Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **22:** 235–247, 1995.

Photochemically Induced Fluorescence Investigation of a β-Cyclodextrin : Azure A Inclusion Complex and Determination of Analytical Parameters

MOUNIR MAAFI, BELKACEM LAASSIS and JEAN-JACQUES AARON*
Institut de Topologie et de Dynamique des Systèmes de l'Université de Paris 7 Denis Diderot, associé au CNRS, URA 34, 1, rue Guy de la Brosse, 75005 Paris, France.

and

M. CARMEN MAHEDERO, ARSENIO MUÑOZ DE LA PEÑA and FRANCISCO SALINAS
Department of Analytical Chemistry, University of Extremadura, 06071, Badajoz, Spain.

(Received: 30 March 1995; in final form: 19 June 1995)

Abstract. The photooxidation of Azure A and fluorescence properties of Azure A and its photoproduct have been investigated in aqueous media and in the presence of β-cyclodextrin (β-CD). The fluorescence intensity of the complex formed between the photoproduct and β-CD was found to be three times higher than that of the uncomplexed Azure A photoproduct. A complex formation constant of $110 \pm 40\ M^{-1}$ was calculated using the Benesi–Hildebrand treatment of the fluorescence emission data. Although the stoichiometry of the Azure A photoproduct: β-CD complex was found to be 1 : 1, it seems that the Azure A structure is only partially included. Calibration graphs were plotted for the free Azure A photoproduct and the photogenerated product included in β-CD. The analytical parameters and quantification limits were determined.

Key words. Azure A, β-cyclodextrin, inclusion complex, photochemically induced fluorescence spectroscopy.

1. Introduction

Cyclodextrins (CDs) and their derivatives are torus-shaped, cyclic oligosaccharides made of six, seven or eight, glucose units (α- β-, and γ-cyclodextrins) joined by α-(1–4) linkages, obtained from the degradation of starch by glucosyltransferase enzyme. The main interest in these compounds rises primarily from their ability to include in their empty cavities, organic, hydrophobic molecules from aqueous media. These inclusion complexes formed between cyclodextrins (host) and organic molecules (guest), are established only by physical forces without covalent bonding. The preparation of cyclodextrin inclusion complexes in solution is simple, and the inclusion can be total or partial according to geometrical rather than

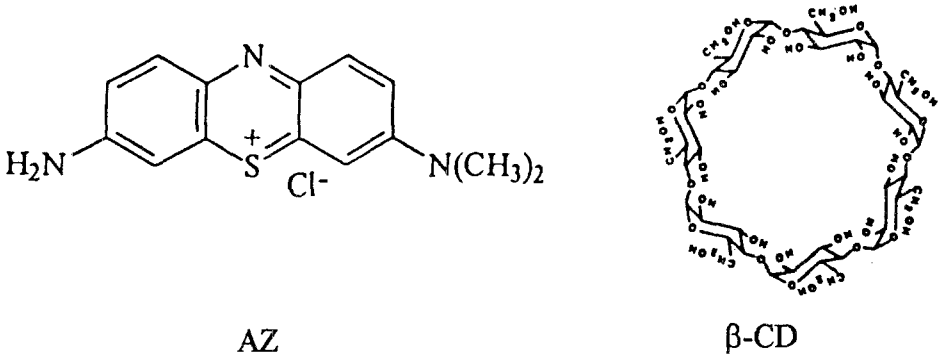
* Author for correspondence.

chemical factors of the guest structure. Although the host: guest ratio is usually 1 : 1, one or more CD molecules can contain one or more guest molecules [1].

Complexed cyclodextrins have a large variety of applications. They have been successfully applied, either in laboratory or even at the industrial scale complex production. In pharmaceutical applications, CDs are used in various drug formulations. CD complexes may enhance the solubility of drugs in solutions [2, 3], improve their chemical and photochemical stability [3, 4], mask unpleasant taste and smell of drugs [5, 6], and minimize side effects [7, 8]. It has been also observed that CD inclusion complexes improve *in vitro* and *in vivo* pharmacological effects and absorption [9, 10]. Moreover, some examples are known in the food industry and in cosmetics where CDs can be used for the preparation of stable water-in-oil emulsions [11].

