Usefulness of cyclodextrins for detection in molecular fluorescence. Application to xenobiotics and drugs

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Cyclodextrins (CDs) present the unique ability to entrap molecules. In analytical chemistry, they have been mainly used for separation purpose. The present review deals with their potential interest towards the detection by molecular fluorescence of the included compounds mainly xenobiotics and drugs.

As some of the included molecules exhibit native fluorescence due to their aromaticity, the addition to their aqueous solution of CDs entails, in some cases, a large enhancement of their emission of fluorescence. This feature appears of particular interest from an analytical point of view, either for direct detection of the guest molecule or as a detection mode after separations techniques (*i.e.:* chromatography or electrophoresis). Decrease in rotation motion of entrapped molecule and (or) decrease in solvent relaxation appear as the main causes of the enhancing emission effect.

Some example picked up from pharmaceutical and bioanalytical literature are presented in order to demonstrate the interest of CDs in the field of the fluorescence techniques.

Introduction

Cyclodextrins (CDs) are cyclic oligomers of α 1-4 linked D-glucose and can form inclusion complexes with a variety of organic compounds in aqueous solution. The α, β, γ, cyclodextrins have six, seven and eight glucose units respectively.

They are called native cyclodextrins as they are issued from *bacillus macerans* fermentation. The size of the internal cavity of native cyclodextrins increase from circa 5 Å for α CD to 8 Å for γ CD. Consequently, the geometry of the guest compound is the first factor that rules the inclusion process. In other words, the internal diameter of the cyclodextrins derivative controls the entire or partial inclusion of the guest molecule. Moreover, the hydrophobicity of the organic compounds is also of crucial importance with regards to the fit of the molecule in the internal cavity of CDs. It is assumed that the driving forces of such interactions are: hydrogen bonding, van der Waals forces or hydrophobic interaction [1,2]. Native CDs give rise to numerous hemisynthetic derivatives by grafting the outer hydroxyls of the molecules by various functional groups either hydrophobic or hydrophilic [3] in order, for instance, to decrease or increase their water solubility. This inclusion capability, common at various degrees, to all CDs derivatives, makes them good candidates for molecular recognition and thus, explain their extensive chromatographic and electrophoretic use especially for chiral separation [4]. On the other hand, the inclusion capability of CDs are of great interest in stabilizing, solubilizing substances [5]. Selective precipitation or extraction can be, also, achieved using CDs [5]. Without claiming to be exhaustive, the aim of this short report is to underline the potential interest of CDs in an other field of analytical chemistry: fluorescence spectrometry to improve the sensitivity and selectivity of the detection of included compounds.

Preliminary considerations

The first observation of fluorescence enhancement upon inclusion in βCD aqueous solutions of the fluorophore: 1 anilino-8-naphtalene sulfonate (ANS) was due to Cramer in 1967 [6]. This prompted many authors to use fluorescence techniques as a spectroscopic tool to study the mechanisms of the inclusion process in solution. As an example, Harada and coworkers [7] as early as 1976 showed that the fluorescence of 2-p-toluidinyl naphtalene sulfonate (TNS) was increased by 25 times with βCD and up to 25 and 30 times with polymers containing cyclodextrins. The authors already stated that the fluorescence increase observed with TNS was mainly due to the restriction of intramolecular rotation in the rigid environment of the cavity and/or to the exclusion of solvent relaxation. The direct consequence is a decrease in the vibrationnal desactivation. Thus, the CDs acts as

Article available at http://analusis.edpsciences.org or http://dx.doi.org/10.1051/analusis:2000280664

spectroscopic shield, protecting the fluorescing singlet state or the phosphoring triplet state (see the article of Muñoz de la Peña *et al.* in this issue) from the external quenchers. Actually, the CDs cavity looks like an organic solvent surrounding the entrapped molecules, which often yields to higher quantum efficiency. Modifying the microenvironment of the caged analyte can, also, be interpreted as increasing the water solubility by complexation of a hydrophobic molecule.

