Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **15:** 131–143, 1993.

Absorptiometric and Spectrofluorimetric Study of the Inclusion Complexes of 2-Naphthyloxyacetic Acid and 1-Naphthylacetic Acid with β-Cyclodextrin in Aqueous Solution

A. MUÑOZ DE LA PEÑA, F. SALINAS, M. J. GÓMEZ, M. I. ACEDO, and M. SÁNCHEZ PEÑA
Department of Analytical Chemistry, University of Extremadura, 06071, Badajoz, Spain

(Received: 28 October 1992; in final form: 10 March 1993)

Abstract. The inclusion complexes of 2-naphthyloxyacetic acid (NOA) and 1-naphthylacetic acid (NAA) with β-cyclodextrin have been investigated in aqueous solution. It has been demonstrated that the naphthalene derivatives form 1 : 1 complexes when included in the cyclodextrin. A possible structure is proposed, having an axial inclusion of the naphthalene derivatives. In the case of the β-CD : NOA complex, the naphthyl moiety is included in the cyclodextrin and the acetic acid group protrudes from the cavity, while NAA is only partially included because of the steric effect of the group in position 1. Association constants of $560 \pm 100\ M^{-1}$ and $100 \pm 50\ M^{-1}$ have been calculated for the β-CD : NOA and β-CD : NAA complexes, making use of the increment in the fluorescene emission produced in the inclusion process.

Key words: β-Cyclodextrin, 2-naphthyloxyacetic acid, 1-naphthylacetic acid, fluorescence spectroscopy, molecular absorption spectroscopy.

1. Introduction

The ability of cyclodextrins (CDs) to act as molecular hosts for a variety of hydrophobic organic species is well known. The three cyclodextrins most commonly used, α-, β- and γ-CD, are composed of 6, 7 and 8 α-(1,4) linked units of glucopyranose, respectively [1]. In spite of being the least soluble in water, β-CD is the one most frequently employed for pharmaceutical and analytical applications. Apart from economic reasons, β-CD seems to be the host of choice when dealing with a variety of compounds that present a compatible size with the interior of its cavity [2]. Cyclodextrin inclusion complexes of fluorophores frequently show differences between the spectra obtained in purely aqueous and aqueous CD solutions, and fluorescence has been extensively used to investigate CD systems [1].

Because of their inherent usefulness in the areas of chemical analysis, pharmaceutics, foods, cosmetics, pesticides, etc., numerous studies have been made to evaluate the complexing ability of CDs [1–4]. Although the host : guest ratio is usually 1 : 1, one or more CD molecules can contain one or more guest molecules. The correct evaluation of stoichiometry is obviously necessary for an accurate

determination of the formation constant. The formation constant provides a measure of complex stability, giving a clearer understanding of the factors affecting complexation, which is of vital importance to the use of these systems for the development of new analytical or industrial methodologies [5–11].

2-Naphthyloxyacetic acid (NOA) and 1-naphthylacetic acid (NAA) are naphthalene derivatives which are widely used as fungicides for controlling diseases on fruits and which are useful as plant growth regulators, to prevent the premature fall of fruits. Naphthalene and naphthalene derivative complexes with CDs have received significant attention in recent years. Hamai [12] reported on the stoichiometry and stability of the complex between naphthalene and β-CD, and Hashimoto and Thomas [13] investigated the interactions of naphthalene and β-CD in the presence of different amphiphiles. Nelson *et al.* [14, 15] reported on cyclodextrin complexes of naphthalene and naphthalene derivatives, using steady-state and fluorescence lifetime measurements. Tran and Fendler [16] investigated the complexation of amine naphthalene derivatives, and Harata and Uedaira [17] used circular dichroism to characterize the inclusion of 1- and 2-substituted naphthalene derivatives. Kano *et al.* [18, 19] reported on the formation of three component complexes of β-CD with naphthalene and naphthalene derivatives, in the presence of different aliphatic and aromatic amines, and Yorozu *et al.* [20] studied the inclusion of β-naphthol with CDs by means of optical absorption and circular dichroism spectrometry.

In luminescence studies, CDs have been employed to enhance fluorescence emission of different luminophors [21–25] and to induce room temperature phosphorescence under appropriate conditions [26–30]. The intensification of luminescent processes of molecules included in the interior of the cavity of the CD is due to the better protection from quenching and other processes occurring in the bulk solvent. CDs have been used to increase the fluorescence intensity of various organic species through a partial encapsulation or total inclusion.

Enhancements of the fluorescence of the pesticide warfarin (3-(α-4-acetonylbenzyl)-4-hydroxycoumarin) [21] and of retinal [22] have been reported to occur upon inclusion in β-CD, as well as several hallucinogenic drugs, including N,N-dimethyltryptamine, the hemisulfate and hydrochloride of mescaline, and the hydrochloride of ibogaine [23]. Also, increments in the fluorescence emission of thiol derivatives of ammonium 7-fluorobenzo-2-oxa-1,3-diazol-4-sulfonate with glutathione, acetylcysteine, cysteine, and several dansyl amino acids [24], and of several *o*-phthaldehyde and fluorescamine derivatives [25] have been reported recently.

