On the absorption and emission properties of three new non-steroidal anti-inflammatory drugs-β-cyclodextrin host-guest inclusion complexes: differentiated sensitivity to the microenvironment upon light excitation

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ABSTRACT. The effects of β-cyclodextrin (β-CD) complexation on the absorption and emission properties of the non-steroidal anti-inflammatory drugs tolmetin (TM), diflunisal (DF), and fenbufen (FB) have been investigated. The absorption spectra of all these compounds are only slightly affected by the addition of β-CD. In contrast, the emission properties were markedly influenced by CD complexation and in a different manner for the three compounds due to a differentiated sensitivity of the excited drugs to the microenvironment. The complexes are all characterised by a 1 : 1 stoichiometry and two different inclusion geometries for TM-β-CD complexes have been observed. Induced circular dichroism (icd) is observed due to the interaction of the compounds with the CD chiral cavity. The icd spectra are characterized by maxima well corresponding to those of the absorption spectra. A nonlinear analysis of the dependence of the icd signal magnitude on the CD concentration provided association constants values $K_{ass} = 1400 \pm 100\,M^{-1}$, $1500 \pm 100\,M^{-1}$, and $2600 \pm 150\,M^{-1}$ for TM, DF, and FB respectively.

1. INTRODUCTION

Cyclodextrins (CD) are cyclic oligosaccharides consisting of six (α-CD), seven (β-CD), or eight (γ-CD) units of α-D-glucose linked together by α-(1,4) bonds and characterised by doughnut shape and a relatively hydrophobic cavity. The most important property of these molecules is the ability to form host-guest inclusion complexes with organic and inorganic substrates [1–5]. A number of factors influence complexation. Of them the "goodness of fit" between host and guest and the hydrophobic effect are probably the most important [6]. The formation of these supermolecules has led to the widespread application of CDs in many different fields such as pharmacology, food science, analytical chemistry and chemical synthesis and catalysis [2]. Cyclodextrins have proven their potential as media for controlling chemical and photochemical reactions [7–10]. As already reported for a large variety of systems [11], the nature of the lowest excited states of the guest molecule, the efficiency of its deactivation pathways, the fate of the reaction intermediates and the opening of new photoreactive channels, are some of the parameters that may be modulated by the CD microenvironment.

Due to their molecular structure, many non-steroidal anti-inflammatory drugs (NSAID) are suitable guests for the CD macrocycle. Photophysics and photochemistry of inclusion complexes of anti-inflammatory drugs with CDs has received considerable attention, especially in the last few years [12–14]. The use of CD as complexing agent represents in fact a useful strategy to minimize the biological damage photoinduced by non-steroidal anti-inflammatory drugs (NSAIDs) [15, 16] as well as a tool to increase drug photostability. Further, the weak binding forces responsible for association to CDs provide a useful model to mimic the interactions of the drug with hydrophobic biological sites. This report deals with the absorption and emission properties of the inclusion complexes of three NSAIDs with β-CD. Such studies are a prerequisite to understand the molecular mechanisms of drug phototoxicity as well as the factors regulating the CD-mediated photoprotective action. Moreover, from the point of view of photophysics and photochemistry, they offers the opportunity to approach new mechanistic aspects.

Tolmetin (TM), 5-(p-toluoyl)-1-methyl-2-pyrrolyacetic acid, diflunisal (DF) 2′,4′-difluoro-4-hydroxy-[1,1′-biphenyl]-3-carboxylic acid and fenbufen (FB), γ-oxo-(1-1′-biphenyl)-4-butanoic acid, are three acids belonging to the NSAID class and bearing the benzophenone (TM)-, and the biphenyl (DF and FB)-like chromophore (see Scheme 1). All these compounds are well known to act as efficient photosensitizers towards biological substrates, inducing red blood cell photohemolysis, liposomes photoperoxidation, and DNA photocleavage [17–20]. Due to the values of the pK_a, the three drugs are present in their carboxylate form at neutral pH.

