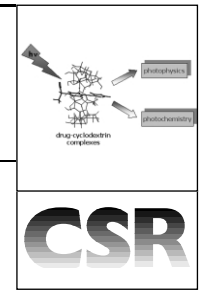
Photoprocesses of photosensitizing drugs within cyclodextrin cavities



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Recently some interest has been focused on the photobehavior of CD-drug inclusion complexes in relation to the problem of the biological photosensitization by drugs. This review is dedicated to the illustration of the mechanistic aspects of the photoprocesses occurring in some non-steroidal anti-inflammatory drugs (NSAIDs), with photosensitising side effects, within CD cavities. It is shown how the photobehavior of the CD-drug associates can help to model the photoreactivity of the drugs in biological sites. The limitations for the use of CDs as protective systems for the clinical administration of photosensitising drugs is also evidenced.

1 Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides with a truncated cone shape. The natural derivatives, synthesized by bacteria, are made by six, seven or eight glycosidic units, linked together by α-1,4 glycosidic bonds (Fig. 1). Due to their structural features, CDs possess cavities of variable size and hydrophobic character able to host a large variety of organic and inorganic substrates.[1]

The association process, assisted by weak interactions like van der Waals and hydrophobic forces and H-bonds, produces novel ground and excited state properties. Consequently, chemistry within CDs has being appealing to investigators in both pure and applied fields for a long time.[2,3]

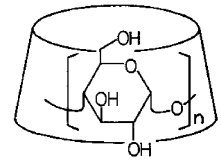

Fig. 1 Schematic representation of the CDs; n = 6, 7 or 8 in α, β and γ CD respectively.

The interaction of CDs with drugs received attention for many reasons. CD derivatives were recognized to be suitable carrier materials for drugs in clinical applications, being able to make up for undesirable drawbacks and provide specific pharmacological effects. Indeed the solubility, stability and bioavailability of several drug molecules can be considerably improved through the formation of CD inclusion complexes.[4] Moreover CD derivatives are used in combination with fluorescence techniques for analytical purposes aimed at optimisation of drug dosages (see, for example, refs. 5,6).

Recently the interest in CD-drug complexes has been related to the problem of the biological photosensitization by drugs. Indeed serious phototoxic reactions (mainly diseases of epidermis and dermis), as well as photoallergic and photomutagenic effects, can be induced in patients subjected to sunlight irradiation while treated with pharmacologically im-

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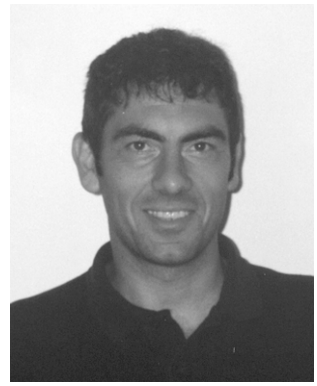


portant chemicals such as antibacterials, tranquillisers, antimicotics and non-steroidal anti-inflammatory drugs (NSAIDs). Such noxious effects, correlated to the drug photochemical reactivity,[7] are substantially decreased in the presence of CDs with phenothiazine drugs[8] and with NSAIDs (in *in vitro* cellular systems).[9–11] Application of CDs in the design of protective systems for the clinical administration of photosensitising drugs was suggested.

A more fundamental interest, also related to the problem of biological photosensitization, is relevant to the possible use of CDs as tools to gain insight into the environmental factors determining the photoreactivity of the drugs and their major photoproducts. Indeed a crucial point for the elucidation of the molecular mechanisms of the biological photosensitization is the understanding of the interaction between the biological targets (lipids, proteins, nucleic acids) and the excited states, radicals, stable products and active oxygen species, generated by photoexcitation of the drugs. To this goal the organized receptor represented by the CD cavity appears to be a suitable model to mimic hydrophobic pockets of cell components.

Light excitation of CD-drug systems has been mostly used for the assessment of the drug photostability or determination of equilibrium constants, mode, stoichiometry and thermodynamic quantities of complexation (by fluorescence methods).[12–14] Only a relatively limited number of studies were dedicated to the mechanistic aspects of the excited state processes occurring within the CD cavities. These investigations were mainly concerned with NSAIDs, a class of molecules with wide therapeutic applications, often actually administrated as β-CD inclusion complexes.[4] A detailed characterization of the emission properties, final photoproducts and transient intermediates formed in aqueous medium both in the absence and presence of CDs is available for the drugs reported in Fig. 2. The investigated compounds are characterized by the presence of chromophores (naphthalene-, biphenyl-, benzophenone-like) that have always attracted the interest of photochemists. Their rich photobehavior in the CD environment will offer us the opportunity to discuss the role of the hydrophobicity, steric constraints, H-donating ability, cage and shielding effects of the CD cavity in the fate of excited states and intermediates; when possible the correlation between the course of the photoprocesses occurring within the CD cavity and the structural features of the CD-drug associate will be evidenced. In light of the available information, we will comment about the actual applicability of CDs as tools to improve drug photostability, protect the biological systems from photodamage and model biological microenvironments.

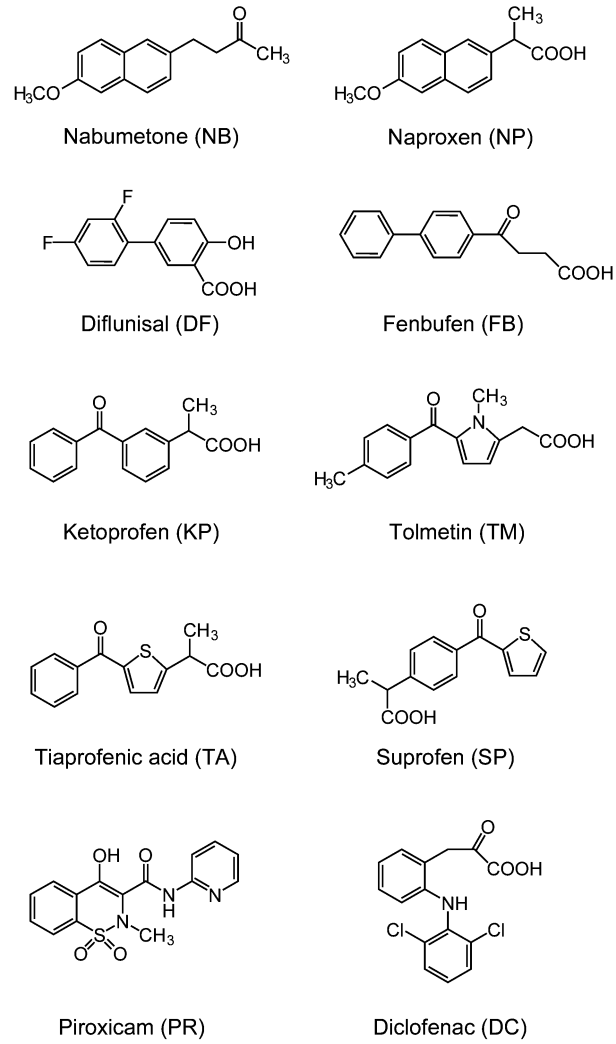


Fig. 2 Non-steroidal anti-inflammatory drugs discussed in the text.