In this paper, the absorption and luminescent characteristics of the inclusion complexes of 2-naphthyloxyacetic acid (NOA) and 1-naphthylacetic acid (NAA) with β-CD have been investigated, with the object of characterizing the inclusion processes involved.

2. Experimental

2.1. Apparatus

Fluorescence measurements were made on a Perkin Elmer Model LS 50 luminescence spectrometer equipped with a xenon discharge lamp equivalent to 20 Kw for 8 μs duration. The instrument is connected via the RS-232 port with an IBM PS/2 80386-SX microcomputer. Data acquisition and data analysis were performed by the use of Perkin Elmer Fluorescence Data Manager Software, Version 2.70. Solutions were excited at 274 nm (λ_{max} of NOA), or at 284 nm (λ_{max} of NAA). Fluorescence measurements were made with excitation and emission band widths of 5 and 3 nm, respectively. The scan rate of the monochromators was maintained at 240 nm min^{-1}. All measurements were made at 20 $\pm$ 0.1 °C by use of a thermostatic cell holder and a Selecta Model 382 thermostatic bath. Absorption measurements were conducted with a Beckman DU-64 spectrophotometer connected via the RS-232 port to an Olivetti PC-286 microcomputer. The Beckman Data Leader Software, Version 3.0, was used for spectral acquisition and analysis of the spectrophotometric data.

2.2. Reagents

All experiments were performed with analytical grade chemicals. Purified LC-grade water (Millipore Milli-Q-system) was used. 2-Naphthyloxyacetic acid (97%) was obtained from Chem Service (West Chester, PA), 1-naphthylacetic acid (99%) was obtained from Aldrich-Chemie and β-cyclodextrin was obtained from Sigma and used as received.

2.3. Methods and sample preparation

A 10^{-2} M stock solution of NOA or NAA was prepared in absolute ethanol. Aqueous 10^{-4} M NOA or NAA solutions were prepared daily by pipetting an aliquot of the stock solution into a 250 mL volumetric flask. The ethanol was evaporated by use of dry nitrogen, deionized water was added and, after sonication, the solution was made up to the mark. Solutions of lower concentrations were prepared by appropriate dilution of the stock aqueous solution.

For the study of the influence of β-CD concentration on the intensity of fluorescence, several solutions were prepared, by maintaining a constant concentration of 2.5×10^{-6} M NOA or 5.0×10^{-6} M NAA and varying the β-CD concentration, by exact weighing of β-CD. After sonication to dissolve the cyclodextrin, the solutions were made up to the mark with deionized water, and were allowed to equilibrate for 15 min prior to analysis. The solutions remain stable for more than 24 h.

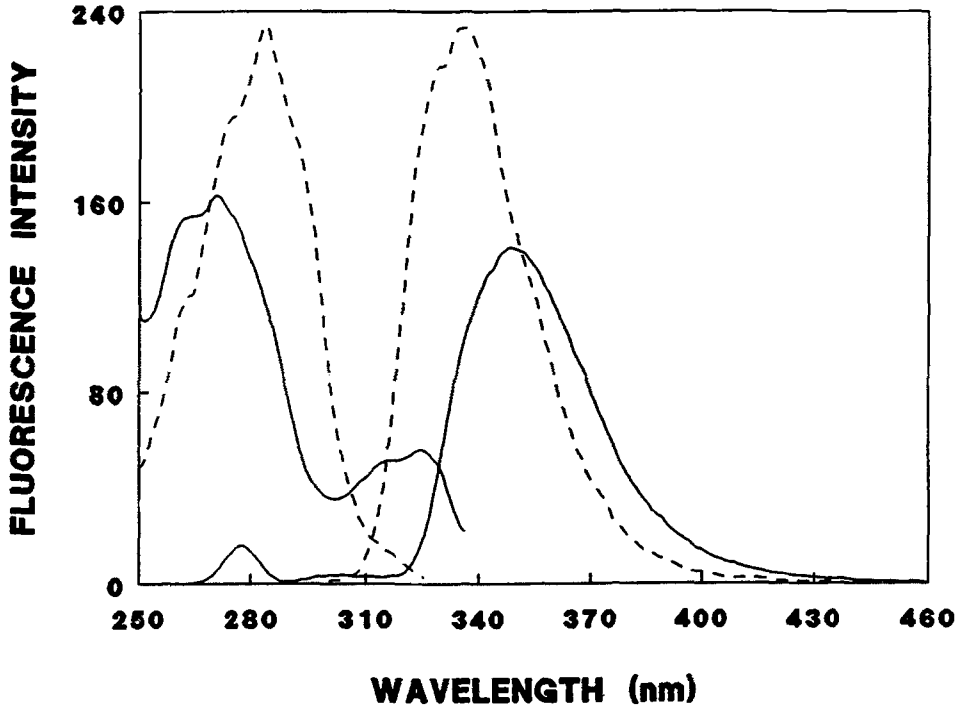

Fig. 1. Excitation and emission spectra of NOA (—), λ_{ex} = 274 nm, λ_{em} = 350 nm, and of NAA (- - -), λ_{ex} = 284 nm, λ_{em} = 336 nm, in aqueous solution.