In that kind of pioneering work, the authors focused their efforts on the quantitative aspects of the inclusion process (*i.e.*: Job's plot for stoichiometry determination, affinity constant calculation). These early studies were confirmed by others [8] for instance in an inclusion study using benzene derivative probes that showed that the fluorescence enhancing was ascribed to the increase of the radiation rate constant (*kr*) of the excited state of the entrapped molecules.

Once more, it should be pointed out that these elegant spectroscopic [9-10] studies were fundamentally conducted with the aim of getting a better insight in the host-guest interaction but without any analytical application purpose. The fluorescence probe acted in such a system only as a "molecular spy" to elucidate the supramolecular interactions involved between various cyclodextrins derivatives and guest organic species. Though, out of scope of our analytical purpose, it is worth noting that this probing approach still remains, of great interest, since it allows rapid, and simple assessment of physicochemical characteristics of newly synthesized cyclodextrins [11-13]. At last, in the case of CDs complexes of native fluorescent compound, fluorescence is often used for the determination of stoichiometry and association constants [14-16].

In the same time, it was rapidly established that such fluorophore/cyclodextrins inclusion complexes did not systematically lead to a fluorescence increase. Indeed, the steric constraints of the guest molecule, the nature and the intensity of the feeble forces involved in the supramolecular interaction rule the fit between host and guest and were responsible of the subsequent emission change of the guest. As an illustration, an increase of about 6 fold of the fluorescence of 5-methoxypsoralen has been reported whereas its structural isomer: 8-methoxypsoralen did not exhibit any fluorescence increase upon addition of βCD [17]. In such a way, the dual analytical interest of using CDs for luminescence detection *i.e.:* sensitivity and selectivity is clearly underlined.

Whatever the exact mechanism involved, in the CDs/organic species interactions, and keeping in mind that our goal is only to depict analytical applications, the following examples will attempt to illustrate the interest of CDs in molecular fluorescence spectrometry.

Increase in molecular fluorescence using CDs by solubilizing effect

This aspect can be clearly illustrated by the work of Garcia-Sanchez [18-19] on the analysis of scandium and beryllium. These two metallic ions were fluorometrically determined by means of authraquinonic ligands, *i.e.*: 1,4–dihydroxyanthraquinone and 1-amino-4 hydroxyantraquinone, respectively. Neither of these ligands was water soluble enough to prepare convenient working solutions of the analytical reagents. In contrast, in the presence of 10^{-2} M βCD, 10^{-4} M aqueous solutions of both ligands could be prepared and thus permitted trace determination of the metallic ions of interest. This resulted from a partial inclusion of the reagent in the β-CD cavity. As analytical consequence, a significative increase in sensitivity was obtained, *i.e.*: for beryllium the limit of detection (LOD), as impurity in organic material, was lowered to 3 ng/ml in the presence of β-CD instead of 17 ng/ml in the absence of CDs.

Similarly, the water insoluble and non fluorescent pigment retinal exhibited, after addition of β-CD, a strong emission due to the solubilizing effect after encapsulation in the interior of the β-CD cavity [20].

Increase in molecular fluorescence by affecting the vibrational motion of the caged analyte

Direct analytical methods

The first analytical uses of cyclodextrins as fluorescence enhancer were reported in the mid-eighties. The emission of luminol derivatives showed a 7 fold increase in terms of chemiluminescence, but only 2 fold in molecular fluorescence in presence of β-CD. Considering the general use of luminol in chemiluminescence techniques, this result is of considerable analytical interest [21].

Another analytical application was due to Garcia-Sanchez and his group concerning the determination of the local anesthesic drug: procainamide hydrochloride in solid dosage forms [22]. In this work, the authors associated the use of β-CD and synchronous second derivative spectrofluorimetry with the aim of simplifying the sample treatment and increasing both the selectivity and the sensitivity of the determination. It was demonstrated that β-CD alleviates the interferences due to some fluorescing excipients and the use of second derivative synchronous fluorimetry instead of direct measurement ensured to the technique a remarkable selectivity. Following these first analytical works, most of the published methods in the field involved β-CD due to its low cost, although having a limited water solubility (~ 0.011 M at room temperature). In this respect, determination of warfarine [23], warfarine and bromadiolone [24], thiols benzoxadiazole derivatives [25] and other *o*-phtalaldelyde derivatives [26] have been reported. Some examples of use of CDs for determination of hallucinogenic drugs were also cited [27].