2 Emission properties

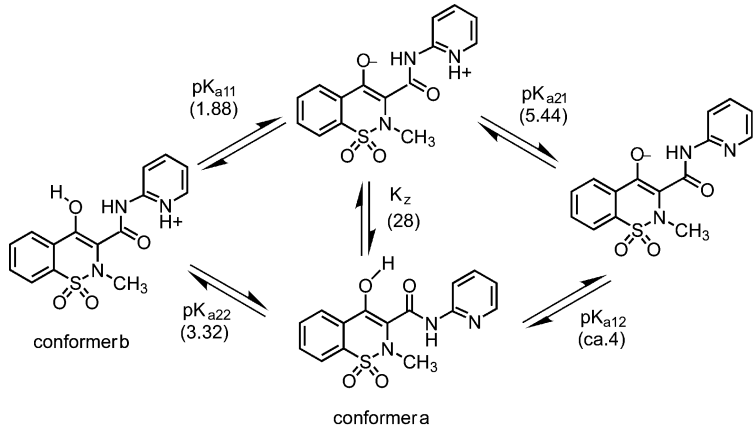
Because of the high sensitivity to the environment, the fluorescence of drugs in aqueous solution exhibits intensity changes and spectral shifts by addition of CDs. These effects can be used as a resource for improving the performance of analytical methods and for determining association constants by least square best fitting of the emission intensity changes at several CD concentrations to Benesi–Hildebrand-like linear equations or non-linear functions.[15]

The main factors determining the modification of the fluorescence properties of common NSAIDs within CD cavities will be illustrated in the following subsections.

2.1 Role of conformational features and mode of inclusion

The emission properties of CD complexes can be controlled by conformational factors and mode of inclusion of the ground state drug. Oxicams offer examples of this. These molecules are characterized by rich dynamic structural features sensitive to chemical substitution, solvent and temperature. Such characteristics reflect in an emission behavior highly sensitive to the environment. The fluorescence of Piroxicam (PR), in particular, was the object of several studies. This molecule is endowed with several prototropic forms resulting from acid–base equilibria, conformers due to inter- or intramolecular H-bonds and tautomers formed by proton transfer either in the ground or in the excited state.[16,17] The emission of PR has been investigated in various media with the twofold goal of understanding molecular structure and proton transfer processes in both the ground and the excited states and clarifying the molecular basis of the drug photosensitizing action. Indeed the phototoxicity of piroxicam is not yet fully understood, being unclear if it is due to a photoproduct, a photoactive metabolite, or a particular tautomer. The results obtained in pure solvents, in dioxane–water mixtures,[18] and in AOT–isooctane–water reversed micelles[19] indicated that the molecular fluorescence of piroxicam is highly sensitive to the solvent H-donor ability. This can be understood on the basis of Scheme 1, which reports the microscopic ionization equilibria of PR in the ground state.

The two interconvertible conformers *a* and *b* are characterized by an intramolecular H-bond (between the hydroxy group of the benzothiazine ring and the *ortho* carboxy group of the carboxamide) and by an 'open' twisted structure, respectively; each of these molecular species is in equilibrium with charged forms due to proton dissociation of the hydroxy group and protonation of pyridine nitrogen, and with the zwitterionic form; in addition, a keto–enol tautomerism is possible (see Scheme 2).[16,17,20] The concentration of each species in solution strongly depends on the medium. Thus



Scheme 1 Ionization behaviour of PR, reproduced from ref. 20 with permission by VHCA-Helvetica Chimica Acta 2002.

polarity, proticity, pH, presence of added salts are extremely important parameters; the total drug concentration is also important, because of the possible occurrence of aggregation.

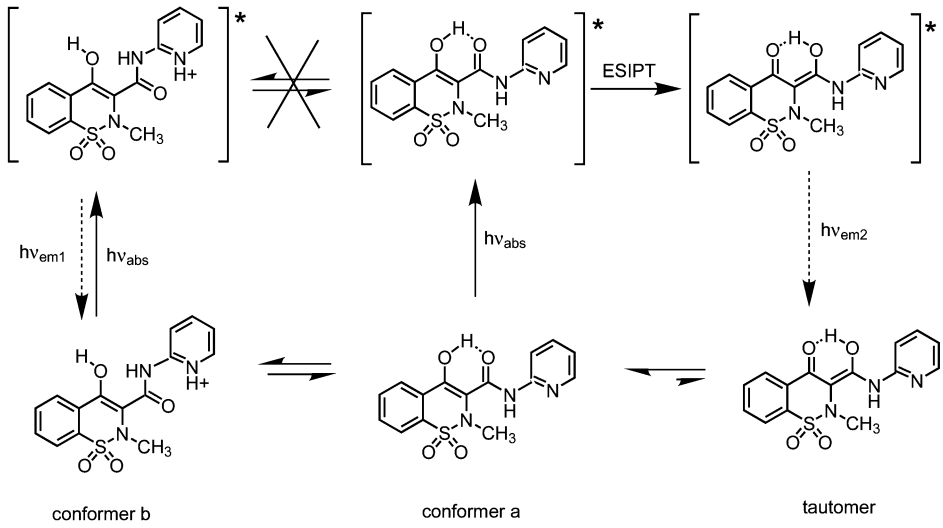
In water at pH 4 the λ_{max} of the absorption spectrum is 360 nm; in presence of β-CD a shift of the maximum to 330 nm was observed and interpreted with a change of the pK_a for the deprotonation of the hydroxy group. A weak fluorescence with maximum at 390 nm and lifetime shorter than 20 ps was attributed to the anionic form. An extremely weak emission in the range 450–550 nm, *i.e*. with a large Stokes shift, was attributed to the presence of a tiny amount of the phototautomer, formed by excited state proton transfer (ESIPT) from the hydroxy group of the benzothiazine ring to the *ortho* carbonyl group (see Scheme 2). Emission in the wavelength region close to 415 nm was assigned to the zwitterion.[16]

In the presence of increasing β-CD concentrations the emission around 460 nm grows in a well defined band, becomes longer lived (*ca*. 60 ps lifetime) and its quantum yield increases from ≪ 0.0001 to *ca*. 0.001.[16] This enhancement was interpreted as being due to enhanced extent of ESIPT, the process being favoured by the formation of a 1:1 drug–β-CD complex. The phototautomer was believed to revert to the starting geometry *via* a keto form of the drug in the ground state (Scheme 2).[16,17] An emission component with *ca*. 130 ps lifetime, observed in the presence of β-CD at 400 nm, was attributed to the inclusion complex of the open conformer *b*. The emission of the zwitterion was not detected in the presence of β-CD.[16]

The hydrophobic, constrained environment represented by the β-CD cavity was thought to facilitate tautomeric emission through inclusion of the benzothiazine moiety of the drug in the 'closed' *a* geometry; this conformation, because of the intramolecular H-bond, was believed to inhibit the proton dissociation of the hydroxy group and favour intramolecular proton transfer in the excited state. On the contrary in the homogeneous environment H-bonding to water was believed to favour the emission of the 'open' conformer *b* and of the charged species. No [16] or very little [17] variations were observed in the emission of PR by addition of α-CD, because of the lack of appreciable interaction. On the other hand, the emission changes induced by γ-CD indicated that encapsulation of conformer *a* takes place to a larger extent than in β-CD.[17]

The relation between the fluorescence properties of PR in the presence of CDs and the conformational features of the molecule helps to clarify the mode of interaction of the drug with the cell components and provides information on the origin of the adverse biological photoeffects. It is indeed conceivable that the 'open' conformer *b* and the charged or zwitterionic species will be located in polar hydrophilic sites, whereas the 'closed' neutral form *a* will be preferentially associated to hydrophobic and lipophilic components. Thus conformer *a* is the most likely candidate for being responsible for drug photobinding to proteins which are the origin of photoallergic effects, and of disruption of the lipidic scaffold of cell membranes, which leads to other phototoxic manifestations.