3. Results and Discussion

3.1. Excitation and Emission Spectra

2-Naphthyloxyacetic acid and 1-naphthylacetic acid fluoresce in aqueous solution. The corrected excitation and emission spectra of these compounds are shown in Figure 1. The maximum of excitation of NOA appears at 274 nm and maxima at 313 and 322 nm are also observed, while the emission appears at 350 nm. The maximum of excitation of NAA appears at 284 nm and the maximum of emission at 336 nm.

3.2. Influence of pH

The influence of the pH on the fluorescence of NOA and NAA is shown in Figure 2. The fluorescence of NOA at 350 nm increases with pH, giving the maximum variation in the range of pH between 2 and 4. For pH between 4 and 9, a constant emission intensity is attained. The fluorescence tiration curve gave a pK_a value of 2.5 ± 0.1 for this compound. The emission fluorescence of NAA at 336 nm increases with pH, showing the maximum variation in the range between pH 3 and 5. The fluorescence titration curve gave a pK_a value of 4.2 ± 0.1 for this compound.

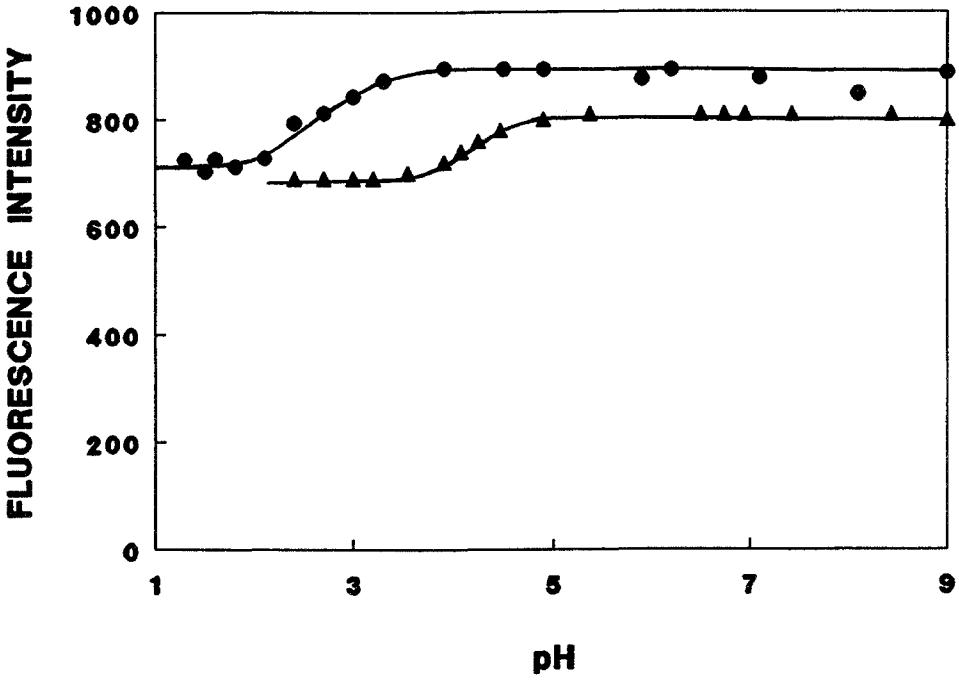

Fig. 2. Influence of pH on the fluorescence emission of (•) NOA, λ_{ex} = 274 nm, λ_{em} = 350 nm, and of (▲) NAA, λ_{ex} = 284 nm, λ_{em} = 336 nm.

3.3. INCLUSION COMPLEX WITH β-CD

Figure 3 shows the absorption spectra of NOA and NAA, in the absence and presence of 10^{-2} M β-CD. The absorption maxima of NOA are localized at 260.5, 271, 312 and 325 nm. A decrease of the absorbance is observed upon addition of the cyclodextrin, together with a small batochromic displacement of around 1 nm. The absorption maxima of NAA are localized at 272.5 and 281 nm. A small increase of the absorbance and displacement of the maxima to longer wavelengths is observed upon addition of the cyclodextrin.

The displacement to longer wavelengths has been described for other compounds and attributed to inclusion in the cyclodextrin cavity [1]. Figure 4A shows the emission spectra of NOA in the absence and in the presence of 10^{-2} M β-CD. Figure 4B shows the emission spectra of NAA in the absence and in the presence of 10^{-2} M β-CD.