Several spectroscopic methods are useful in the analysis and characterization of the CD complexes. The quantitative determination of the guest content can be performed by using current analytical methods such as UV-vis, GLC and HPLC. Inclusion is also characterized by changes in various spectral properties of both the guest and host. A circular dichroism signal can be observed on adding a CD to the aqueous solution of an achiral potential guest. The induced signal intensity and sign are sensitive to the orientation of the guest chromophore included in the CD cavity [1]. Frequently, a bathochromic shift of several nm and/or band broadening are observed in the UV absorption spectra of guests. At sufficiently high CD concentration these UV properties are similar to those obtained in ethanolic solutions [1, 12]. Luminescence spectroscopy is an interesting means to characterize CD complexes, because of the significant signal increment that is frequently induced. Fluorescent organic compounds in organic solvents, usually present poor fluorescence in aqueous media. Adding CDs to such solutions results in an important enhancement of fluorescence [13,14]. The CD cavity behaves similarly to an organic solvent; it affords an apolar environment and a nonhydrated state for the included chromophore. Cramer *et al.* [15], reported a ten-fold enhancement of the fluorescence emission on addition of 0.01 M of β-CD to 1-anilino-8-naphthalensulfonate aqueous solution. Park and Park [16] showed that inclusion of (aminostyryl)-l-methylpyridinium dyes by β-CD provoked an important increase of fluorescence, and they used these results for fluorescent-probe studies on association of cationic and neutral organic molecules with β-CD.

Azure A (AZ) is a charged phenothiazine derivative (see Scheme 1). This class of compounds is widely used in pharmaceutical preparations and employed as psychotropic tranquillizing drugs [17]. Phenothiazine dyes such as Azure A, have been investigated for their phototherapeutic effects against carcinomas [18]. Phenothiazine derivatives have other applications such as insecticides, anthelmintics, pigments, and oxidation-reduction indicators [19].



Scheme 1. Structures of Azure A (AZ) and β-cyclodextrin (β-CD).

The photochemical oxidation of phenothiazines is also interesting for quantitative analytical purposes, because of the relatively strong fluorescence of the oxidation photoproducts [20–26].

In a previous study, we have investigated the formation of an inclusion complex of unsubstituted phenothiazine with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). A 1 : 1 stoichiometry was found for the complex with HP-β-CD, which has a formation constant of 80 M^{-1} [27].

In this work, the influence of the presence of β-cyclodextrin on the fluorescence properties and photooxidation reaction of Azure A in aqueous media has been studied. Also, the improvement of the analytical parameters, based on this new, β-CD-enhanced, photochemically-induced fluorescence approach, has been considered for potential applications.

2. Experimental

2.1. Reagents

Azure A (AZ, 3-amino-7-dimethylaminophenothiazinium chloride), was purchased from Fluka AG (Bush, Switzerland). β-cyclodextrin (β-CD) was obtained from Janssen Chemica. Buffer solutions were prepared from NH_3 and NH_4Cl purchased from Panreac (Spain). These analytical grade compounds were used as received without further purification. Distilled water was used throughout.

2.2. Instrumentation and experimental conditions

Fluorescence excitation and emission spectra were recorded 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 was connected via an RS-232 interface to an IBM PS\2 80386-SX microcomputer. Data acquisition and data analysis were performed by using the Perkin–Elmer fluorescence data manager software, version 2.70. The instrument working conditions were: excitation and

emission bandwidth 15 nm, photomultiplier voltage 700 V, and scan rate of spectral acquisition 240 nm/min. All measurements were performed in a 1-cm quartz cell at 20 °C, using a thermostatic cell holder and a Selecta Model 382 thermostatic bath. An Osram 200 W high pressure mercury lamp with an Oriel Model 8000 power supply was used for sample irradiation. The photolysis quartz cell was placed at a distance of 30 cm from the lamp and the solution was stirred magnetically during the irradiation. The formation constant of the complex was determined by a non-linear regression method, based on an iterative Marquard-type process. First order apparent rate constants were determined by applying the Guggenheim method [28] to the fluorescence emission spectral data.

2.3. Procedures and sample preparation

A 10^{-3} M stock solution of Azure A was prepared by dissolving the compound in various buffer solutions of pH ranging from 6 to 13. An optimal pH value of 10.5 was found to give the largest photochemically induced fluorescence signals and was used for all measurements. 10^{-5} M solutions were obtained by serial dilutions in a total volume of 25 mL containing 9 mL of buffer, and making up to the mark with distilled water. Under these conditions, AZ presented the same photophysical properties as in a solution of total buffer solvent. An aliquot of the working solution of AZ was irradiated with UV light of the mercury lamp for suitable time intervals.

For the study of the influence of the β-CD concentration on the fluorescence intensity, several solutions were prepared, by maintaining the concentration of AZ (10^{-5} M) and the buffer volume (9 mL) constant, and varying the volume of the stock β-CD solution (10^{-2} M) added. An irradiation time of 16 min was used.

3. Results and Discussion

3.1. Fluorescence properties of Azure A and its photoproduct in aqueous media

The native fluorescence of Azure A in aqueous medium (pH = 10.5) is characterized by a very low intensity. The excitation spectrum presents only one maximum located at 351 nm, and the emission spectrum shows a maximum at about 385 nm, and a shoulder in the higher wavelength region (about 422 nm).