A further example of the influence of the mode of inclusion on the emission behavior of a drug is represented by the case of Tolmetin (TM), a photosensitising molecule in which both photolability (see below) and *in vitro* phototoxicity are decreased by complexation with β-CD.[21] In the absence of CD



Scheme 2 Schematic diagram of photoprocesses of PR, reproduced with some changes from ref. 16 with permission by Elsevier 2002.

the fluorescence of TM is characterized by an extremely weak emission band located in the region 370–550 nm with a very short lifetime (*ca*. 0.5 ns). In the presence of β-CD the emission intensity becomes larger and the lifetime longer (Fig. 3). Indeed

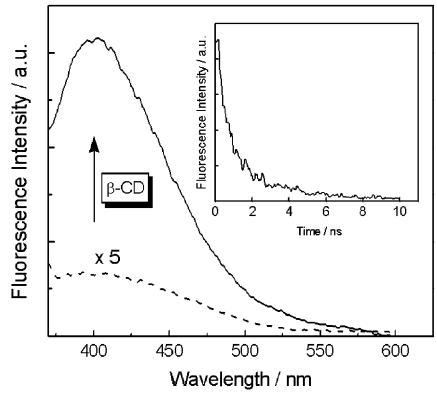


Fig. 3 Fluorescence spectra of TM (- - -) in the absence and (——) in the presence of 10^{-2} M β-CD. The inset shows the decay trace observed in the region 370–450 nm. Data from ref. 21.

two complexes, both with 1:1 stoichiometry but different inclusion modes, were proposed and identified by the inclusion of either the tolyl or the pyrrole ring in the CD cavity. With respect to the free drug, one of the two excited complexes has an almost unchanged lifetime (*ca*. 0.5 ns with 70% weight) whereas the other one is longer lived (*ca*. 2.7 ns with 30% weight) (see inset Fig. 3). Lengthening of the singlet lifetime in the second complex is accompanied by a less efficient photoconversion to the triplet state. These facts directly relate to an increased photostability of the drug in the presence of CD, because the triplet state is the precursor for adiabatic decarboxylation (see below).[21,22]

Another system, whose fluorescence properties within CDs are determined by the mode of inclusion, is Nabumetone (NB), a naphthalene derivative with a butanone side chain. It is worth examining the behavior of NB in parallel to that of Naproxen (NP), a parent propionic acid derivative. NB has a very weak fluorescence in water, peaking at 355 nm. This fact is due to a folded conformation which favors quenching of the emission by an intramolecular process (either a charge transfer or a radical-like reaction, involving the excited carbonyl and the naphthalene moiety).[23,24] Addition of α-CD to aqueous solutions of the drug induces a strong enhancement of the emission intensity and appearance of a structure in the spectrum. This effect is accompanied by drastic changes in the absorption spectrum, which is red-shifted by *ca*. 9 nm. NP also has a fluorescence band peaked at 355 nm which, however, exhibits very little sensitivity to the α-CD environment. The emission of both drugs is enhanced in a similar way by addition of β-CD. Comparative examination of the values of ΔH and ΔS and ^{1}H NMR shifts for the complexation of both NB and NP to α- and β-CD indicated that the butan-2-one side chain of NB is preferentially inserted in the α-CD cavity, whereas the methoxynaphthalene moiety of both drugs is accommodated in the larger β-CD cavity. A conformational control by the α-CD cavity was thus proposed to be active and determine the emission behavior of the NB complex.[25] Indeed inclusion of the butanone chain in the α-CD cavity hinders attainment of the folded conformation responsible for emission quenching.[23–25]

Inclusion of the aryl moiety holding the acetic acid group accounts well for the changes in the fluorescence intensity of Diclofenac (DC), in both the dissociated and protonated forms, in the presence of β-CD. The emission enhancement is not accompanied by significant spectral change. This is in agreement with formation of a 1:1 CD–drug complex in which the acid–base properties of the carboxylic group are substantially unchanged (the pK_a* is 3.68 in the absence of CD and 4.01 in the presence of CD, Fig. 4). On the other hand the association constants for the neutral and dissociated form are similar (Log K = 3.11 and 3.15 M^{-1}, respectively), in agreement with a location of the carboxylic moiety outside and of the aryl moiety inside the cavity.[6]

The fluorescence intensity of DC drops at alkaline pH's, because of an OH-induced quenching. In the presence of β-CD this quenching process still takes place but occurs at higher pH's, in agreement with a protective action of the CD against attack by the base (Fig. 4).[6]

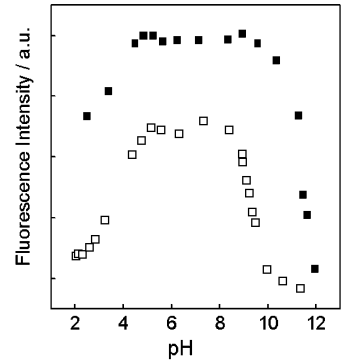


Fig. 4 Influence of the pH on the fluorescence intensity of 5.5×10^{-6} M DC (□) in the absence and (■) in the presence of 10^{-2} M β-CD, λ_{exc} = 289 nm, λ_{em} = 362 nm. From ref. 6 with permission by the Royal Society of Chemistry 2002.

The acid and dissociated forms of DC also interact with α-CD giving 1:1 complexes of similar stability and similar inclusion geometry as with β-CD. A substantial fluorescence intensity enhancement with some spectral modifications is observed (Fig. 5).[5]

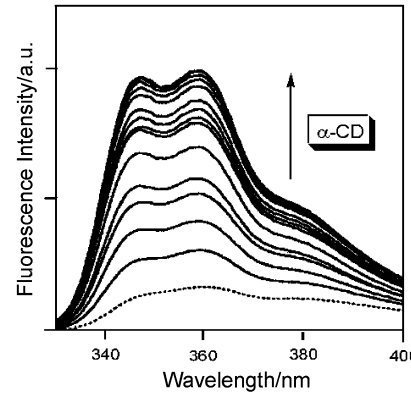


Fig. 5 Fluorescence spectra of 2.8×10^{-6} M DC, (- - -) in the absence and (——) in the presence of increasing amounts of α-CD (from 1.6×10^{-4} M to 7.2×10^{-3} M) in neutral aqueous media, λ_{exc} = 289 nm. From ref. 5 with permission by Elsevier 2002.