On addition of β-CD, the relative fluorescence emission of NOA and of NAA increase and the excitation and emission maxima remain unshifted. Although it has been reported that a red shift in the excitation and a blue shift in the emission

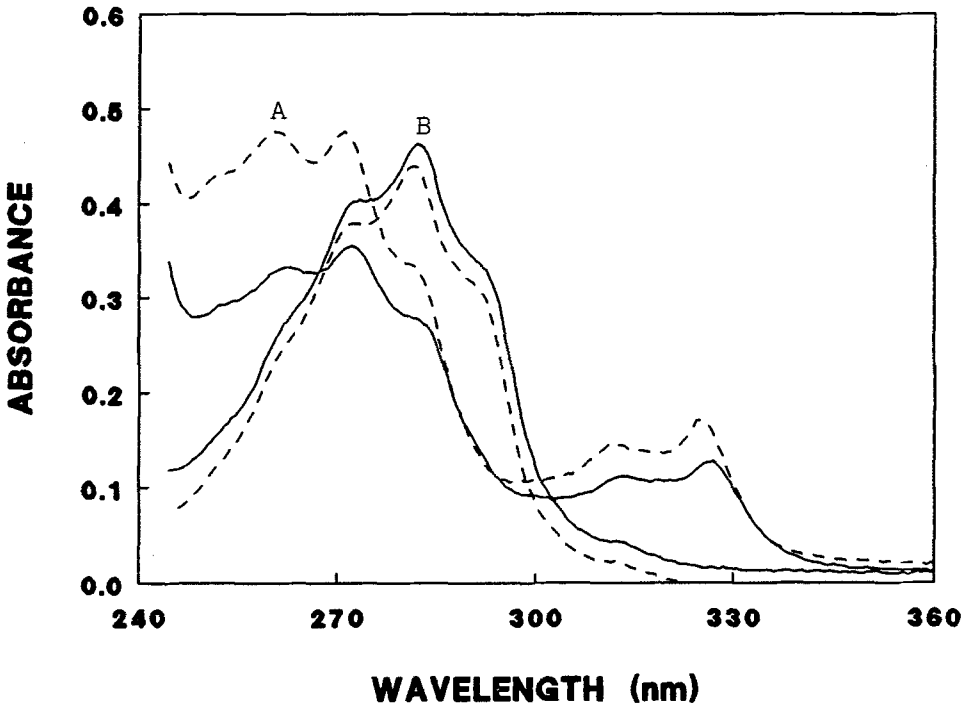

Fig. 3. (A) Absorption spectra of NOA in the absence (- - -) and presence (——) of 10^{-2} M β-CD. (B) Absorption spectra of NAA in the absence (- - -) and presence (——) of 10^{-2} M β-CD.

maxima are usually produced upon inclusion [1, 24], it is also well documented that not all the molecules included inside β-CD show perceptible changes of the emission wavelength to lower values. For example, Baeyens *et al.* [25] found increases of between 10% and 65% in the fluorescence emission of mexiletine- and lysine hydrochloride-*o*-phthaldehyde and mexiletine hydrochloride-, cysteine-, cysteamine-, homocysteine- and lysine hydrochloride-fluorescamine derivatives, in the presence of β-CD, with a negligible influence of the enhancing reagent on the excitation and emission maxima.

3.4. Influence of β-cyclodextrin concentration on the β-CD : NOA inclusion complex

The fluorescence intensity of NOA increases as the β-CD concentration is increased (Figure 5). The stoichiometry and the formation constant of the β-CD : NOA complex were calculated as follows. Assuming a 1 : 1 stoichiometric ratio, according to the following equilibrium:

$$[CD] + [NOA] \rightleftharpoons [CD : NOA] \quad (1)$$

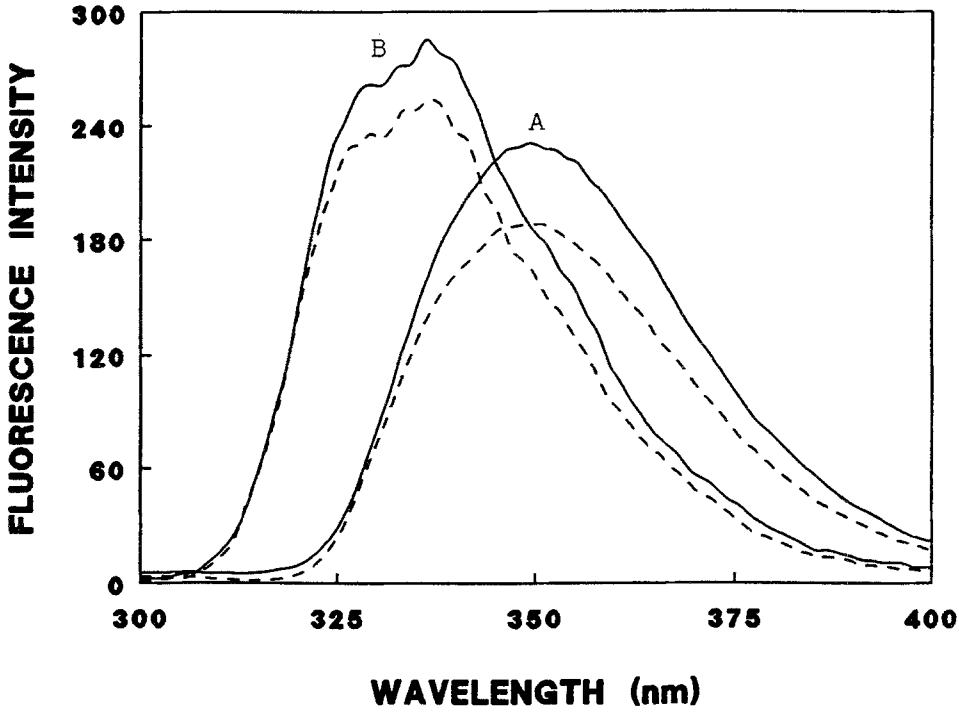

Fig. 4. (A) Fluorescence emission spectra of NOA in the absence (- - -) and presence (——) of 10^{-2} M β-CD. (B) Fluorescence emission spectra of NAA in the absence (- - -) and presence (——) of 10^{-2} M β-CD.