2. MATERIALS AND METHODS

Tolmetin sodium salt dihydrate, diflunisal, and fenbufen, from Sigma Chemical Company (St. Louis MO,

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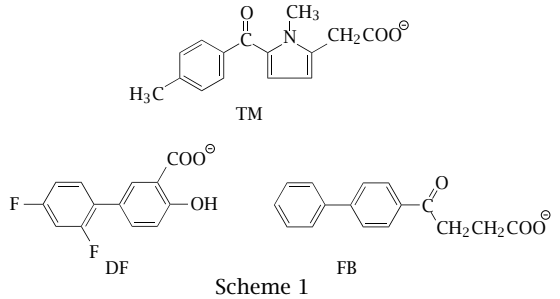


Scheme 1

USA) and β-CD from Serva (Heidelberg, Germany) were used as received. Water was purified through a Millipore Milli-Q system. All the experiments were performed in phosphate buffer 10^{-2} M at pH 7.4. The pH of solution was measured with a glass electrode.

Ultraviolet absorption was measured by a Perkin-Elmer Lambda 9 spectrophotometer; fluorescence and circular dichroism spectra were recorded with a Spex Fluorolog-2 (mod. F-111) spectrofluorimeter and with a Jasco J-715 dicrograph, respectively. Fluorescence lifetime measurements were performed by using a Hamamatsu C-4334 streak camera and an IBH Consultants LTD time correlated single photon counting system.

3. RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of the three compounds recorded in the presence of increasing amount of β-CD. In all cases, a decrease in the molar absorption coefficients upon addition of CD was observed. Furthermore, a blue shift of the two main absorption maxima was noticed for TM. These changes provide a first indication of the incorporation of the drugs in the hydrophobic cavity also verified by the presence of induced circular dichroism (icd) signals (Figure 2). In fact due to the absence of chiral centres, the three compounds are not optically active by themselves in aqueous solutions. Addition of β-CD induces optical activity as a consequence of the complexation with the optically active macrocycle. The icd bands are characterized by maxima well corresponding to those of the absorption bands, thus ruling out the formation of complexes with 2 : 2 stoichiometry. The nonlinear analysis of the dependence of the intensity of the absorption (A) and the icd signal (CD, for 1 cm pathlength) on the β-CD concentration was performed according to equations (1) and (2) (corresponding to conditions where the β-CD concentrations are in large excess with respect to guest concentration), respectively

$$A = \varepsilon_0 c_0 \left(\frac{(1 + (\varepsilon_c/\varepsilon_0) K_{ass}[\beta\text{-CD}])}{(1 + K_{ass}[\beta\text{-CD}])} \right), \qquad (1)$$

$$\text{CD} = \frac{10\theta c_0 K_{ass}[\beta\text{-CD}]}{(1 + K_{ass}[\beta\text{-CD}])}, \qquad (2)$$

