IS THE DETERMINATION OF THE ASSOCIATION CONSTANT OF CYCLODEXTRIN INCLUSION COMPLEXES DEPENDENT ON THE TECHNIQUE

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ABSTRACT

Differences in association constants values of cyclodextrin inclusion complexes can be found in the literature. Most of the times, different experimental conditions have been used leading to different results. This paper reports the association constants (K_a) values of two different cyclodextrins (CD), namely native beta-CD (βCD) and hydroxypropyl-βCD (HP-βCD) with bentazon (BTZ, herbicide used in control of broadleaf weeds and sedges in many crops). These constants were determined by spectrophotometric and fluorescence measurements carried out under the same experimental conditions, and they were compared with those previously obtained by electrochemical techniques. The association constant values for the BTZ/HP-βCD inclusion complex obtained by fluorescence measurements were lower than those expected taking into account the values obtained from the other techniques.

Differences in the complexation of the guest with CD in the excited and basal state could explain these results.

Keywords: cyclodextrin, inclusion complexes, bentazon, association constant.

1. INTRODUCTION

In supramolecular chemistry, the formation of a complex between a host and a guest is a measure of selectivity. When a supramolecular complex is employed as a drug carrier the association constant is a direct measure of host bioavailability. As mentioned by Chadha and col. *"high values of equilibrium constant (>5000 kg/mol) may lead to very slow release of drug from complex while a low value (<200 kg/mol) reduces the effect that inclusion complexation has on the bioavailability of the drug"* [1]. Thus, determination of the association constant (K_a) of inclusion complexes involving cyclodextrins is an important topic in different areas of application.

It is well-known that different techniques can be used for determining association constants. A full review with the description of the methodologies and techniques was made by Chadha and col[1]. However, sometimes, it seems that the results obtained for the association constants are dependent on the technique used for their determination. We have used previously electrochemical and spectrophotometric techniques to obtain the association constant of a bentazon/cyclodextrin inclusion complex using b-cyclodextrin (βCD)[2] and hydroxypropyl-bcyclodextrin (HP-βCD)[3]. Our results are similar to those reported by Porini and Escandar[4] who used fluorescence measurements in the case of a native βCD inclusion complex, but differ from the values reported in the case of BTZ/ HP-βCD inclusion complex.

As far as we know, most of the times the studies where association constants are determined employ one or another technique, but few comparative studies can be found [5,6] which allow associating the differences observed in the determination of K_a to the use of a particular methodology. Thus, in this paper, with the purpose of verifying the association constant values of cyclodextrin inclusion complexes, spectrophotometric and fluorescence measurements have been carried out and compared with previous results. These experiments are aimed at determining if the differences observed by other authors using fluorescence measurements could be ascribed to the methodology employed.

2. EXPERIMENTAL

2.1. Chemicals

β-cyclodextrin (βCD) and (2-hydroxypropil)-β-cyclodextrin (HP-βCD, Mw ~1,460) were obtained from Calbiochem and Sigma-Aldrich, respectively, and used without prior purification. Bentazon (BTZ) was supplied by Sigma-Aldrich. All other reagents employed were analytical grade. All solutions were prepared with ultrapure water (18.2 MW cm) obtained from a Millipore Milli-Q system.

2.2. Apparatus

Spectrophotometric measurements were carried out using a Hitachi U-2910 spectrophotometer with 1 cm quartz cell. The data were recorded using UV Solutions Application software 2.2. Fluorescence emission measurements were performed in a PC1 spectrofluorimeter from ISS, thermostated at 25°C. λ_{ex} 334 nm, λ_{em} 435 nm, slits 1mm excitation and emission. Data were processed with Vinci software.

2.2. Methods

For all experiments, 0.5 mM aqueous solutions of BTZ were prepared. Homogeneity of the initial solutions was assured by sonicating them in an ultrasonic bath for one hour followed by constant agitation for two hours using a magnetic stirrer. In order to avoid analyte dilution, working solutions, where BTZ concentration was kept constant while CD varied from 0 to 10 mM, were prepared taking the appropriate mass of CD and using the adequate volume of initial BTZ solution. Each solution was kept under agitation for 12 hours and stored at 4 °C for 24 hours. All the solutions were protected from light by keeping them in dark flasks and the absorbance and/or fluorescence emission was monitored as a function of CD concentration. All the measurements were made in triplicate.