The formation constant of the complex (K_1) is given by

$$K_1 = \frac{[\text{CD} : \text{NOA}]}{[\text{CD}][\text{NOA}]} \tag{2}$$

where [CD], [NOA] and [CD : NOA] are the corresponding equilibrium concentrations of these species, respectively. As the inital concentration of cyclodextrin $[\text{CD}]_0$ is in a large excess over the complex concentration,

$$[\text{CD}] = [\text{CD}]_0 - [\text{CD} : \text{NOA}] \approx [\text{CD}]_0 \tag{3}$$

and from the mass balance,

$$[\text{NOA}]_0 = [\text{NOA}] + [\text{CD} : \text{NOA}] \tag{4}$$

where $[\text{NOA}]_0$ is the initial concentration of NOA. Consequently, Equation 4 can be simplified to

$$K_1 = \frac{[\text{CD} : \text{NOA}]}{[\text{CD}]_0([\text{NOA}]_0 - [\text{CD} : \text{NOA}])} \tag{5}$$

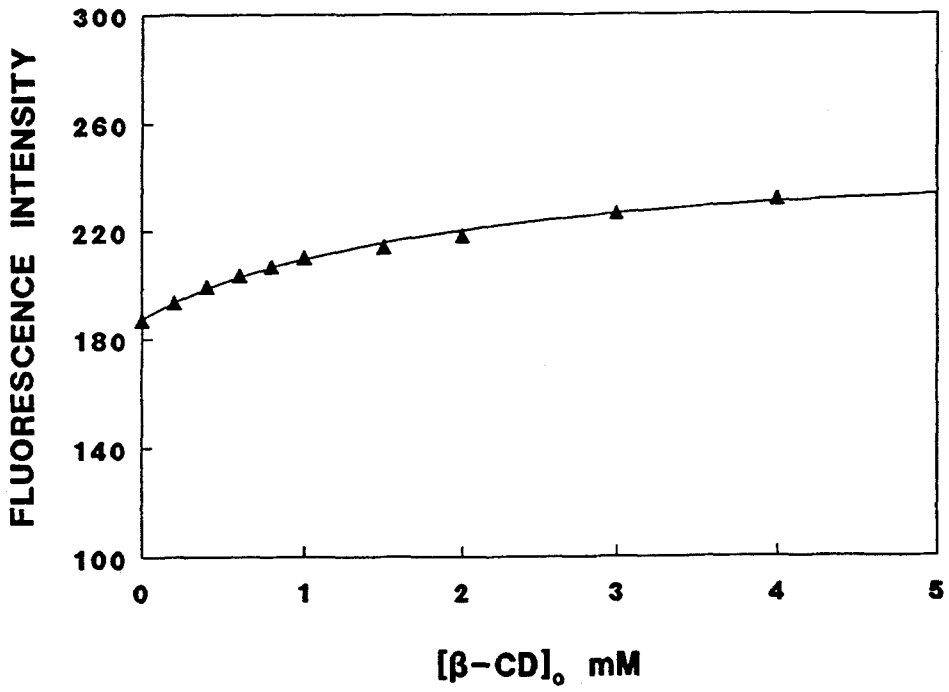

Fig. 5. Influence of β-CD concentration on the fluorescence intensity of NOA. The solid line was calculated through the use of Equation 8, assuming a 1 : 1 stoichiometry and using the values F_∞ and K_1 obtained by nonlinear regression analysis.

As can be observed in Figure 5, the quantum yield of fluorescence of NOA increases on interacting with β-CD. Thus, fluorescence intensity increases, as [β – CD] increases, up to a constant value for a sufficient reagent excess. By using similar expressions to those in ref. [9], the stoichiometry and the formation constants of the complex have been evaluated. The relation between the fluorescence increment and the β-CD concentration is given by the following equation:

$$\frac{F - F_0}{[\mathrm{CD}]_0} = (F_\infty - F_0)K_1 - (F - F_0)K_1 \tag{6}$$

where $[\mathrm{CD}]_0$ denotes the initial CD concentration, F_0 denotes the fluorescence intensity of NOA in the absence of β-CD, F_∞ denotes the fluorescence intensity when all of the NOA molecules are essentially complexed with β-CD, and F is the observed fluorescence at each CD concentration tested. The representation of $(F - F_0)/[\mathrm{CD}]_0$ against $(F - F_0)$ is known as a Scatchard plot [31] and allows the determination of the stoichiometry and the determination of the formation constant. If the stoichiometry is really 1 : 1, a linear plot should be obtained. The application of the method to our experimental data gives a linear graph, suggesting a 1 : 1 stoichiometry for the complex.