It is conceivable that a deep insertion of the phenylacetic moiety in the CD cavity, hinders important molecular degrees of freedom and slows down the non-radiative deactivation rate, thus determining the observed increase of the emissive ability of the DC excited state.

2.2 Role of steric constraints and environmental polarity

Two NSAIDs with biphenyl-like structure provide examples of how the CD environment can influence the emission behavior by directly affecting some excited state property of the drug. Diflunisal (DF) and Fenbufen (FB) in their carboxylate form, interact with β-CD forming 1:1 complexes with similar association constants (1700 and 2700 M^{-1}, respectively).[26]

This indicates that in both cases the aromatic moiety is inserted in the cavity and that the features of the inclusion complexes are similar in the ground state. However a differentiated effect is observed in the fluorescence of these drugs by addition of β-CD: a slight decrease of the intensity, accompanied by a blue shift of the emission maximum in the case of DF and a remarkable decrease of the emission intensity with no spectral changes in the case of FB (Fig. 6).

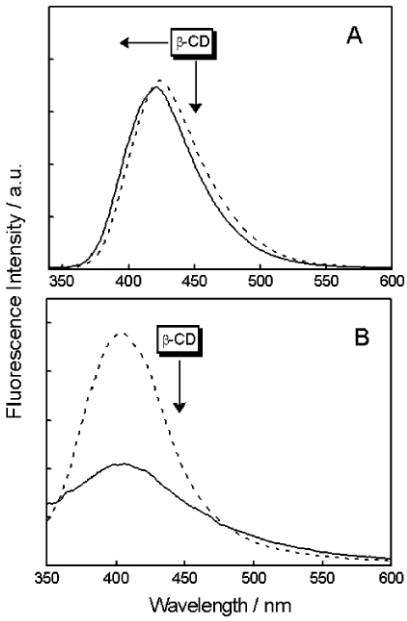


Fig. 6 Fluorescence spectra of (A) DF (λ_{exc} = 318 nm) and (B) FB (λ_{exc} = 290 nm) in the absence (- - -) and in the presence of 10^{-2} M β-CD (——) at pH 7.4. Data from ref. 26.

The rationale for such findings relies on the geometrical features and electronic character of the emitting states. A large Stokes shift, indicating a marked change in the excited state equilibrium geometry, characterizes the emission of DF. This effect was attributed to a change in the dihedral angle between the planes of the two phenyl rings upon electronic excitation and/or to an intramolecular excited state proton transfer from the hydroxy group to the carboxylate moiety, connected to each other in the ground state by an H-bond. Because proton transfer is relevant to a molecular site most likely located outside the cavity, it was not reasonable to expect the process is perturbed by the inclusion. The blue shift in the emission spectrum induced by β-CD inclusion was thus attributed mainly to a steric hindrance to the twisting movement about the central single bond in the excited state (corroborated also by the increase of the degree of fluorescence polarization observed in the presence of CD).[26] This constraint does not appreciably influence the emission ability of the drug, thus leaving the fluorescence quantum yield almost unchanged. However, it will be seen in the following that it is able to strongly affect a photochemical pathway and lead to increased photostability.[26]

On the other hand the spectroscopic and photophysical features of FB are dominated by the existence of low lying states of n,π* character.[27] The fluorescence quantum yield in water is low because of efficient conversion of the excited molecule to the triplet manifold. This process is related to the forbidden character of the lower excited singlet S_1, ^{1}n,π* in nature, and its favourable coupling to an upper triplet T_3 of π,π* character, close in energy to S_1. In the lower polarity environment of the CD cavity the n,π* states are stabilized, whereas the π,π* states are destabilized. Thus the electronic coupling S_1–T_3 becomes more favourable, further increasing the intersystem crossing efficiency and decreasing the emission ability of the drug. Increased photostability is observed, despite the lowest triplet is the precursor state in the Norrish I photocleavage of the drug (see below).[26]

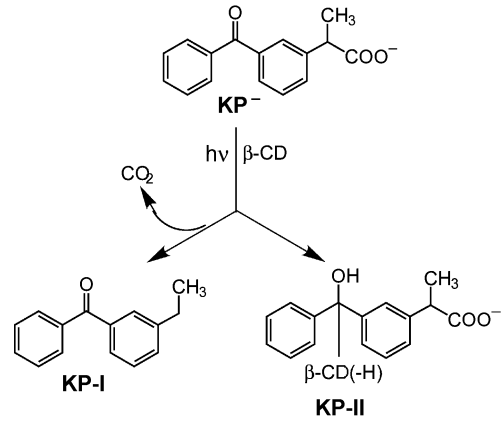
3 Photoreactivity

Although photostability is a main requirement for drugs for both optimization of preparative and conservative processes and minimization of photosensitizing effects, in most NSAIDs chemical functions responsible of light absorption at λ > 330 nm do introduce photoreactivity. Because the microenvironment represented by the CD cavity is able to deeply modify the photochemical properties of the guest molecules,[3] CD inclusion was proposed as a tool for the control of drug photodegradation.

The factors determining the photoreactivity changes of drugs in CD cavities will be illustrated in the following, on the basis of the detailed product and mechanistic investigations available for a series of NSAIDs.

3.1 Photodegradation quantum yields and stable photoproducts

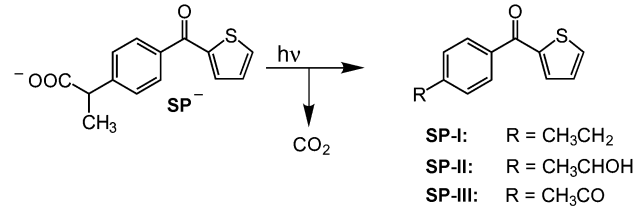
The 2-arylpropionic acids with benzophenone-like chromophores Ketoprofen (KP), Suprofen (SP) and Tiaprofenic Acid (TA), under UVA irradiation in aqueous medium at neutral pH, undergo photodecarboxylation from the dissociated forms as the sole photoreaction. KP is photobleached with a quantum yield of 0.75[28] and converts in anaerobic conditions to the corresponding ethyl derivative KP-I (see Scheme 3). In aerated



Scheme 3

solutions, in addition, a series of oxygenated derivatives are formed.[28,29] In the presence of β-CD the quantum yield of photodecarboxylation is depressed from 0.75 to 0.42 but an additional photoprocess, likely reductive, contributes to keep the global photodegradation quantum yield of the drug close to the high value of the homogeneous aqueous medium.[30,31] Indications for the formation of a β-CD photobinding product KP-II (Scheme 3) have been obtained by both UV absorption spectroscopy and NMR.[30]

Decarboxylation appears to be the only reaction in the photolysis of SP also. The ethyl derivative SP-I is formed as the main photoproduct in the absence of oxygen; the oxidation products SP-II and SP-III add in aerobic conditions (Scheme 4).[32,33] The photodegradation quantum yield of the drug in