It was only in the early nineties that authors paid attention to the other CDs derivatives, *i.e.*: the more soluble native α and γ CDs; but also the hemisynthetic derivatives:

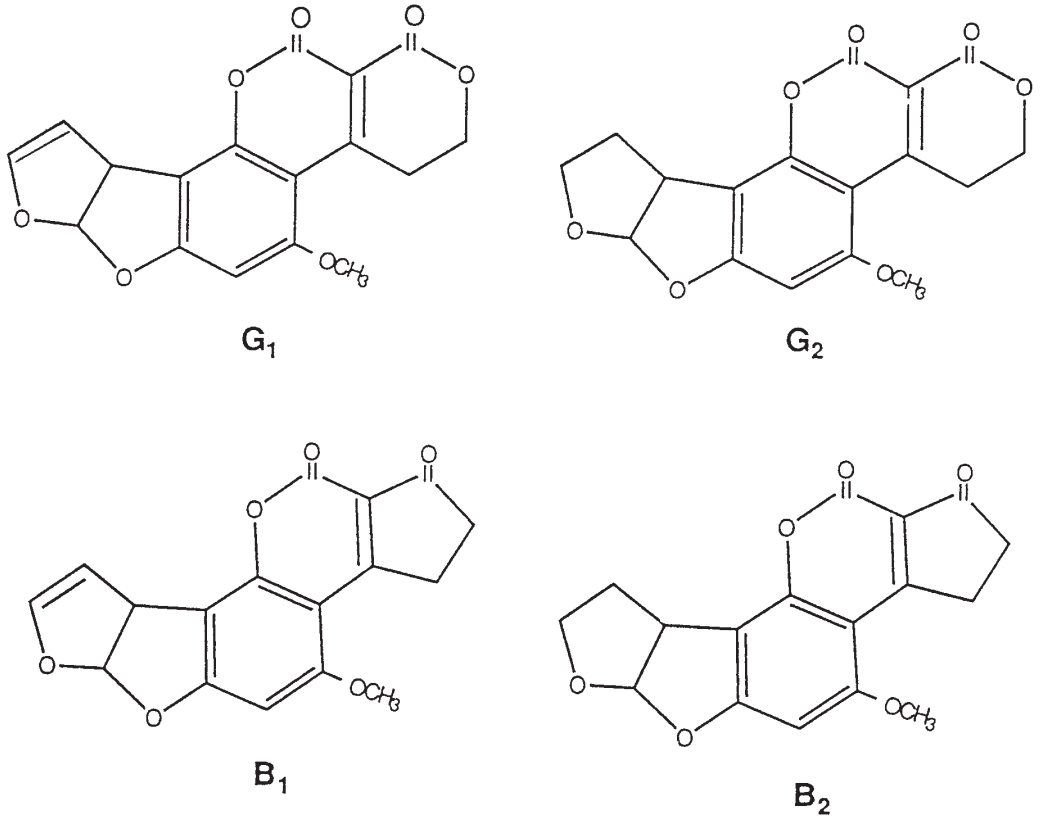

Figure 1. Chemical formulae of the insaturared B_1, G_1 and satured B_2 and G_2 aflatoxins.

hydroxypropyl β-CD (HP β-CD) heptakis *o*-dimethyl β-CD (DM β-CD). As a common feature, all these compounds exhibit a higher water solubility than the parent β-CD, allowing the use of a larger excess of reagent available for the complexation process.

As an illustration, Vazquez *et al.* [28] demonstrated the superiority of the DM-β-CD over all the other tested CDs derivatives for enhancing the fluorescence of B_1 and G_1 aflatoxins.

In the same paper, the selective effect of the inclusion process was, once more, demonstrated by the inefficiency of the addition of CDs on the fluorescence of the furanic saturated B_2 and G_2 aflatoxins which are distinguished from their saturated homologues B_1 and G_1 only by a small structural difference, as shown in figure 1.

