic acids with β-CD [37]. In the case of complexes of benzoic acids with α-CD, it was concluded that the carboxylic group is located at the narrow end of the cavity [25]. A similar finding was reported by Hamilton *et al*. in the case of complexes of halogen substituted benzoic acids and β-CD [38]. The present spectra

do not allow an unambiguous rejection or confirmation of one or the other mode of inclusion in both cases. Correlation cross-peaks from protons on both sides of the naproxen molecule, i.e., H-1, H-3 and H-5, H-7 with proton H-5 of β-CD are present. This suggests that inclusion is not specific with respect to the substituent groups of naproxen. The results do not confirm the conclusion drawn recently as concerns the preferred mode of inclusion [15]. This is not unexpected, however, as the carboxylate group may form hydrogen bonds equally strongly with the β-CD OH groups present on both edges of the host molecule. One hint may be found in the case of the carboxylic form which points to the first mode, i.e., the crosspeaks between H-4 and H-8 of naproxen-acid to H-3 of β-CD are much weaker than those to H-5 and H-6, whereas they are of comparable intensity in the anionic form as shown in Figures 3–5. The results of molecular modelling also indicate the possibility of two mutual orientations of naproxen and HP-β-CD [12].

It is also worth mentioning that dipolar contacts are not symmetrical on both sides of naproxen substituent groups, i.e., they are stronger for protons H-1 and H-5 vs H-3 and H-7. In view of the optimized structure of lowest energy, obtained as reported in Section 3, the protons giving stronger interactions are found in eclipsed conformations with the methyl of the OCH_3 substituent (H-5) and with the CH proton of the carboxy methyl substituent (H-1).

5. Conclusions

Fluorometric and 2D ^{1}H NMR investigations support the formation of inclusion complexes between naproxen and four cyclodextrins. ^{1}H NMR ROESY and NOESY cross-peaks suggest that two mutual orientations of naproxen and β-cyclodextrin are possible. Application of the Benesi–Hildebrand, Scott and Scatchard linearizations showed the stoichiometric ratio of the complexes studied to be 1 : 1. The association constants of the complexes are related to the cyclodextrin cavity dimensions, and are lower for the larger cavity of γ-cyclodextrin than those for β-cyclodextrin. They are also related to pH and are several times greater for naproxen acid than for naproxen base. For β-CD, where association constants were measured at the same temperature (35°C) by phase solubility, the present values are higher than the latter.

References

1. N. Sadlej-Sosnowska: *J.Fluorescence* **7**, 195 (1997).
2. J. M. Madrid, F. Mendicuti, and W. L. Mattice: *J. Phys. Chem. B* **102**, 2037 (1998).
3. M. El Baraka, R. García, and E. Quiñones: *J. Photochem. Photobiol. A: Chem.* **79**, 181 (1994).
4. G. C. Catena and F. V. Bright: *Anal.Chem.* **61,** 905 (1989).
5. I. Durán-Merás, A. Muñoz de la Peña, F. Salinas, and I. Rodríguez-Cáceres: *Appl. Spectrosc.* **51**, 684 (1997).
6. A. Örstan and J. B. A. Ross: *J. Phys. Chem.* **91**, 2739 (1987).
7. Y. L. Loukas: *J. Phys. Chem. B* **101**, 4863 (1997).