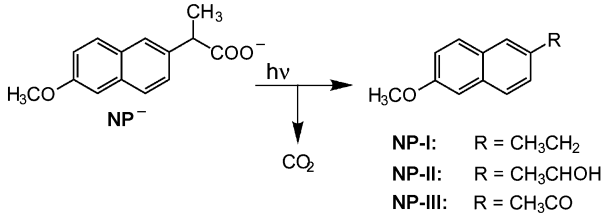
Scheme 4

phosphate buffer at pH 7.4 is both concentration and temperature dependent. At 20 °C the photodegradation quantum

yield of a suprofen solution 5×10^{-5} M is $\Phi_{-SP} = 0.076$. In the β-CD inclusion complex at the same concentration, decarboxylation remains the only photoreaction but its quantum yield increases to $\Phi_{-SP}^{CD} = 0.23$; the formation of the oxidation products SP-II and SP-III is significantly inhibited.[34,35]

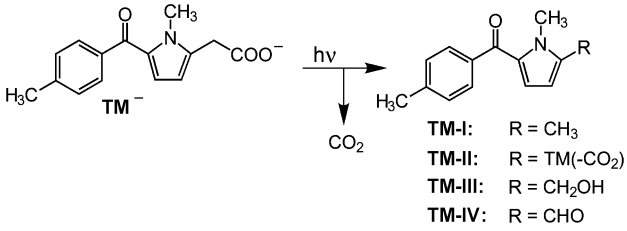
The photolysis of TA in neutral buffer presents strong analogies with that of SP, exhibiting a temperature dependent quantum yield ($\Phi_{-TA} = 0.25$ at 20 °C) and similar photoproducts.[33] Inclusion of the drug in β-CD does not change the nature of the photoreaction but strongly increases its efficiency ($\Phi_{-TA}^{CD} = 0.43$).[35]

Photodegradation of other members of the 2-arylpropionic acid family was also found to be affected by β-CD. The rate of NP photodecarboxylation is enhanced by CD complexation and the product distribution shows the predominance of the alcohol (NP-II) over the ketone derivative (NP-III), which on the contrary prevails in homogeneous media (Scheme 5).[36]



Scheme 5

The arylacetic acid analogue TM also undergoes photodecarboxylation with formation of several photoproducts (Scheme 6).[37] The efficiency of the reaction drops 50% down



Scheme 6

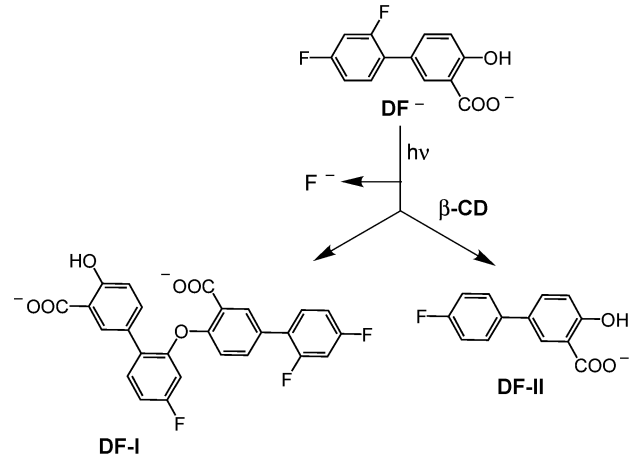
by β-CD inclusion and the product distribution is significantly changed (Table 1).[21,22]

Table 1 Percentage product distribution of TM and its photoproducts present after UV irradiation in phosphate buffer pH 7.4 in different experimental conditions. [TM] = 9×10^{-5} M; [β-CD] = 1×10^{-2} M. From ref. 21.

Conditions	TM	TM-I	TM-II	TM-III	TM-IV
N_2	81.3	17.5	0.0	0.0	1.2
N_2–β-CD	90.4	9.6	0.0	0.0	0.0
Air	81.7	0.0	4.2	14.1	0.0
Air–β-CD	90.2	3.3	1.8	4.7	0.0

DF in neutral aqueous solution undergoes photodefluorination, a rather uncommon reaction in fluoroaromatics, due to the strength of the C–F bond (125 kcal mol^{-1}). The main photoproduct isolated in the absence of oxygen DF-I is shown in Scheme 7.[38] If DF is irradiated as a β-CD inclusion complex a *ca*. 4-fold reduction of the quantum yield of photodegradation occurs; the formation of the photoproduct DF-I is suppressed[26] and a new photoproduct DF-II is observed (S. Sortino, unpublished results).

For the biphenyl-like analogue FB the effect of β-CD inclusion is a decrease by a factor *ca*. 3 in the photodegradation quantum yield with respect to that in aqueous solution.[26,27] The



Scheme 7

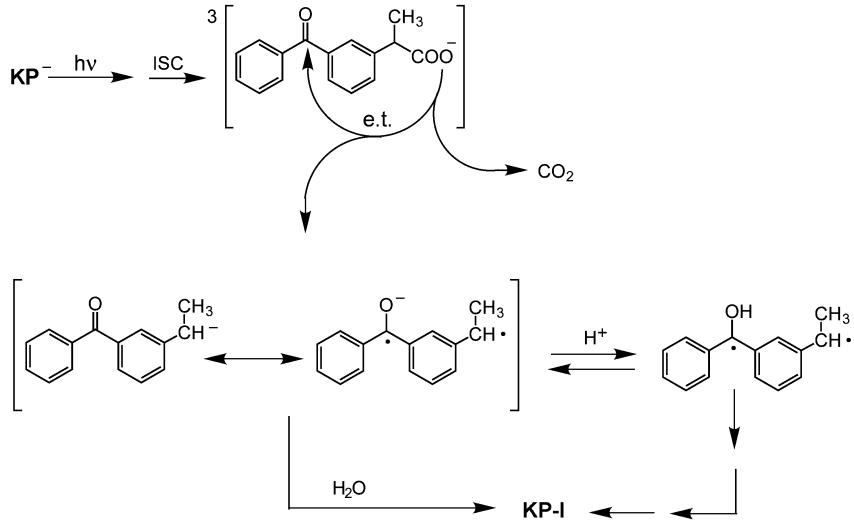
final photoproduct, resulting from α-cleavage (Norrish I fragmentation) in the absence of oxygen, *i.e*. 4-biphenylcarboxyaldehyde, remains the same.

3.2 Transient intermediates and mechanistic aspects

This section is devoted to highlighting the main modifications induced by the CD microenvironment on the spectroscopic behavior and kinetic evolution of photogenerated transient intermediates. The systems illustrated below offer variegated mechanistic aspects and provide valid rationale for the photochemical changes observed in the steady-state photolysis.