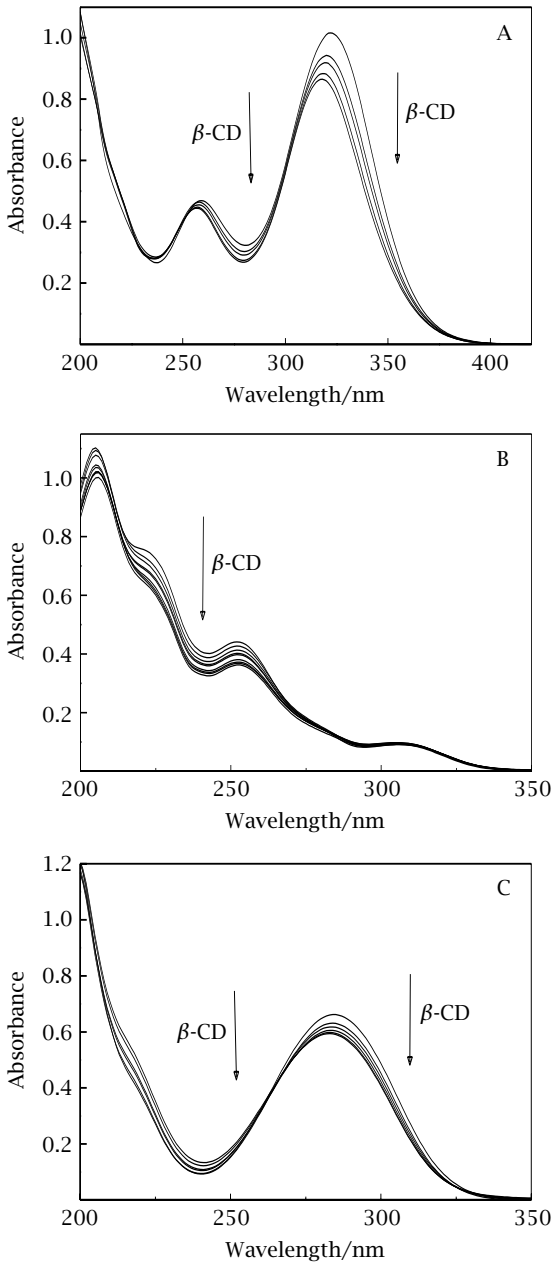


Figure 1. *Absorption spectra of (A) TM, (B) DF, and (C) FB in phosphate buffer solution, pH 7.4, in the presence of increasing amount of β-CD up to* 10^{-2} *M. Cell path: 1 cm.*

where c_0 is the initial concentration of the drug, ε_0 the absorption coefficient of the free molecule, ε_c that of the complex, θ is the molar ellipticity of the complex and K_{ass} is the association constant. Both absorption and icd are consistent with the formation of complexes with 1 : 1 stoichiometry according to the following equilibrium:

$$\text{drug} + \beta\text{-CD} \rightleftharpoons \text{drug}-\beta\text{-CD} \qquad (3)$$

Association constants of $1400 \pm 100\,\text{M}^{-1}$, $1500 \pm 100\,\text{M}^{-1}$, and $2600 \pm 150\,\text{M}^{-1}$ were found for TM, DF, and FB respectively. The value obtained for TM-β-CD inclusion complex compares well with those of others

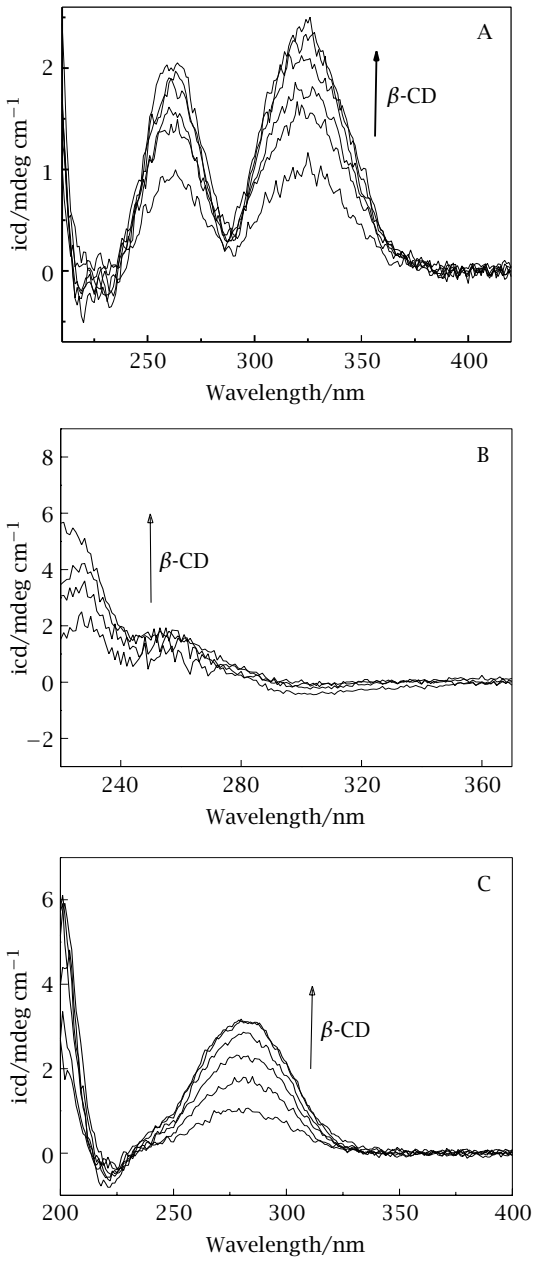


Figure 2. *Induced circular dichroism observed in phosphate buffer solutions,* pH *7.4, of (A) TM, (B) DF, and (C) FB in the presence of increasing amount of β-CD up to* 10^{-2} *M. Cell path: 1 cm.*