Equation 6 can be regrouped in the more familiar Benesi–Hildebrand form [31].

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K_1[\mathrm{CD}]_0} + \frac{1}{F_\infty - F_0}. \tag{7}$$

Typical double-reciprocal plots for the β-CD : NOA complex are shown in Figure 6. A linear relationship is obtained when $1/(F - F_0)$ is plotted against $1/[\mathrm{CD}]_0$. This indicates that the stoichiometry of the complex is 1 : 1. In contrast, a downward concave curvature is obtained when these data are fitted to a 2 : 1 complex. The linear plot can be used to obtain K_1, by simply dividing the intercept by the slope, but Benesi–Hildebrand plots tend to place more emphasis on lower concentration values than on higher concentration values. Consequently, the slope of the line is more sensitive to the ordinate value of the point having the smallest concentration. A better estimation is obtained by nonlinear regression analysis [31]. Rearranging the data, we obtain the direct relationship between the observed fluorescence intensity, F, and the β-CD concentration, $[\mathrm{CD}]_0$,

$$F = F_0 + \frac{(F_\infty - F_0)K_1[\mathrm{CD}]_0}{1 + K_1[\mathrm{CD}]_0}. \tag{8}$$

The experimental data can be directly fitted by the use of this equation. The initial parameter estimates needed for the nonlinear regression (NLR) method have been obtained from the linear plots. The NLR analysis of the data has been performed by an iterative Marquardt-type process. An NLR plot of the experimental data is depicted in Figure 5. An association constant of 560 ± 110 M^{-1} was calculated for β-CD : NOA through the application of the NLR method. Similar calculations gave a 1 : 1 stoichiometry and an association constant of 100 ± 50 M^{-1} for the β-CD : NAA complex.

3.5. Structure of the Inclusion Complexes

The dimensions of the naphthalene molecule are approximately 6.8 Å in height and 8.4 Å in width, and the thickness is around 3 Å, according to the Corey–Pauling–Kolton (CPK) space filling molecular models [32]. Accordingly, it seems that naphthalene cannot be included equatorially in β-CD, which presents a cavity length of around 7.8 Å [1]. In consequence, only a partial inclusion is expected if this is equatorial, but this will not be energetically favored. An axial inclusion is thus the energetically favoured orientation.

For monosubstituted naphthalene derivatives, as in this case, the mode of inclusion of the complexes will depend on the position of the substituted group. If the naphthalene is substituted at position 1, a complete axial inclusion is not possible, because the substituted group could not be included inside the cyclodextrin cavity due to steric effects. However, the nonsubstituted naphthalene ring could be axially included with the substituted ring and the substituent group outside the β-cyclodextrin cavity. In contrast, naphthalene substituted in position 2 can form

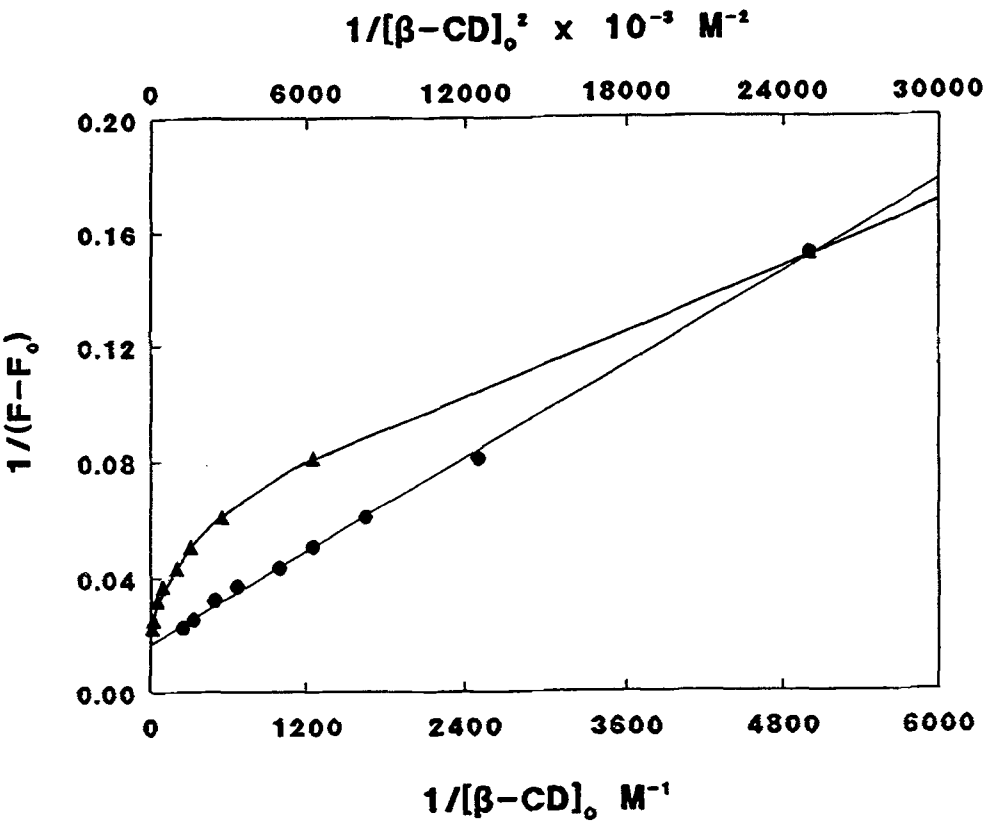

Fig. 6. Double-reciprocal plot between NOA and β-CD in aqueous solution. A linear relationship when the data are plotted assuming a 1 : 1 β-CD : NOA stoichiometry (•) and a downward concave curvature when the data are plotted assuming a 2 : 1 β-CD : NOA stoichiometry (▲).

complexes with the axial orientation, which are almost totally included inside the β-cyclodextrin cavity.