It is well known that the fluorescence intensities and maxima wavelengths of phenothiazines increase upon UV irradiation [20–26]. This behaviour can be explained by the formation of a photoproduct which has been identified as a sulphoxide derivative produced by the oxidation of the phenothiazine sulfur group [26]. The electronic structure of the first excited singlet-state of the phenothiazine neutral photoproduct is stabilized, producing a red-shift of the excitation and emission maxima, and a higher fluorescence quantum yield. In the case of AZ, we found that, after an irradiation time (t_{irr}) of six minutes, the excitation spectrum exhibited

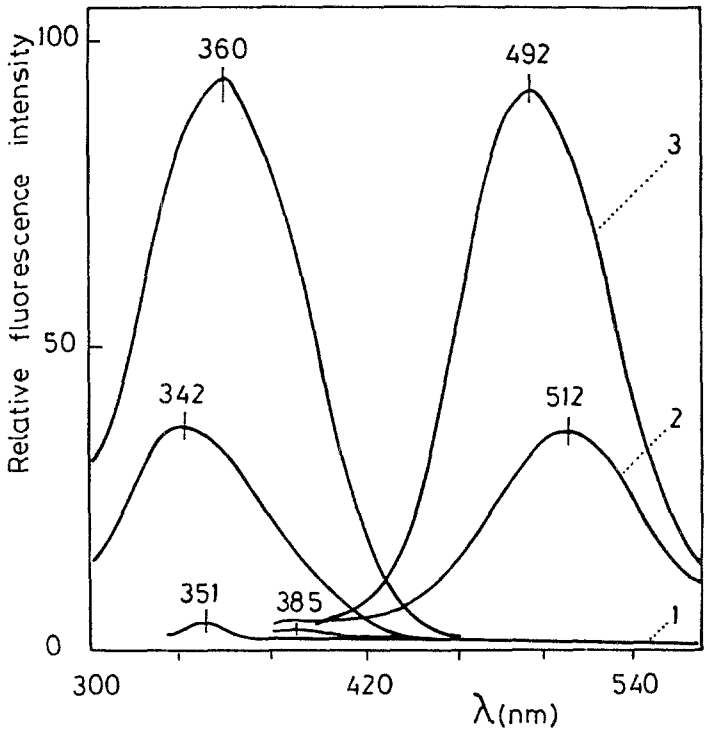


Fig. 1. Fluorescence excitation and emission spectra of Azure A (10^{-5} M) in aqueous solutions (pH = 10.5), at 20 °C: (1) before irradiation, (2) irradiated for 6 min, and (3) irradiated for 16 min, in the presence of 6.4×10^{-3} M β-cyclodextrin.

a slightly blue shifted maximum at about 342 nm, while the emission spectrum was strongly red shifted at 512 nm; both bands were strong, broad, and structureless. The fluorescence intensity was increased by about 30 times, relative to that of a non-irradiated AZ solution (Figure 1).

3.2. Effect of β-CD on the fluorescence spectra

It is well documented that aromatic compounds with one or two benzene rings can be included in the β-CD hydrophobic cavity, in aqueous media [1, 29, 30]. Usually, one of the specific characteristics of the inclusion complex formed between β-CD and the organic compound, is an enhancement of its fluorescence quantum yield relative to the original luminescence of the analyte [1]. Moreover, several authors [1, 16, 31–33], have reported that the fluorescence maxima of such inclusion complexes are shifted, to higher wavelengths for excitation main peaks, and to lower wavelengths for emission ones.

All these features are in agreement with the fluorescence experimental data we have found in our investigation of the irradiated Azure A : β-CD aqueous buffer solutions. Indeed, the photochemically-induced fluorescence spectrum of

the formed complex exhibits, relative to that of the non-complexed photoproduct of Azure A, a red-shift of about 18 nm for the excitation peak maximum, which is centered at 360 nm, and a blue shift ($\approx$ 20 nm) for the emission maximum, which appears at 492 nm (Figure 1). The two fluorescence bands remain broad and structureless as for the AZ photoproduct. In addition, the fluorescence intensity of the complex presents a three-fold increase relative to the intensity of the free Azure A photoproduct in aqueous solution (Figure 1). The latter pronounced wavelength shifts and fluorescence intensity enhancement indicate that the analyte should be included in the β-cyclodextrin host.

3.3. KINETICS OF THE OXIDATION PHOTOREACTION

In order to quantitatively evaluate photooxidation kinetics, we have studied the evolution of photoproduct fluorescence intensity with irradiation time, both for free Azure A and the Azure A photoproduct : β-cyclodextrin complex.

In fact, the fluorescence intensity increases exponentially with increasing irradiation time, reaching a plateau at about 6 min for the free Azure A photoproduct solutions, and at 16 min for AZ photoproduct : β-CD (Figure 2). It is worthwhile to note that the shape and position of emission bands remain unchanged during the photolysis time (Figure 3), indicating that the fluorescence emission mainly arises from AZ photoproduct. Also, because complexation with β-CD is known to reduce or even to inhibit the photochemical degradation of pharmaceutical drugs [34], the formation of an inclusion complex must act as an inner filter against UV irradiation. Indeed, the photostability of Azure A is improved in the presence of β-CD, as shown by the observed longer irradiation time (16 min). After the plateau region, a decrease of fluorescence intensity is noted. The last part of the kinetic plot could be explained by a secondary reaction of the Azure A photoproduct, giving a non fluorescent compound.