The association constant of the inclusion complexes were determined employing the Benesi-Hildebrand method[7], where a plot of the inverse of difference between emission from complex and free BTZ against the reciprocal of βCD concentration, allows to obtain the association constant from the slope, and the linearity of the plot assures 1:1 stoichiometry

3. RESULTS AND DISCUSSION

As previously mentioned, we have studied complexation of BTZ with βCD and HP-βCD (figure 1) using both electrochemical and spectrophotometric techniques[2,3]. The association constants (K_a) for BTZ/βCD and BTZ/HP-βCD obtained before by us using differential pulse voltammetry were 118 ± 20[2] and 244 ± 19[3], respectively. Spectrophotometric technique had been used only for complex BTZ/βCD. The K_a value obtained was 140 ± 7[2]. All the K_a values obtained by these techniques are collected in Table 1. The stoichiometry 1:1 of the complexes has been determined in these previous studies and is supported by the results of the fitting performed with the Benesi-Hildebrand method[7].

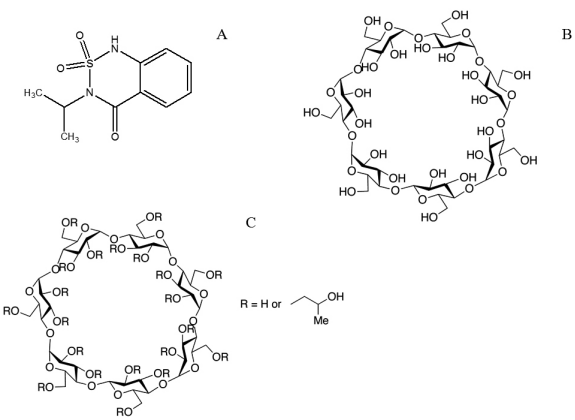

Figure 1: Molecular structure of bentazon (A), β-cyclodextrin (B) and (2-Hydroxypropil)-β-cyclodextrin (C)

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In our current study, the K_a for BTZ/ HP-βCD has been determined by UV-Vis spectrophotometry and also K_a for both BTZ/βCD and BTZ/HP-βCD have been obtained by fluorescence, all of them under the same experimental conditions

In the spectrophotometric titration employing UV-visible spectrophotometry, a decrease of the absorbance is observed as the concentration of the CD derivative is increased (figure 2A). The absorption maximum at 332 nm shows no shift with the increase in CD concentration. The induced changes in absorbance are attributed to the formation of inclusion complexes[8,9], because, although small changes are observed, they suggest that the chromophore of the guest is carried from the aqueous medium to the non-polar cavity of the CD similarly to the effect provoked by changes in polarity of solvent. According to previous discussions[1,9], direct interaction of the guest with the CD or exclusion of solvating water molecules, or a combination of both effects, could promote the perturbation of the electronic energy levels resulting in a change in absorbance. The analysis of the data using the Benesi-Hildebrand equation permits obtaining the association constant. Figure 2B shows the reciprocal of the absorbance variation as a function of the inverse of the BTZ/HP-βCD complex concentration together with the linear fit obtained from the Benesi-Hildebrand method. The association constant determined from these data considering a 1:1 inclusion complex is 260 ± 20 M^{-1}. The inset of figure 2B shows the non-linear plot of absorbance variation against CD concentration.