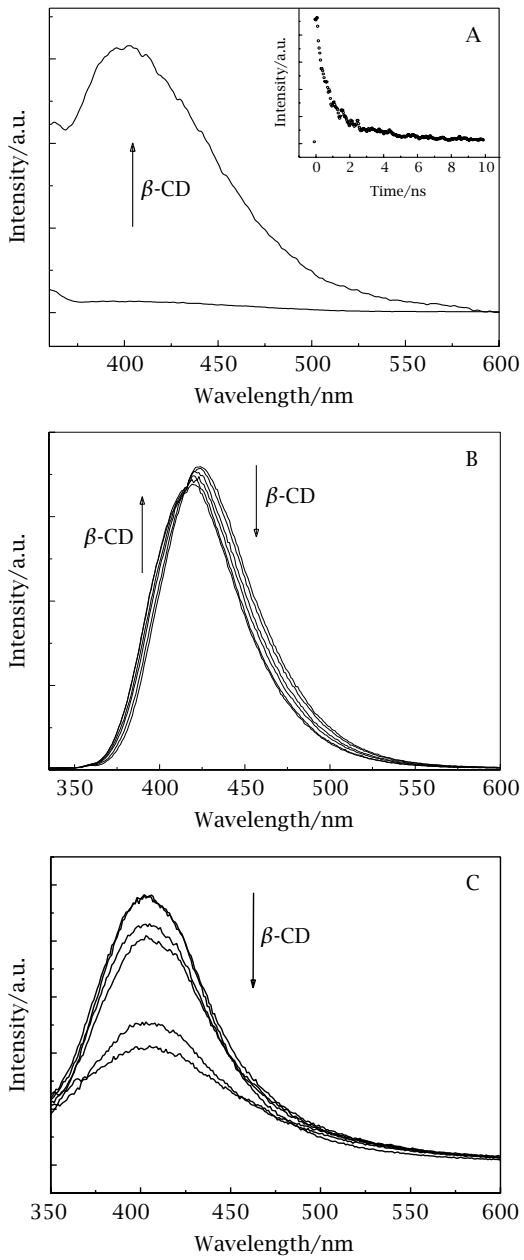


Figure 3. *Fluorescence spectra of (A) TM, (B) DF, and (C) FB in phosphate buffer solutions, pH 7.4, in the presence of increasing amount of β-CD up to* 10^{-2} *M. The inset of Figure 3A shows the decay trace observed for the TM-β-CD system in the region 370–450 nm.*

anti-inflammatory drugs containing the benzophenone-like chromophore [13, 14]. Despite the fact that DF and FB are characterized by the presence of the same chromophoric unity, the higher association constant obtained for FB accounts for a better affinity of this drug for the CD cavity. This can be reasonably due to the more hydrophobic nature of FB compared with DF.

The addition of β-CD also affects the fluorescence behavior. In contrast with the small changes induced on the absorption spectra, the emission intensity of TM is markedly increased by the addition of β-CD (Figure 3a), indicating that the excited drug is highly sensitive to the change in the microenvironment. The emission decay profiles is reported in the inset of Figure 3a. The kinetic analysis showed that the trace is well fitted by a biexponential decay with rate constants $k_1 = 1.8 \times 10^9\ s^{-1}$ and $k_2 = 3.7 \times 10^8\ s^{-1}$ and normalised pre-exponential factors $a_1 = 0.7$ and $a_2 = 0.3$. These results can be rationalised with the presence of two inclusion complexes of very different lifetimes, both with 1 : 1 stoichiometry. Indeed the two different aromatic moieties, the tolyl and the pyrrol rings, could interact with the macrocycle in different inclusion geometries.