The treatment of the variation of fluorescence data with time, before the plateau region, was performed using the Guggenheim method [28] for first-order kinetics. Straight lines were obtained (correlation coefficients $\geq$0.98) when plotting $\ln(I' - I)$ vs. irradiation time. However, the slopes of these lines were concentration dependent, suggesting that the oxidation photoreaction follows pseudo-first-order kinetics. It was found that for 10^{-5} M solutions, the photooxidation apparent rate constant of AZ in the presence of β-CD ($k_1' = 0.20$ min^{-1}), was smaller than that of free Azure A ($k_1 = 0.28$ min^{-1}). Similarly, Flamigni [35] established recently that the photoreactivity of triplet and radical intermediates of Rose Bengal and Erythrosin B was slower in complexes with α- and γ-cyclodextrins than in water. Park *et al.* [36] found also that the hydration reaction of 1,4-dihydronicotinamide analogues was retarded by the presence of β-CD.

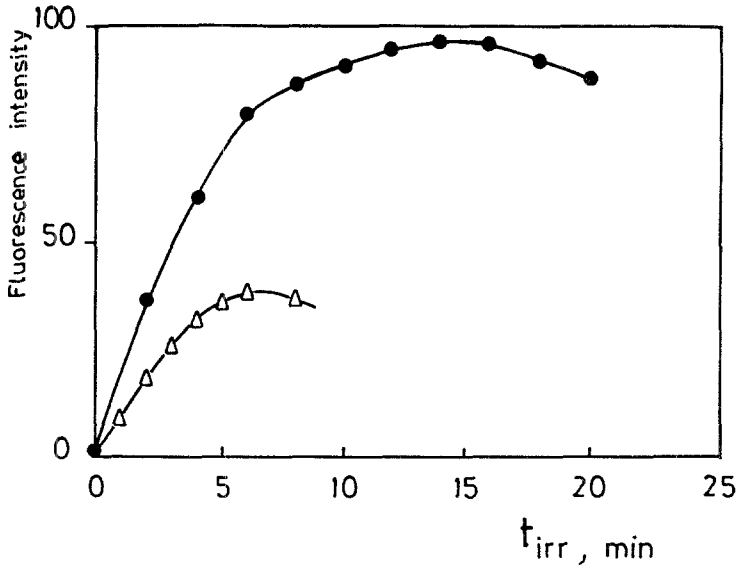


Fig. 2. Evolution of the fluorescence intensity of the Azure A photoproduct with irradiation time. (△) Free Azure A, (●) AZ : β-CD complex.

3.4. Effect of the β-CD concentration

The addition of β-CD to the Azure A solution produces an enhancement of the photochemically induced fluorescence emission of the complex. This intensity depends on the added β-CD concentration (Figure 4). Indeed, for a constant analyte concentration (10^{-5} M) and a 16-min irradiation time, the fluorescence emission intensity increases significantly with increasing β-CD concentrations from 4×10^{-4} M to 6.4×10^{-3} M. It was found that the maximum fluorescence intensity of the complex could not be reached, because of the low β-CD solubility limit. However, these fluorescence data can be used for determining the stoichiometry and formation constant of the AZ photoproduct : β-CD complex.

3.5. Characteristics of the AZ photoproduct : β-CD inclusion complex

The stoichiometry and the formation constant (K_f) of the Azure A oxidation photoproduct : β-cyclodextrin (AZ : β-CD) complex were calculated as previously [27], assuming a 1 : 1 stoichiometry ratio, according to the following equilibrium:

$$[\beta\text{-CD}] + [\text{AZ}] \rightleftarrows [\beta\text{-CD} : \text{AZ}] \quad (1)$$

It was assumed that the AZ photoproduct concentration decrease due to photodegradation was negligible under the experimental conditions used.

As can be observed in Figure 4, the fluorescence intensity of Azure A photoproduct increased on interacting with β-CD and forming the inclusion complex.