The use of other native cyclodextrins than β-CD was finally rare. This may be due to the steric hindrance brought by the smaller α CD cavity, which limits the inclusion capability. On the other hand, the wider cavity of γ CD limited the enhancing effect certainly by lack of fit between the host and various guest compounds. Some exceptions were, nevertheless, reported as for the antibacterial agent: nalidixic acid whose enhanced fluorimetric determination in pharmaceuticals and urine was made possible by using the native γ CD [29]. Other further applications confirmed the interest of DM β-CD and allowed the detection of the antioxidant agent hesperidine [30]. Some other attractive approaches combined photochemically induced fluorescence (PIF) and CDs addition. In this case, βCD and HP βCD were used for the determination in tap and river water of pesticides: coumatetralyl, pirimiphos-methyl, chlorpyriphos, deltamethin and fenvalerate [31]. In that approach, the fluorescent photoproducts exhibited a drastic increase in their fluorescent signal after CDs derivatives addition, which permitted the determination of pesticides traces with limits of detection ranging between 0.2 and 54 $ng.ml^{-1}$ depending on the compound.

Tetracycline determination in urine was performed using an ethyl substituted β-cyclodextrin fluorosensor, consisting of a flow cell packed with 2, 6 *O*-diethyl β-CD immobilized on silica gel. In such a way, limits of detection were 4, 7.5 and 9 mM for tetracycline, oxytetracycline and chlotetracycline, respectively. The authors noted the lack of specificity of the method when a large excess of bivalent cations complexed tetracycline and directly enhanced the native fluorescence of the antibiotic. In contrast, most of the endogenous species are ineffective towards the selectivity of the detection [32].

Detection in separation methods

Although direct analytical methods based on the CDs derivative fluorescence enhancement effect should be preferred due to their simplicity and rapidity in many cases the complexity of the matrix (*i.e.*: in biological media) requires the use of a separation technique. In that context, the way of CDs derivative addition in the chromatographic system must be carefully considered.

If the CDs derivative are directly added in the eluent, it must be kept in mind that spiking the mobile phase with such compounds can lead to drastic changes in the elution profile in comparison to what happens in absence of cyclodextrins. This chromatographic influence is largely plurifactorial and depends on various factors, including the CD concentration, the polarity of both mobile and stationnary phases, etc. In some cases, it can benefit to the detection by reducing the band spreading; conversely, selectivity and resolution can be altered.

When CDs are added in a post-column mode, a pure spectroscopic effect is expected and the convenient mixing of the eluent with the post-column reagent and its duration is the keypoint. Moreover, a post-column device generates band spreading due to extra column effect which should be minimized by using appropriate mixing tee, coils and small inner diameter tubings, in order to limit the void volumes in the whole system. As in the previous paragraph, some examples will illustrate the interest and limitation of the use of CDs derivatives as sensitizer of molecular fluorescence in separation sciences.

Cepeda-Saez *et al.* [33] reported the enhancing effect of 10^{-2} M β-CD addition in methanol/water eluent to separate, in reverse phase liquid chromatography, the antipsoriatic drug: 5-methoxypsoralen (5-MOP) from its parent compound: psoralen (PSO), used here, as internal standard. Figure 2 summarizes quite well the spiking effect of βCD on detection: first, a decrease in retention time (Fig. 2b and c) reducing the band spreading but also the resolution; second a whole enhancing effect on the emission (Fig. 2c) concomitant with its selectivity (*i.e.*: absence of influence on PSO itself) and a signal increase due to the chromatographic effect discussed above and to the spectroscopic enhancement resulting from inclusion inside of the β-CD cavity. The proposed method leads to a LOD of 2.25×10^{-9} M for 5-MOP and exhibits a circa 5 fold gain in sensitivity in comparison to the same method used without βCD.