This fact was experimentally demonstrated by Harata and Uedaira [17] by using circular dichroism. They studied several naphthalene derivatives, mono and di-substituted in positions 1 and 2: 1-naphthylacetic acid, 2-naphthylacetic acid, 1-naphthoic acid, 2-naphthoic acid, 1-naphthylamine, 2-naphthylamine, 1,8-diaminonaphthalene and 2,3-diaminonaphthalene. They found a very notable difference in the circular dichroism spectra of both types of complexes, concluding that the steric effect of the substituents is so strong that the structures should be different. The calculation of the rotational strength, by the Kirkwood–Tinoco method, revealed that the structure of the naphthalene complex, substituted in position 2, was an axial inclusion. However, they did not find conclusive results on the structure of the naphthalene complex substituted in position 1.

Tran and Fendler [16] proposed a partial axial inclusion for a naphtha-

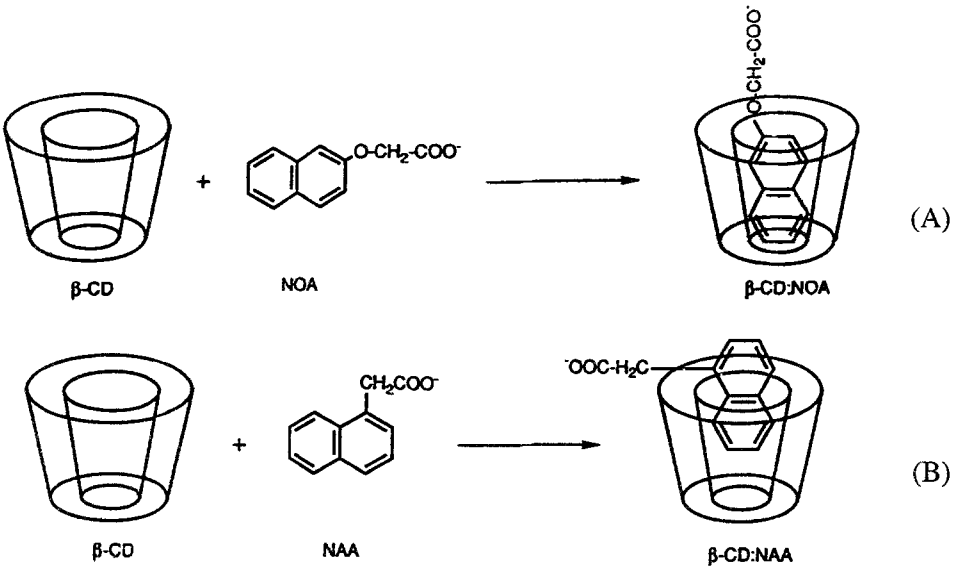

Fig. 7. Diagrammatic representation of the structure of the complexes of (A) NOA and (B) NAA with β-CD.

lene substituted derivative in position 1 (1-naphthylethylamine), and Catena and Bright [33] proposed an axial inclusion as the more probable structure for 2,6-diaminonaphthalensulfonate.

Nelson *et al.* [15] studied the inclusion of methyl- and ethylnaphthalene substituted in positions 1 and 2 using life-time measurements. They noticed that the lifetimes of the 1-substituted naphthalenes are shorter than those of the 2-substituted naphthalenes, suggesting that β-CD can only include part of the 1-substituted naphthalene molecules, while the 2-substituted naphthalenes can completely enter the cavity. When more of the naphthalene ring resides in the interior of the β-CD, it is likely that more protection from deactivation pathways is offered.

Taking these considerations into account, the proposed structure for the inclusion of NOA and NAA with β-CD is an axial inclusion, as represented in Figure 7. For the β-CD : NOA complex (Figure 7A), the two benzene rings will be included in the β-cyclodextrin cavity and the substituent group protrudes from the cavity. For the β-CD : NAA complex (Figure 7B), the inclusion is only partial, with the nonsubstituted ring inside the CD cavity, and the substituted ring and the substituent group outside the β-CD cavity. The hypothesis is further strengthened by the acid–base behaviour of NOA and NAA included in β-CD. The variation of the fluorescence emission of the inclusion complexes with pH was evaluated, and

pK_a values of 2.5 and 4.2 for NOA and NAA complexes, respectively, were found in accordance with the values found in the absence of β-CD.