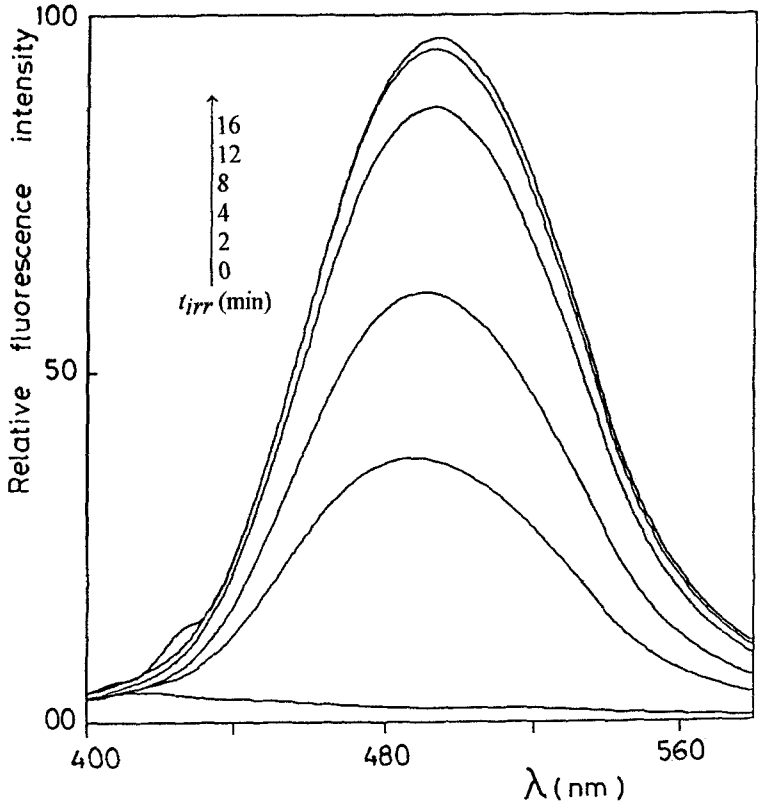


Fig. 3. Spectral changes, at various UV irradiation times, of Azure A (10^{-5} M) aqueous solutions (pH = 10.5) at 20 °C, in the presence of 6.4×10^{-3} M β-CD.

In the case of a 1 : 1 stoichiometry, the relation between this fluorescence increase and the β-CD concentration, can be represented by the following equation [37]:

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K_f[\beta - \mathrm{CD}]_0} + \frac{1}{F_\infty - F_0} \tag{2}$$

where $[\beta\text{-CD}]_0$ is the initial β-CD concentration, F_0 denotes the fluorescence intensity of AZ in the absence of β-CD, F_∞ is the fluorescence intensity when all of the AZ photoproduct molecules are essentially complexed with β-CD, F is the measured fluorescence at each β-CD concentration tested, and K_f is the formation constant of the complex. The representation of $1/(F - F_0)$ vs $1/[\beta\text{-CD}]_0$, known as a double reciprocal plot [37], allows the determination of the stoichiometry and the formation constant. If the stoichiometry is 1 : 1, a linear plot should be obtained. In the case that a 2 : 1 stoichiometry is predominant, the application of Equation (3) should give a linear plot:

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K'_f[\beta\text{-CD}]_0^2} + \frac{1}{F_\infty - F_0} \tag{3}$$

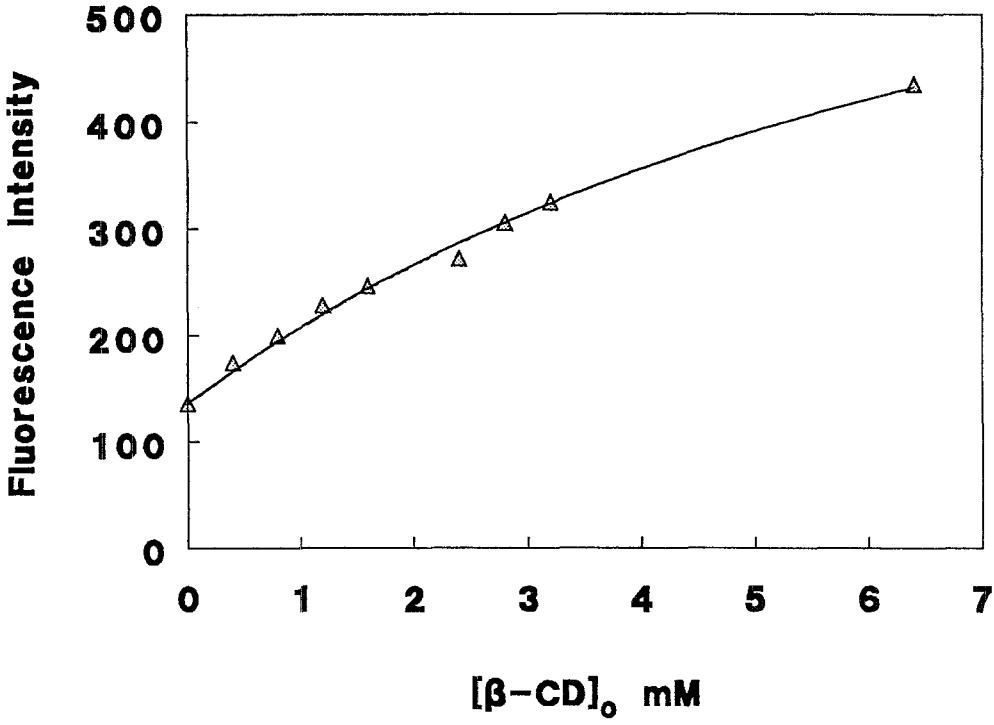


Fig. 4. Influence of β-CD concentration on the photochemically induced fluorescence intensity of a 10^{-5} M Azure A aqueous solution (pH = 10.5, t_{irr} = 16 min, T = 20 °C). The solid line was plotted according to Equation 4, assuming a 1 : 1 stoichiometry.