Ketoprofen. Despite a benzophenone-like behavior being expected for KP on the basis of the very close structural analogy with the parent molecule, the photoreactivity of this drug in neutral aqueous solution is quite different.[31,39] Scheme 8 summarizes the mechanism that accommodates the results of time-resolved transient absorption and photoacustic investigations.[31,40]

The triplet state of KP in neutral aqueous medium was detected by picosecond laser flash photolysis (see Fig. 7). The maximum position, shape and intensity of its absorption spectrum, similar to those obtained from an optically matched solution of benzophenone in comparative experiments, point to a n,π* triplet populated with a quantum yield near to unity. In spite of the spectral analogies, the kinetic evolution of the KP triplet state is different from that of a benzophenone-like triplet. The former decays with a time constant of 250 ps whereas the second is very long-lived and basically unreactive in an aqueous environment (the lifetime is of the order of tens of microseconds in oxygen-free solution). As illustrated in Fig. 7 and the related inset, the triplet decay of KP matches well the build up of a new transient absorption with λ_{max} around 580 nm. This behavior was rationalised with a decarboxylation reaction, promoted by an intramolecular electron transfer between the carboxy and the aromatic-carbonyl moieties and favored by a mixed n,π*–π,π* charge distribution in the KP excited state.[31] The involvement of the triplet state in the photoreaction was questioned on the basis of results in basic water–acetonitrile solutions,[41] which, however, in our opinion, can be irrelevant to a purely aqueous medium. The 580 nm transient was assigned to a carbanion, incorporating spectroscopic features reminiscent of a ketyl radical anion and a benzylic radical (see Scheme 8).[31,39] The time constant for the decay of this species is in the order of 120–150 ns and reflects either formation of the stable photoproduct KP-I *via* direct reaction with solvent[31,39] or formation of a neutral (triplet) biradical intermediate with λ_{max} 520 nm, depending on pH.[31,40]



Scheme 8 Mechanistic pathways for KP photodegradation in neutral aqueous solution.

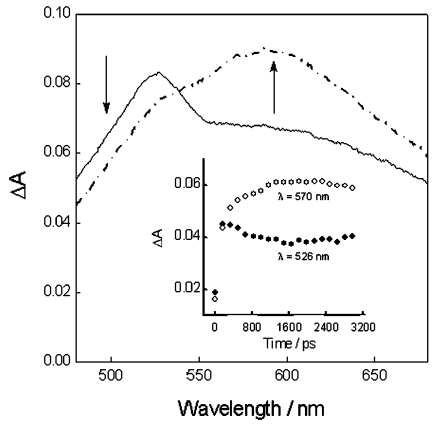


Fig. 7 Transient absorption spectra observed in a 7×10^{-5} M neutral aqueous solution of KP (——) 99 ps and (–·–·–) 693 ps after a 35 ps laser pulse (266 nm). The inset shows the absorbance changes monitored at (●) 526 nm and (○) 570 nm. Data from ref. 31.

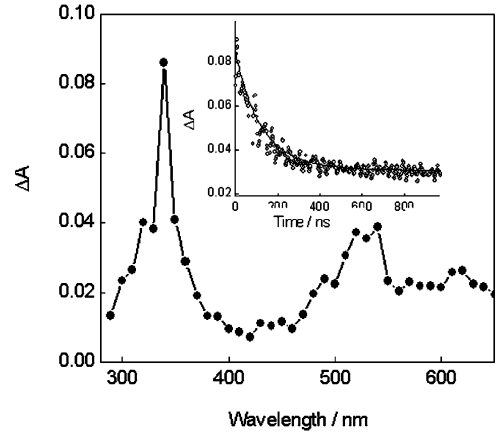


Fig. 8 Transient absorption spectrum observed in a 3.5×10^{-5} M N_2-saturated neutral aqueous solution of KP containing 10^{-2} M β-CD and recorded 4 ns after a 35 ps laser pulse (266 nm). The inset shows the time profile monitored at 340 nm and the related fitting according to eqn. (1). Data from ref. 30.

Inclusion of the KP molecule in the β-CD cage does not modify significantly the efficiency of the triplet state population but leads to dramatic effects on the triplet kinetic evolution. Indeed, contrary to what is observed in water solution, the triplet absorption, characterized by a maximum at 525 nm, is much more longer lived, being recognizable in the difference spectrum taken 4 ns after the laser pulse and displayed in Fig. 8.[30]

A rate constant k_1 of *ca.* 10^7 s^{-1} is measured for the triplet decay, on the basis of a double exponential kinetics analysis (shown in the inset), affording time constants $1/k_1 = 100$ ns and $1/k_2 = 250$ ns.

$$\Delta A(t) = a_1\exp(-t/\tau_1) + a_2\exp(-t/\tau_2) + a_3 \qquad (1)$$

Such a remarkable lengthening of the triplet lifetime was attributed to the low polarity character of the CD cavity that makes the intramolecular charge transfer process, responsible for decarboxylation, thermodynamically disfavored. This proposal is fully corroborated by the structure of the complex (Fig. 9) in which a deep embedding of the aromatic carbonyl moiety in the cavity is clearly evidenced.[30]

The $1/k_2$ time constant is relevant to a new photoreactivity pathway open in the complex, consisting of the intrinsically slow process of H-abstraction from the CD macrocycle by the carbonyl triplet. This reaction, quite common for benzophenone-like derivatives incorporated in CD cavities, leads to a radical pair, formed by a ketyl radical and a cyclodextrin counter-radical, which, finally, converts to reduced photo-

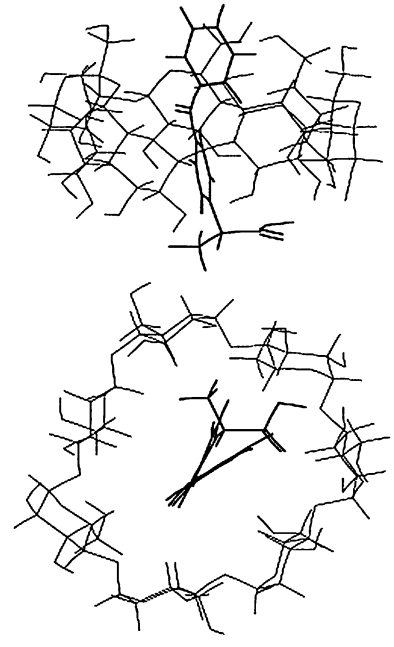
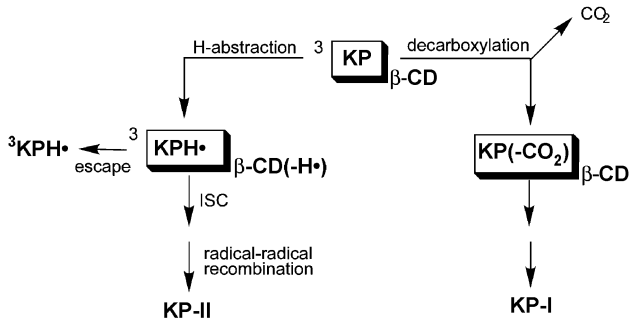


Fig. 9 Side and top view of the structure of the KP-β-CD complex obtained by combining conformational calculations with induced circular dichroism spectra. Details in ref. 30.

products. The mechanism depicted in Scheme 9 was proposed where two parallel reactivity pathways occur from the lowest triplet state of the KP-β-CD inclusion complex.[30]



Scheme 9 Schematic mechanistic pathways for KP-β-CD photodegradation.

According to this view, the 250 ns time constant was assigned to the decay of the triplet radical pair, recombining upon ISC to form the β-CD-covalently-linked product (see previous section). The presence of such an adduct indicates that the constrained CD environment favors ISC within the pair and recombination over radical exit from the cavity.[30]

The rich photobehavior of KP in the β-CD microenvironment is governed by an harmonic synergism of effects and illustrates the role of the CD as (i) apolar microenvironment that dramatically decreases the reactivity of the lowest triplet state toward decarboxylation and makes competitive an intrinsically slower chemical process; (ii) reactant that provides a source of 14 abstractable hydrogen atoms activating a reductive photochemical channel (iii) constrained medium that reduces the mobility of the radical centers generated by H-abstraction, allowing efficient cage recombination processes.