4. Conclusions

The stoichiometry and stability constants of the complexes of NOA and NAA, with β-CD, in aqueous solutions, have been determined by using the changes in the fluorescence intensity of the naphthalene derivatives upon inclusion. A possible mode of inclusion is proposed and discussed.

The fact that NOA is completely included while NAA is only partially included into the CD cavity is in agreement with the smaller variations found in the absorption and emission spectra of NAA upon inclusion and with the smaller value found for the association constant. A partial inclusion will give a weaker association and smaller variations in the spectral characteristics of the guest. The results of these studies can be used to implement designs of specific analytical or pharmaceutical methodologies.

Acknowledgements

The authors acknowledge the D.G.I.C.Y.T. of the Ministery of Education and Science of Spain (Project PB91-0856) for financial support of this work.

References

1. J. Szeijtli: *Cyclodextrins and Their Inclusion Compounds,* Akademiai Kiado, Budapest (1982).
2. J. Szeijtli: *Pharm. Technol. Int.* **3**, 15 (1991).
3. J. Szeijtli: *Cyclodextrin Technology*, Kluwer, Dordrecht (1988).
4. D. Duchene (Ed.): *Cyclodextrins and Their Industrial Uses,* Ed. de Santé, Paris (1987).
5. A. Muñoz de la Peña, T. T. Ndou, J. B. Zung, K. L. Greene, D. H. Live, and I. M. Warner: *J. Am. Chem. Soc.* **113**, 1572 (1991).
6. A. Muñoz de la Peña, T. T. Ndou, J. B. Zung, and I. M. Warner: *J. Phys. Chem.* **95**, 3330 (1991).
7. A. Muñoz de la Peña, V. Anigbogu, T. T. Ndou, and I. M. Warner: *Anal. Chem.* **63**, 1018 (1991).
8. J. M. Schuette, T. Ndou, A. Muñoz de la Peña, K. L. Greene, C. K. Williamson, and I. M. Warner: *J. Phys. Chem.* **95**, 4897 (1991).
9. V. K. Smith, T. T. Ndou, A. Muñoz de la Peña, and I. M. Warner: *J. Incl. Phenom.* **10**, 471 (1991).
10. J. B. Zung, A. Muñoz de la Peña, T. Ndou, and I. M. Warner: *J. Phys. Chem.* **95**, 6701 (1991).
11. V. Anigbogu, A. Muñoz de la Peña, T. T. Ndou, and I. M. Warner: *Anal. Chem.* **64**, 484 (1991).
12. S. Hamai: *Bull. Chem. Soc. Jpn.* **55**, 2721 (1982).
13. S. Hashimoto and J. K. Thomas: *J. Am. Chem. Soc.* **107**, 4655 (1985).
14. G. Nelson and I. M. Warner: *J. Phys. Chem.* **94**, 576 (1990).
15. G. Nelson, G. Patonay, and I. M. Warner: *Appl. Spectrosc.* **41**, 1235 (1987).
16. C. D. Tran and J. H. Fendler: *J. Phys. Chem.* **88**, 2167 (1984).
17. K. Harata and H. Uedaira: *Bull. Chem. Soc. Jpn.* **48**, 375 (1975).
18. K. Kano, I. Takenoshita, and T. Ogawa: *Chem. Lett.* 1035 (1980).
19. K. Kano, I. Takenoshita, and T. Ogawa: *J. Phys. Chem.* **86**, 1833 (1982).

20. T. Yorozu, M. Hoshino, M. Imamura, and H. Shizuka: *J. Phys. Chem.* **86**, 4422 (1982).
21. J. C. Márquez, M. Hernandez, and F. Carcía Sánchez: *Analyst* **115**, 1003 (1990).
22. D. A. Lerner, B. Del Castillo, and S. Muñoz Botella: *Anal. Chim. Acta* **227**, 297 (1989).
23. O. Jules, S. Scypinski, and L. J. Cline Love: *Anal. Chim. Acta* **169**, 335 (1985).
24. W. Baeyens, B. Lin, and V. Corbisier: *Analyst* **115**, 359 (1990).
25. W. Baeyens, B. L. Ling, V. Corbisier, and A. Raemdonck: *Anal. Chim. Acta* **234**, 187 (1990).
26. S. Scypinski and L. J. Cline Love: *Anal. Chem.* **56**, 322 (1984).
27. S. Scypinski and L. J. Cline Love: *Anal. Chem.* **56**, 331 (1984).
28. H. J. Casal, J. C. Netto-Ferreira, and J. C. Scaiano: *J. Incl. Phenom.* **3**, 395 (1985).
29. S. Hamai: *J. Am. Chem. Soc.* **111**, 3954 (1989).
30. A. Muñoz de la Peña, I. Durán-Merás, F. Salinas, T. T. Ndou, and I. M. Warner: *Anal. Chim. Acta* **255**, 351 (1991).
31. K. A. Connors: *Binding Constants, the Measurement of Molecular Complex Stability,* John Wiley & Sons, New York (1987).
32. C. D. Gutsche: *Calixarenes,* p. 184, The Royal Society of Chemistry, Cambridge (1989).
33. C. Catena and F. V. Bright: *Anal. Chem.* **61**, 905 (1989).