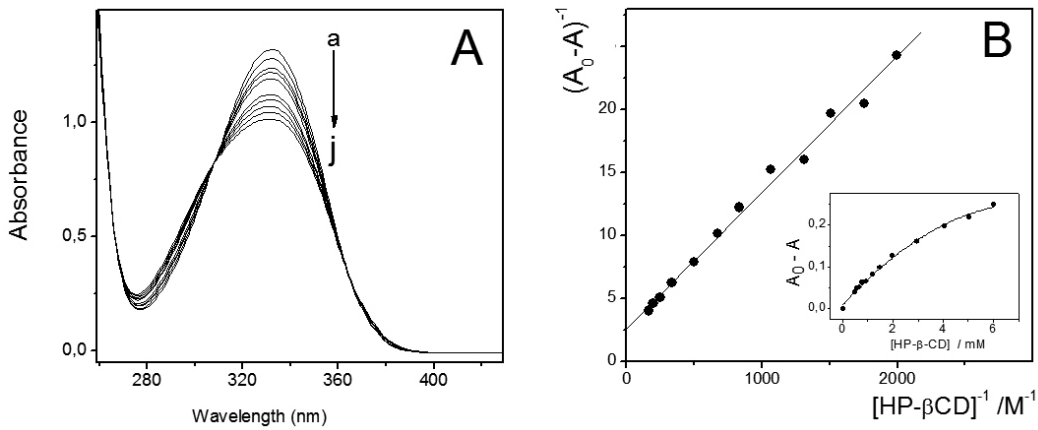

Figure 2. (A) Absorption spectra of bentazon, at constant concentration (0.5 mM) in absence and presence of different concentrations of HP-βCD. Curves a – j: 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10 mM of HP-β-CD. (B) Benesi-Hildebrand plot for BTZ/HP-βCD 1:1 inclusion complex. Inset: Absorbance variation (ΔA) as a function of HP-βCD concentration. Inset: Plot of variation of absorbance against CD concentration.

As we can see in Table 1, the K_a values are slightly higher than those obtained by differential pulse voltammetry (DPV). However, this difference is not significative. Besides, as it has been noted by Liu et al[10], the errors in the values of the association constants determined via spectrophotometric titration are usually larger than those obtained by other techniques (calorimetry, for example)

Table 1: Association constant for BTZ/β-CD and BTZ/HP-β-CD systems by different techniques,

Standard deviations are calculated on triplicate trials.

*:quadruplicate trails

	K_a /M^{-1}			
Complex	***DPV***	***UV-Vis***	***Fluorescence***	***Literature value*** [ref 4]
BTZ/β-CD	***118 ± 20*** [ref 2]	***140 ± 7*** [ref 2]	***102 ± 5***	***105 ± 8***
BTZ/HP-β-CD	***244 ± 19*** [ref 3]	***260 ± 20***	***103 ± 30****	***134 ± 14***

At this respect, it is also interesting to note that while the K_a values for BTZ/HP-βCD obtained from both electrochemical and spectrophometric measurements are not too different, they are almost twice larger than the value reported by Porini and Escandar determined by fluorescence procedures[4]. In many occasions, the values of the association constants obtained within a same study are in reasonable agreement even if different techniques are used for their determination[2,5]. The use of different experimental conditions has been invoked[6] to be the origin of the discrepancies observed with the values obtained by other authors.

Therefore, we carried out fluorescence titration measurements on both BTZ/βCD and BTZ/HP-βCD systems under the same experimental conditions employed for spectrophotometric determinations to check if different values were also observed.

In figure 3A we show the BTZ fluorescence spectrum in the presence of increasing amounts of βCD. The increase of cyclodextrin (either native or its propyl derivatives) induces an enhancement of the emission intensity accompanied by an important hypsochromic shift of the emission band (shift of ~40 nm). The appearance of an isoemissive point is not observed. The double reciprocal plot of $1/ I_{440} - I_0$ against 1/ [CD] is showed in figure 3B, where I_{440} is the intensity of fluorescence at 440 nm in the presence of increasing amounts of CD, and I_0 is the emission intensity in the absence of CD. The inset shows the non-linear behavior of fluorescence of BTZ as a function of CD concentration.

This information indicates that BTZ is inside the less polar cavity of the CD, where it has a higher fluorescence quantum yield and emits at shorter wavelength. As can be seen, the fluorescence intensity is drastically increased while smaller changes can be observed in the UV spectrum with the same concentrations of HP-βCD

On the basis of our results, it seems difficult to conclude if the association constant obtained is dependent on the technique used for its determination. While similar association constant values were obtained by the three different techniques in the case of βCD inclusion complex, much larger K_a values were obtained by DPV and UV-Vis than by fluorescence titration in the HP-βCD case. According to the tendency observed in the βCD complex, a K_a value similar to those obtained by DPV and UV-Vis would be also expected for BTZ/HP-βCD when measured by fluorescence procedures. However, this is not the case, and a much smaller association constant value was obtained. Even more, the values that we have determined in this paper using fluorescence titration are almost identical for βCD and HP-βCD. If the results reported by Escandar[4], who also used fluorescence techniques determinations, are taken into account we observed a similar trend: reported constants for βCD and HP- βCD are 105 ± 8 and 134 ± 14, respectively, no showing a distinct effect as consequence of the modification of the CD rim.