where K'_f is the formation constant of the complex with a 2 : 1 stoichiometry, and the remaining symbols have the same meaning as in Equation (2). Typical double-reciprocal plots for the AZ photoproduct : β-CD complex are shown in Figure 5. A linear relationship is obtained when $1/(F - F_0)$ is plotted against $1/[\beta\text{-CD}]_0$, (r = 0.99), indicating that the stoichiometry of the complex is 1 : 1 (Figure 5a). In contrast, a downward concave curvature is obtained when these data are fitted to a 2 : 1 complex, using Equation (3) (Figure 5b). The linear plot can be used to obtain K_f, by simply dividing the intercept by the slope, but Benesi–Hildebrand plots tend to place more emphasis on lower concentration values than on higher ones. As a result, the slope of the line is more sensitive to the ordinate value of the point having the smallest concentration. Therefore, a better estimation can be made using nonlinear regression analysis [38]. Rearranging the data, we obtain the direct relationship between the observed fluorescence intensity, F and $[\beta\text{-CD}]_0$:

$$F = F_0 + \frac{(F_\infty - F_0)K_f[\beta\text{-CD}]_0}{1 + K_f[\beta\text{-CD}]_0} \tag{4}$$

Equation (4) allows a direct fit of the exponential data. The initial parameter estimates needed for the nonlinear regression method have been obtained from the