Tolmetin. TM photodecarboxylation represents a further example of chemistry mediated by an unusually short lived triplet state.[22] The overall mechanism in neutral aqueous solution is shown in Scheme 10. Triplet deactivation involves an intramolecular electron transfer between the electron rich pyrrole ring and the carbonyl moiety to give a resonance-stabilized biradical that fast releases CO_2 with a rate constant $k_{-CO2} > 2 \times 10^7\ s^{-1}$. The reaction occurs adiabatically and leads to a triplet carbanion as the key intermediate.

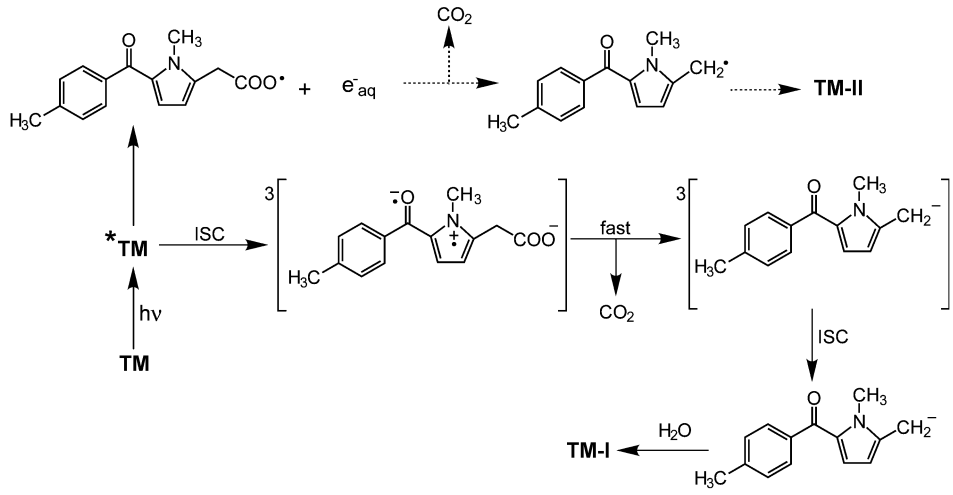
Such a species (with two main absorption bands peaked at 640 and 380 nm) decays *via* ISC with a rate constant of $1.5 \times 10^5\ s^{-1}$ to the ground-state carbanion. This latter, absorbing predominantly in the UV region, decays with a rate constant of $3 \times 10^4\ s^{-1}$ to form, *via* protonation by solvent, the main stable photoproduct TM-I.

A secondary process, also leading to decarboxylation, is photoionization. Electron photoejection likely occurs in the singlet manifold by a mixed mono- and biphotonic mechanism, and is followed by fast loss of carbon dioxide. The generated pyrrolyl radical, by self-recombination, gives rise to the dimer TM-II, found as a minor photoproduct in the steady-state photolysis.[22]

Binding of TM to β-CD does not alter the nature of the intermediates formed, but considerably modifies the efficiency of their formation as well as their time evolution.[21] As shown in Fig. 10, the transient absorption, recorded at neutral pH in N_2-saturated solution upon laser excitation of the TM-β-CD complex, is dominated at early times by the typical broad band extending beyond 700 nm due to hydrated electrons. The virtually complete disappearance of this absorption in the presence of an electron scavenger such as N_2O reveals the well-defined band of the triplet carbanion centered at *ca*. 600 nm. The yield of this transient is *ca*. 50% lower than that found with the free TM under the same experimental conditions, nicely accounting for the *ca*. 50% decrease of the photodegradation quantum yield in the TM–β-CD complex. This finding is more than coincidental and is related to a reduction in the efficiency of formation of the precursor TM triplet by CD complexation, consistent with the remarkable increase of the fluorescence quantum yield, illustrated in Section 2.

It is interesting to note that in this system the short range intramolecular charge transfer triggering the CO_2 detachment, does not appear to be perturbed by the inclusion, whereas the ISC efficiency is. The difference is striking with respect to the KP-β-CD complex, in which the efficiency of population of the triplet state is not perturbed, whereas a marked decrease of the decarboxylation efficiency occurs.

Comparison of the spectral and kinetic behavior of the triplet and ground state carbanions in aqueous and CD medium (*ca*. 30 nm blue-shift of the visible band of both species along with a 5-fold lengthening of their lifetime in presence of CD) affords useful information about the residence environment of these transients. The mechanism is that depicted in Scheme 11 in which both the excited and the ground-state carbanions remain entrapped within the β-CD cage. Indeed the lengthening of the lifetime of unstable intermediates is quite typical for species incorporated in CD cavities and may be, at least in part, related to the protection by the cavity walls against attack by external agents. Accordingly, the bimolecular constant for quenching of the triplet carbanion by oxygen drops down by a factor 3 with respect to water and a sensible reduction of the yields of the oxidation products TM-III and TM-IV is observed.



Scheme 10 Mechanistic pathways for TM photodegradation in neutral aqueous solution.

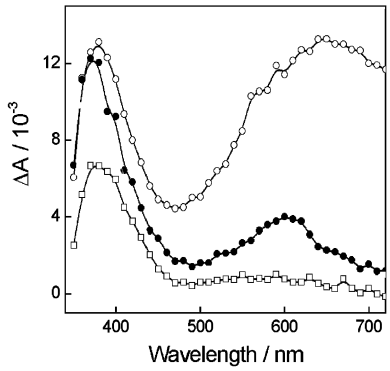


Fig. 10 Transient absorption spectra observed in a 3×10^{-5} M neutral aqueous solution of TM containing 10^{-2} M β-CD upon 6 ns laser pulse (308 nm) in different experimental conditions: (○) 0.15 μs after pulse in N_2-saturated solution; (●) 0.15 μs and (□) 150 μs after pulse in N_2O-saturated solution. Data from ref. 22.

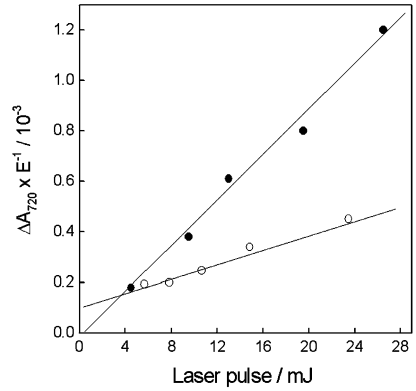


Fig. 11 Laser power dependence of the ratio between the absorbance changes, at 720 nm 0.1 μs after a 308 nm laser pulse, and the pulse energy according to eqn. (2), observed in a TM 3×10^{-5} M N_2-saturated solution (○) in the absence and (●) in the presence of 10^{-2} M β-CD. Data from ref. 22.