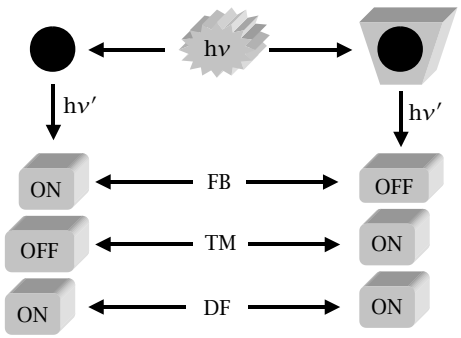
A different behavior was observed in the case of DF. As reported in Figure 3b, addition of β-CD provoked

a slight decrease of the fluorescence intensity accompanied by a blue shift of the emission maximum. A recent study on the excited state properties of DF in aqueous medium [21] pointed out a large Stokes shift between the absorption and the emission maxima, indicating a large difference between the geometry of the ground and that of the excited state. Biphenyl derivatives are known to undergo upon excitation a large change in the angle between the planes of the phenyl rings (twisted in the ground and coplanar in the excited state (22)). However the Stokes shift in DF is *ca.* 20 nm bigger than that observed for 4-biphenylcarboxylic acid used as a model compound, suggesting that it cannot be simply rationalized by a change in the molecular degree of planarity. The additional contribute was attributed to an intramolecular proton transfer. The proton involved in the ground state in the intramolecular hydrogen bond from the hydroxyl to the carboxyl moieties could in fact shift in the excited state toward the carboxylic oxygen according with the behavior of other salicylic acid derivatives [22, 23]. Two different hypotheses may account for the photophysical behavior observed in the presence of β-CD. Actually, the inclusion of DF in the inner cavity of CD could influence the prototropic shift as well as the twisting around the central single bond leading to the change in the angle of the two phenyl rings upon excitation. We believe the most likely one is the latter. In fact it is reasonable to expect that the hydrophylic hydroxyl and carboxyl moieties are not deeply included in the macrocycle which, as a consequence, basically does not affect the proton shift. This hypothesis is in good agreement with the changes in the absorption spectra under CD complexation (Figure 1b) which where the most significant in the 260 nm band, corresponding to transitions localized on the phenyl rings (25). Therefore, on the basis of these considerations, the blue shift observed in the fluorescence spectra in the DF-β-CD host guest complex, may be attributed to a hindrance to reach the planar conformation once the two phenyl rings are constrained in the cavity. This is in agreement with the behavior of the 4-biphenylcarboxylic acid-CD inclusion complex [24].

The fluorescence of FB in aqueous solution is characterised by a maximum around 390 nm. A remarkable decrease of the emission intensity was noticed upon complexation with CD but no shift in the spectra was observed (Figure 3c). This result are consistent with the formation of a non-emissive inclusion complex. In order to rationalize this result we have to take in account of the value of the fluorescence quantum yield in the absence of CD. By taking into account that the fluorescence quantum yield of FB in aqueous medium is much lower (*ca.* 0.001) compared to that of biphenyl in polar solvent (*ca.* 0.2), we believe that the presence of the butanoic side-chain is responsible for the behavior observed. Indeed, such a chain may introduce lower lying n,π^* transitions deactivating the aromatic biphenyl-like singlets. Similar cases are reported in the literature [25, 26]. The decrease of the fluorescence intensity in the FB-β-CD inclusion complex, can be thus attributed to a strong influence of the hydrophobic microenvironment on the relative energy of the excited states involved. As a consequence other non-radiative deactivation channels may be opened or take place with higher efficiencies. In this respect a control of the molecular conformation by the CD could be also envisaged.

4. CONCLUSIONS

This preliminary overview on the spectroscopic properties of three new host-guest inclusion complexes has provided a further example of how complexation with CD can be a valid tool in controlling the photophysical properties of drugs. The accommodation of the guest molecules in the hydrophobic environment lead to new properties, *i.e.*, optical activity. The emission properties of the three drugs are modified in a different manner upon CD complexation. Tolmetin becomes fluorescent upon complexation with CD whereas the formation of a non-emissive complex is observed for FB. Finally, only a slight decrease of the fluorescence quantum yield but accompanied by a significant increase in the energy of the lowest singlet state was noticed for DF-β-CD inclusion complex. As reported pictorially in Scheme 2, the obtained results illustrate the ability of CD in influencing the photophysical properties of the three drugs and their differentiated sensitivity to the hydrophobic microenvironment upon light excitation. In the light of these results, changes in the photochemical and photobiological pathways of the drugs under complexation are expected.



Scheme 2

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