Taken together, all these facts suggest that there are inherent characteristics to the fluorescence measurements that can influence the determination of the association constants. Indeed, when fluorescence techniques are employed, the different binding ability in the ground state and in the excited state might explain the differences found for the constant values. According to Valuer[11] excitation upon light absorption could induce conformational changes or the charges in the excited state might suffer a redistribution modifying the electrostatic interactions in the host-guest complex, affecting association phenomena. Besides, different constants might also be explained if during the lifetime of the excited state, association and/or dissociation processes occur. Additionally, we would like to mention that dynamic studies on the entry/exit rate of guest from the CD cavity have allowed determining association rate constants of excited states, and in the specific case of xantones, differences of several orders of magnitude can be observed when compared with ground state[12]

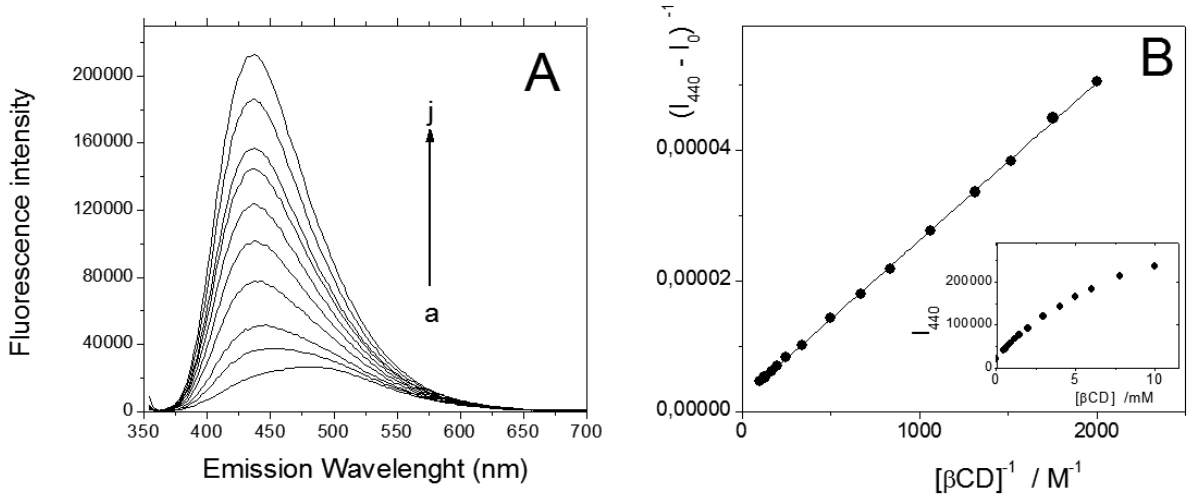

Figure 3 (A) Emission fluorescence of bentazon at constant concentration (0.5 mM) in absence and presence of different concentrations of HP-βCD. Curves a – j: identical concentrations as figure 2. (B) Curve-fitting plots for the fluorometric titration: Inset: Fluorescence intensity as a function of -βCD concentration

4. CONCLUSIONS

Although the same experimental procedures and data analysis, using a previous experimentally determined 1:1 stoichiometry of the complexes, have been applied to obtain association constants, we have found differences in the values observed mainly when fluorescence procedures have been employed. Since association constants of guest with cyclodextrins are obtained evaluating the changes of a particular property of the isolated guest and of the host-guest complex, conformational changes or modifications in electrostatic interactions in the complex could affect the association phenomena. These changes might be more significant in the case of fluorescence techniques. In these latter techniques new species are involved (excited state guest) which can associate with CD in a different way resulting in different association constants. If dynamic information about excited state processes is not available, a good practice is to compare constant values with a method which only involves ground state guest participation.

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