The field of the aflatoxins determination is also a good example of the attention paid by analytical chemists to the enhancing effect of CDs on fluorimetric detection of xenobiotics due to their very similar structure and their high toxicity. Francis and coworkers were the first to report the

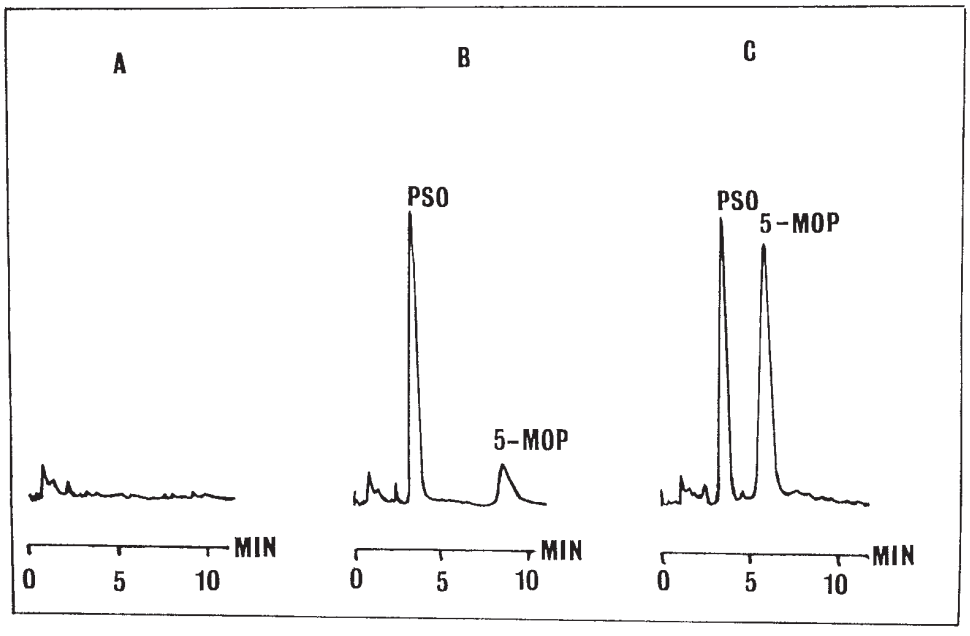

Figure 2. Typical chromatogram of spiked serum with 5-methoxypsolaren (5-MOP) and Psoralen (PSO, Internal Standard) from ref. [33]. (a): blank serum (b): fluorimetric detection with mobile phase free of β-CD (c): fluorimetric detection with the same eluent with 10^{-2} M β-CD added.

potential interest of a post column addition of β-CD to increase the detection of B_1 and G_1 aflatoxins fluorescence. They showed the selective increase in fluorescence signal of the unsaturated B_1 and G_1 aflatoxins [34].

Similarly to the direct methods reported in the previous paragraph, it was demonstrated that DM β-CD gave rise to the best gain in fluorescence as shown for the determination of B_1 and G_1 aflatoxins in food analysis [35] or more recently, for B_1 aflatoxin and its metabolites P_1 in urine [36] as illustrated in figure 3.

Finally, in capillary zone electrophoresis (CZE), if the addition of CDs derivatives to the electrolyte buffer is one of the most popular way of improving the separations, there are a few examples using of CDs with the goal of fluorescence detection enhancement.

βCD and HP βCD slighly enhance the fluorescence of 1-cyano-2-substituted benz[*f*] isoindole fluorescent derivatives of glutamate and aspartate issued from rat brain dialysate [37]. This fluorescence increase remains limited and is of the order of 1.70, for both derivatives, relative to a solution without CD. Moreover, addition of CD yields an optimum resolution between the two derivatized aminoacids. Recently, γ-CD was used in micellar electrokinetic chromatography (MEKC) to entrap a visualizing fluorescent amino agent belonging to the oxarine series. The latter compound is displaced from the γ CD cavity when water pollutants such as amino substituted polycyclic aromatic hydrocarbons (amino-PAH), are injected.

Due to the pH value of the buffer (about 4-5) amino-PAH are protonated and thus, easily compete with oxarine 750®, the encapsulated visualizing agent the subsequent displacement leads to a reduction in fluorescence and thus, to negative peaks. The real interest of introducing γ-CD in the buffer is to increase the fluorescence emission of the visualizing compound and so, to increase the sensitivity of indirect detection when the displacement occurs.