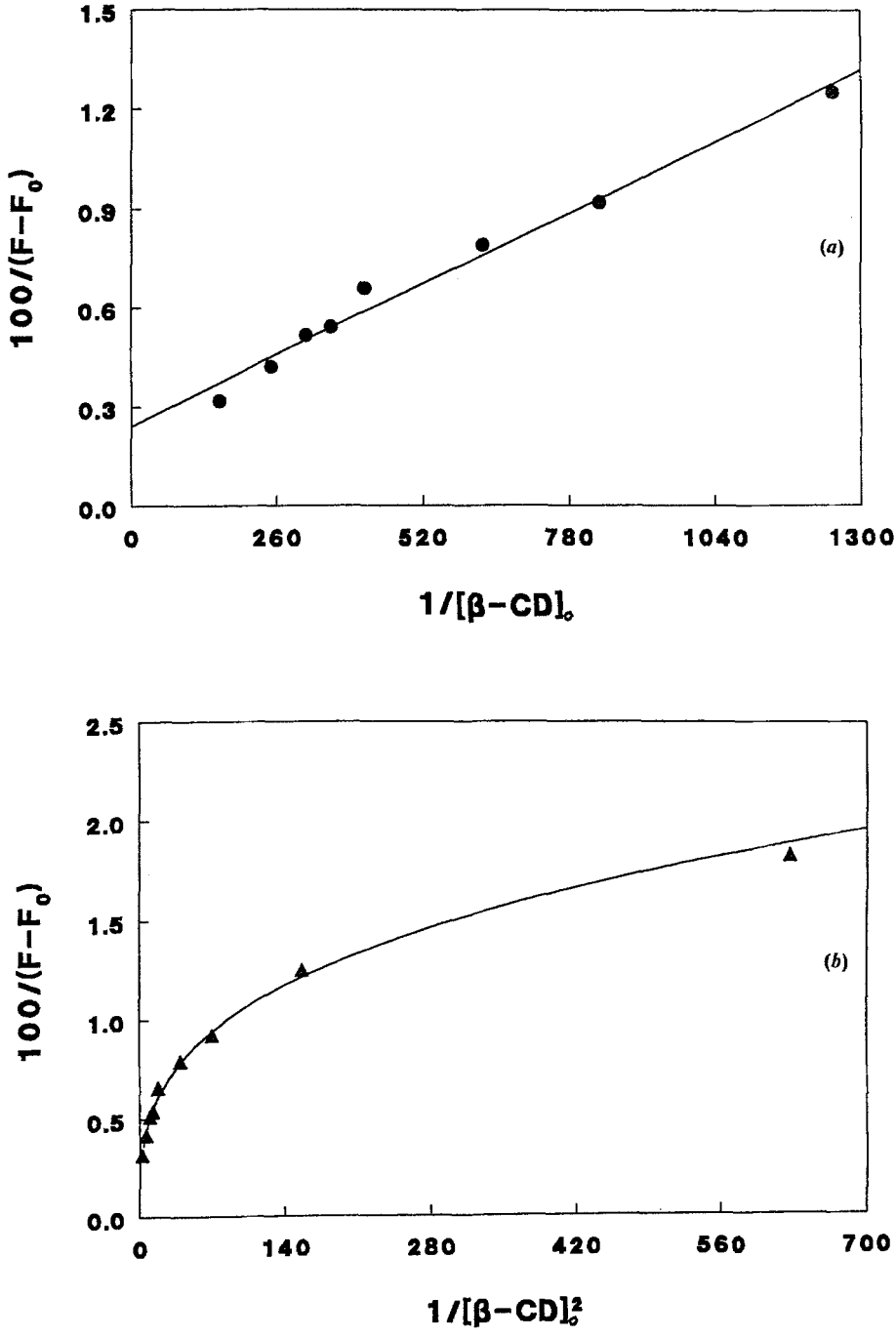


Fig. 5. Double reciprocal plots. A linear relationship was obtained when the data are plotted assuming a 1 : 1 AZ photoproduct: β-CD stoichiometry (5a), and a downward concave curvature when the data are plotted assuming a 1 : 2 AZ photoproduct: β-CD stoichiometry (5b).

linear plots. The calculated association constant is $110 \pm 40\ M^{-1}$. This formation constant of relatively low value is of the same order of magnitude as that obtained for the complex formed between unsubstituted phenothiazine photoproduct and 2-hydroxypropyl-β-cyclodextrin ($K_f = 80 \pm 40\ M^{-1}$, [27]). This result indicates that both phenothiazine photoproducts are included partially in the β-cyclodextrin cavity, as was concluded for the complex formed between β-CD and proscillaridin [1]. The latter characteristic may be due, in our case, to steric hindrance or electrostatic repulsive interaction caused by the lateral sulphoxide chromophore, formed after UV irradiation.

3.6. Analytical studies

In order to evaluate the analytical interest of this approach, we have established the calibration graphs for uncomplexed and complexed Azure A photoproduct, in aqueous media (pH = 10.5). The analytical figures of merit were also determined. The calibration graphs were constructed by plotting the fluorescence signal against initial AZ concentration, under the following conditions: λ_{ex} = 342 nm, λ_{em} = 510 nm and t_{irr} = 6 min, for free AZ, and λ_{ex} = 360 nm, λ_{em} = 491 nm and t_{irr} = 16 min, for the AZ photoproduct : β-CD complex. The ranges of linearity were 0.1–1.5 $\mu g\ mL^{-1}$ and 0.05–1.5 $\mu g\ mL^{-1}$, for free AZ and AZ photoproduct : β-CD in aqueous solutions, respectively. The limits of quantification (LOQ) and of detection (LOD) were found to be 43 and 14 $ng\ mL^{-1}$, respectively, in the absence of β-CD, and 11 and 4 $ng\ mL^{-1}$, respectively, in the presence of β-CD. This shows that the photochemical-fluorimetric detectability of AZ is significantly enhanced when complexed with β-CD. For a series of six fluorescence measurements, the relative standard deviations of the method were 5.5 and 4.6%, for free AZ and complexed AZ, respectively.

4. Conclusions

The Azure A photoproduct : β-CD inclusion complex was characterized by an important (three-fold) fluorescence signal enhancement, and a longer irradiation time (16 min), relative to the free Azure A photoproduct in aqueous buffer (pH = 10.5) solutions. The stoichiometry of the complex, obtained by a Benesi–Hildebrand plot, was 1 : 1, and a formation constant of $110 \pm 40\ M^{-1}$ was determined using the changes of fluorescence intensity upon adding different β-CD concentrations. The formation of this complex presents also an interest from the analytical standpoint, since it allows better sensitivity for quantification of Azure A.

Work is in progress in our laboratories to develop an analytical method based on complexation of 2-hydroxypropyl-β-CD and γ-CD with Azure A photoproduct. The latter self-assembled species are expected to be more fluorescent than the Azure A photoproduct : β-CD complex.

Acknowledgements

This work was supported by a Spanish–French integrated action (HF-41) of the Spanish and French governments.