8. N. Erden and N. Celebi: *Gazi Univ. Eczacilik Fak. Derg.* **5**, 91 (1988); *Chem. Abstr.* **110**, 13440h.
9. G. Bettinetti, P. Mura, A. Liguori, G. Bramanti, and F. Giordano: *Il Farmaco*, **44**, 195 (1989); *Chem. Abstr*. **112**, 104742r.
10. G. Bettinetti, F. Melani, P. Mura, R. Monnani, and F. Giordano: *J. Pharm. Sci.* **80**, 1162 (1991).
11. H. W. Frijlink, E. J. F. Franssen, A. C. Eissens, R. Oosting, C. F. Lerk, and D. K. F. Meijer: *Pharm. Res.* **8**, 380 (1991).
12. F. Melani, G. P. Bettinetti, P. Mura, and A. Manderioli: *J. Incl. Phenom.* **22**, 131 (1995).
13. I. Orienti, A. Fini, V. Bertasi and V. Zecchi: *Eur. J. Pharm. Biopharm.* **37**, 110 (1991).
14. *Clarke's Isolation and Identification of Drugs*, A. C. Moffat (ed.), Pharmaceutical Press, London, 1986.
15. A. Ganza-Gonzalez, J. L. Vila-Jato, S. Anguiano-Igea, F. J. Otero-Espinar, and J. Blanco-Méndez: *Int. J. Pharm.* **106**, 179 (1994).
16. F. J.Otero-Espinar, S.Anguiano-Igea, N. García-Gonzalez, J. L.Vila-Jato, and J. Blanco-Méndez: *Int. J. Pharm.* **79**, 149 (1992).
17. K. Okimoto, R. A. Rajewski, K. Uekama, J. A. Jona, and V. J. Stella: *Pharm. Res.* **13**, 256 (1996).
18. R. I. Gelb, L. M. Schwartz, R. F. Johnson, and D. A. Laufer: *J. Am. Chem. Soc.* **101**, 1869 (1979).
19. R. J. Bergeron, M. A. Channing, and K. A. McGovern: *J. Am. Chem. Soc.* **100**, 2878 (1978).
20. R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson, and D. A. Laufer: *J. Am. Chem. Soc.* **103**, 1750 (1981).
21. K. A. Connors, S.-F. Lin, and A. B. Wong: *J. Pharm. Sci.* **71**, 217 (1982).
22. D. Pendergast and K. Connors: *J. Pharm. Sci.* **73**, 1779 (1984).
23. R. I. Gelb, L. M. Schwartz, and D. A. Laufer: *Bioorg. Chem.* **9,** 450 (1980).
24. M. R. Eftink, M. L. Andy, K. Bystrom, H. D. Perlmutter, and D. S. Kristal: *J. Am. Chem. Soc.* **111**, 6765 (1989).
25. D. M. Davies and M. E. Deary: *J. Chem. Soc., Perkin Trans.2* **1996**, 2415.
26. D. M. Davies and J. R. Savage: *J.Chem.Soc., Perkin Trans. 2* **1994**, 1525.
27. K. Pitchumani and M. Vellayappan: *J. Incl. Phenom.* **14**, 157 (1992).
28. F. Cramer, W. Saenger, and H.-C. Spatz: *J. Am. Chem. Soc.* **89,** 14 (1967).
29. A. Buvári and L. Barcza: *J. Chem. Soc., Perkin Trans. 2* **1988**, 543.
30. K. A.Connors: *J. Pharm. Sci.* **84**, 843 (1995).
31. A. Gadre, V. Rüdiger, H.-J. Schneider, and K. A. Connors: *J. Pharm. Sci.* **86**, 236 (1997).
32. F. Djedaini and A. Perly: *Nuclear Magnetic Resonance of Cyclodextrins, Derivatives and Inclusion Compounds*, Chapter 6 in *New Trends in Cyclodextrins and Derivatives*, D. Duchêne (ed.), Editions de Santé, Paris, 1991.
33. M. J. Frisch, G. W. Trucks, H. B. Schlegel, P. M. W. Gill, B. G. Johnson, M. A. Robb, J. R. Cheeseman, T. A. Keith, G. A. Petersson, J. A. Montgomery, K. Raghavachari, M. A. Al-Laham, V. G. Zakrzewski, J. V. Ortiz, J. B. Foresman, J. Cioslowski, B. B. Stefanov, A. Nanayakkara, M. Challacombe, C. Y. Peng, P. Y. Ayala, W. Chen, M. W. Wong, J. L. Andres, E. S. Replogle, R. Gomperts, R. L. Martin, D. J. Fox, J. S. Binkley, D. J. Defrees, J. Baker, J. P. Stewart, M. Head-Gordon, C. Gonzales, and J. A. Pople: *Gaussian 94 (Revision D.4)*; Gaussian Inc.: Pittsburgh, PA, 1995.
34. K. A. Connors: *Binding Constants*, John Wiley & Sons, 1987.
35. H.-J. Schneider, T. Blatter, and S. Simova: *J. Am. Chem. Soc.* **113**, 1996 (1991).
36. R. J. Bergeron, D. M. Pillor, G. Gibeily, and W. P. Roberts: *Bioorg. Chem.* **7**, 263 (1978).
37. K. A. Connors: *Chem. Rev.* **97**, 1325 (1997).
38. J. A. Hamilton, M. N. Sabesan, and L. K. Steinrauf: *Carbohydr.Res.* **89**, 33 (1981).