Conclusion

This brief overview on the analytical interest of CDs in molecular fluorescence spectroscopy was an attempt to illustrate,

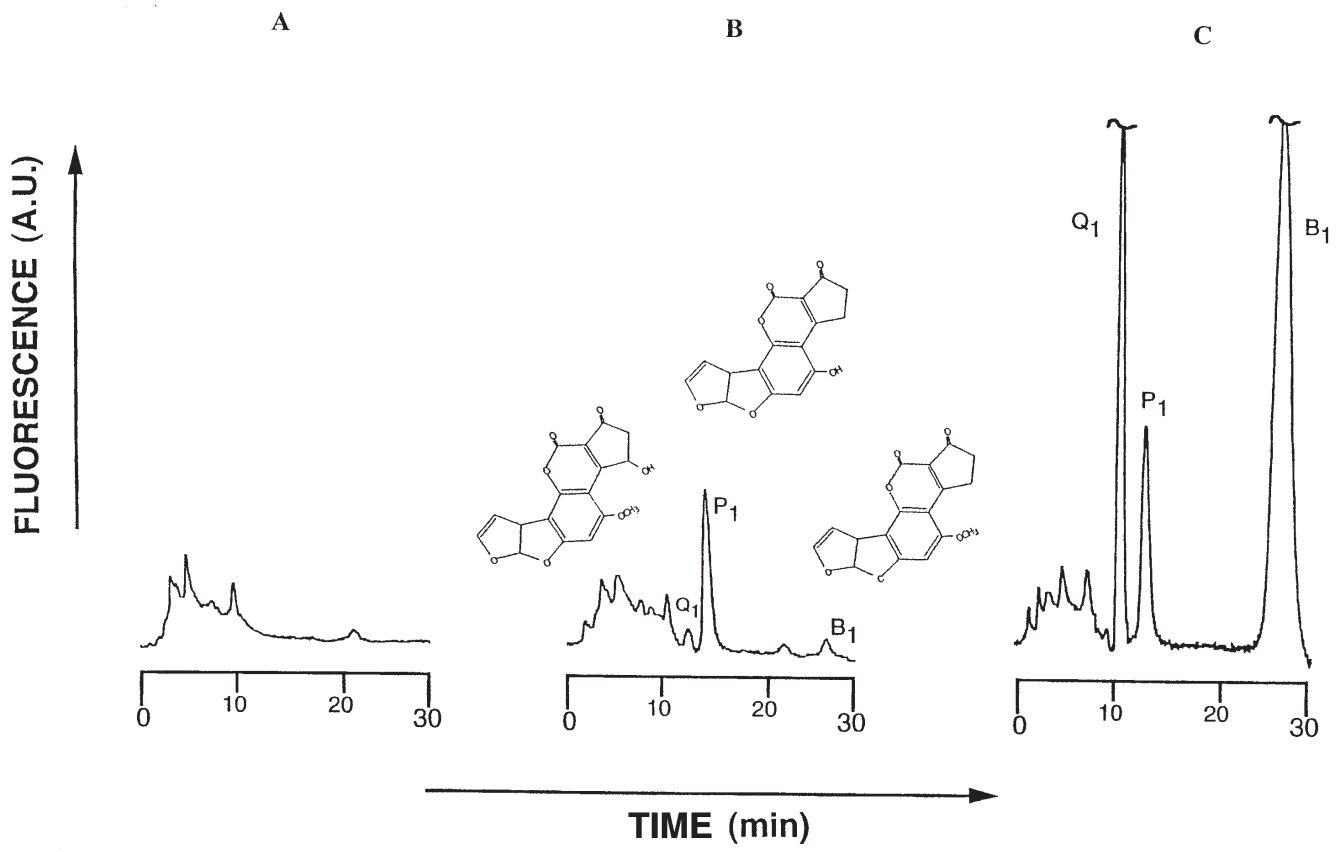

Figure 3. Typical chromatogram of Q_1, B_1, P_1 aflatoxins determination in human urine sample from ref [36]. (a) blank sample (b) the same urine spiked with the three aflatoxins Q_1, B_1, P_1 using eluent free of DM β-CD (c) the same urine detected with an eluent spiked with 10^{-2} M DM β-CD.

by some selected examples, the potentiality of the inclusion complex concept in this spectroscopic area and also to remind that the use of CDs derivatives in analytical chemistry is not exclusively limited to separation problems.

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