References

1. K.H. Frömming and J.Szejtli: in J.E.D. Davies (ed.), *Topics in Inclusion Science: Cyclodextrins in Pharmacy*, Kluwer Academic Publishers, Dordrecht–Boston–London (1994).
2. B.W. Müller and U. Brauns: *Int. J. Pharm.* **26**, 77 (1985).
3. K.S. Esters, M.E. Brewster, A.I. Webb and N. Bodor: *Int. J. Pharm.* **65**, 101 (1990).
4. K. Uekama, M. Otagiri, H. Seo and M. Tsuroka: *Pharm. Acta Helv.* **58**, 338 (1983).
5. F.M. Andersen, H. Bundgaard and H.B. Mengel: *Int. J. Pharm.* **21**, 51 (1984).
6. V. Weiszfeiler and J. Szejtli: in O. Huber and J. Szejtli (eds.), *Proceedings of the Fourth International Symposium on Cyclodextrins*, Kluwer Academic Publishers, Dordrecht–Boston–London (1988), p. 359.
7. K. Uekama, M. Otagiri, Y. Uemura, T. Fujinaga, K. Arimori, N. Matsuo, K. Tasaki and A. Sugi: *J. Pharmacobio.-Dyn.* **6**, 124 (1983).
8. K. Dietzel, K.S. Esters, M.E. Brewster, N.S. Bodor and H. Derendorf: *Int. J. Pharm.* **59**, 225 (1990).
9. K. Uekama, M. Otagiri, A. Sakai, T. Irie, N. Matsuo and Y. Matsuoka: *J. Pharm. Pharmacol.* **37**, 532 (1985).
10. L. Szente, I. Apostol, A. Gerlóczy and J. Szejtli: *Pharmazie* **40**, 408 (1985).
11. K. Miyajma, K. Tamita and M. Nakagaki: *Chem. Pharm. Bull.* **33**, 2587 (1985).
12. R.L. VanEtten, J.F. Sebastian, G.A. Clowes and M.L. Bender: *J. Am. Chem. Soc.* **89**, 3242 (1967).
13. J.C. Márquez, M. Hernández and F. Garcia Sánchez: *Analyst* **115**, 1003 (1990).
14. (a) I. Durán-Merás, A. Muñoz de la Peña, F. Salinas and I. Rodriguez Cáceres: *Analyst* **119**, 1215 (1994). (b) A. Muñoz de la Peña, F. Salinas, M.J. Gomez, M.I. Acedo and M. Sanchez Peña: *J. Incl. Phenom.* **15**, 131 (1993).
15. F. Cramer, W. Saenger and H.-CH. Spatz: *J. Am. Chem. Soc.* **89**, 14 (1967).
16. J.W. Park and K.H. Park: *J. Incl. Phenom.* **17**, 277 (1994).
17. S.G. Dahl: in E.Usdin, S.G. Dahl and L.F. Gram (eds.), *Clinical Pharmacology Psychiatry: Neuroleptic Antidepressant Research*, Macmillan, London (1988), pp. 125–137.
18. G.J.S. Foncer, R.C. Reej and R. Devonshire: *Photochem. Photobiol.* **52**, 489 (1990).
19. R.R. Gupta: *Phenothiazines and 1,4-Benzothiazines: Chemical and Biomedical Aspects*, Elsevier, Amsterdam (1988).
20. J.J. Aaron: in S.G. Schulman (ed.), *Molecular Luminescence Spectroscopy*, Part 3, Wiley, New York (1993), p. 85.
21. D. Chen, A. Rios, M.D. Luque de Castro and M. Valcarcel: *Analyst* **116**, 171 (1991).
22. D. Chen, A. Rios, M.D. Luque de Castro and M. Valcarcel: *Talanta* **38**, 1227 (1991).
23. J. Martinez Calatayud and C. Gomez Benito: *Anal. Chim. Acta* **256**, 105 (1992).
24. B. Laassis, J.J. Aaron and M.C. Mahedero: *Anal. Chim. Acta* **290**, 27 (1994).
25. B. Laassis, J.J. Aaron and M.C. Mahedero: *Talanta* **41**, 1985 (1994).
26. E.O. Fadiran and A.G. Davidson: *J. Chromatogr.* **442**, 363 (1988).
27. J.J. Aaron, B. Laassis, M.C. Mahedero, A. Muñoz de la Peña and F. Salinas: *J. Incl. Phenom.* **18**, 69 (1994).
28. E.A. Guggenheim: *Phil. Mag.* **1**, 538 (1926).
29. W. Baeyens, B. Ling, V. Corbisier and A. Raemdonck: *Anal. Chim. Acta* **234**, 187 (1990).
30. J. Szejtli: *Cyclodextrin Technology*, Kluwer Academic Publishers Dordrecht–Boston–London (1988).
31. J. Szejtli: *Cyclodextrins and Their Inclusion Compounds*, Akademic Kiado, Budapest (1982)
32. W. Baeyens, B. Ling and V. Corbisier: *Analyst* **115**, 359 (1990).
33. R.P. Frankewich, K.N. Thimmaiah and W.L. Hinze: *Anal. Chem.* **63**, 2924 (1991).
34. M.F. Andersen and H. Bundgaard: *Int. J. Pharm.* **19**, 189 (1984).
35. L. Flamigni: *J. Chem. Soc. Faraday Trans.* **90**, 2331 (1994).

36. J.W. Park, S.Y. Cha and K.K. Park: *J. Chem. Soc. Perkin Trans. 2* 1613 (1991).
37. V.K. Smith, T.T. Ndou, A. Muñoz de la Peña and I.M. Warner: *J. Incl. Phenom.* **10**, 471 (1987).
38. K.A. Connors: *Binding Constants: The Measurement of Molecular Complex Stability*, J. Wiley and Sons, New York (1987).