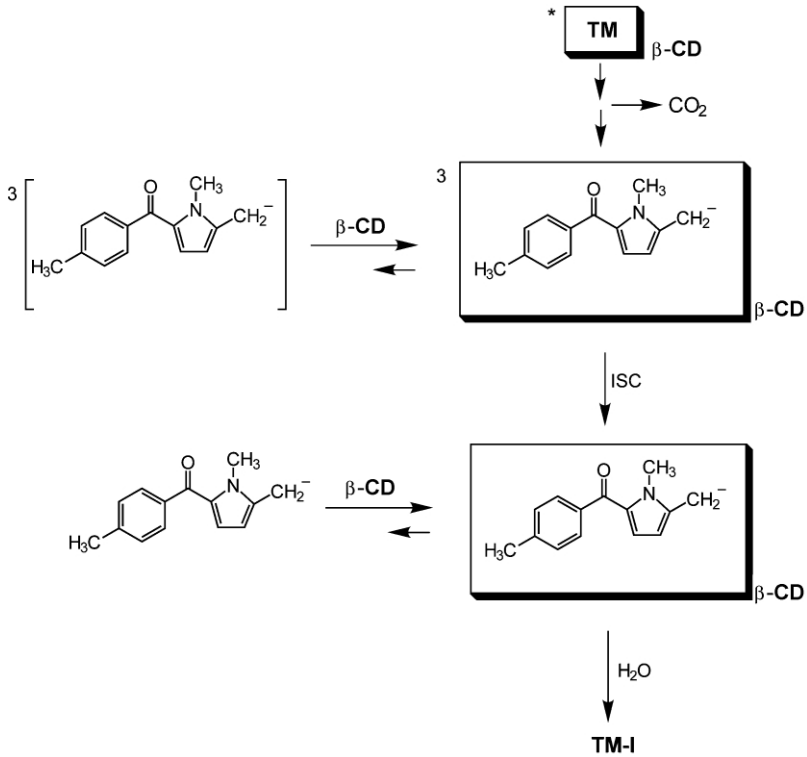
The features of the photoionization process in the TM–β-CD complex offers the opportunity to illustrate the consequences of CD association on one and two-photon photoejection events. The absorbance of the solvated electron *vs.* the laser pulse energy is usefully described by the following equation

$$\Delta A/E = a + bE \qquad (2)$$

where E is the energy of the laser pulse, a is a coefficient depending on the quantum yield of the one-photon process and b is a factor depending on the extinction coefficients and yields of the intermediate steps of the consecutive two-photon process. Fig. 11 shows that the data relevant to TM fit quite well to the above equation. The slope in the presence of β-CD, three times greater than that in aqueous environment under the same experimental conditions, indicates a considerable increase of the efficiency of the two photon photoionization process, whereas the basically zero value of the intercept is in agreement with a total suppression of the one-photon path.

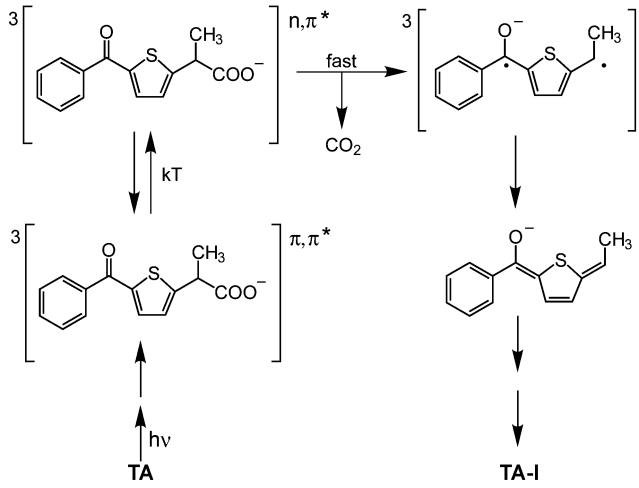
This behavior is fully understood in the light of the kinetics of the fluorescence decay, described earlier, in which a long-lived component was present upon CD complexation. This component accounts for a considerably higher probability of absorption of a second photon by the singlet state during the nanosecond laser pulse and alters the relative contribution of the two photoionization pathways. It also explains why, despite a higher production of the pyrrolyl radical (see Scheme 10), the dimeric stable product TM-II, formed by self-recombination of this radical, is not observed in the presence of β-CD. This finding is indeed fully consistent with generation of the pyrrolyl radical exclusively *via* a two-photon pathway, that under steady-state irradiation conditions is not important because of the low intensity of the excitation light.[21]

Tiaprofenic acid. As anticipated in the previous section, TA photodecomposition also involves decarboxylation. The photoreaction is in this case strongly temperature dependent. Indeed laser flash photolysis studies well established that photoextrusion of carbon dioxide is mediated by the π,π* lowest triplet state, but actually proceeds from a n,π* triplet, higher in energy by *ca*. 40 kJ mol^{-1}.[42] The lowest triplet is efficiently populated (Φ_{ISC} = 0.9), and is characterized by absorption maxima at 370



Scheme 11 Proposed scenario for triplet and ground state carbanions formed after TM–β-CD photodecarboxylation.

and 600 nm and its lifetime is 0.8 μs at room temperature. Scheme 12 summarizes the reaction mechanism proposed in aqueous solution.[43]



Scheme 12 Mechanistic pathways for TA photodegradation in neutral aqueous solution.

Loss of carbon dioxide leads to a triplet biradical which converts with τ = 1.6 μs to a ground-state enolate intermediate. This transient decays in the millisecond time domain to the stable photoproduct TA-I. Laser excitation of the complex TA–β-CD leads to the spectral changes displayed in Fig. 12,[35]

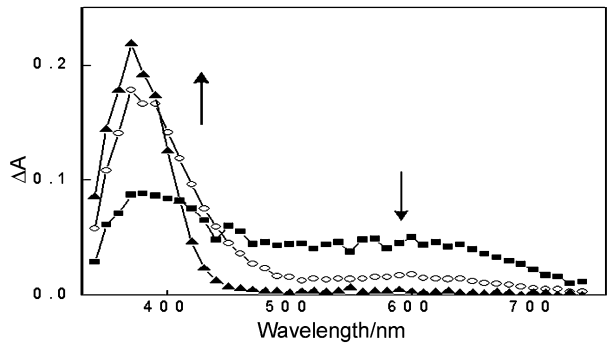


Fig. 12 Transient absorption spectra observed in a 1.2 × 10^{-4} M Ar-saturated neutral aqueous solution of TA containing 10^{-2} M β-CD upon 6 ns laser pulse (355 nm) (■) 0.06 μs, (○) 1.3 μs and (▲) 4.5 μs after pulse. Data from ref. 35.

closely similar to those observed in the absence of the host molecule, and attributed to the sequential formation of the lowest triplet of the complexed TA, of the decarboxylated triplet biradical and of the ground state long-lived enolate. The analogies are in agreement with the occurrence of photodecarboxylation as the only process also in the case of the complexed drug and are consistent with the formation of the same stable photoproducts (see earlier). As far as the lowest triplet of the complexed TA is concerned, it is populated with the same efficiency observed for the free drug, but its lifetime is significantly shortened to 0.36 μs. This fact relates to the increase of the decarboxylation quantum yield and can be understood in the light of the structural features of the inclusion complex (Fig. 13). The thenoyl moiety is deeply embedded in the CD cavity and a substantial lowering of the environmental polarity is experienced by the included guest. The energy of the lowest π,π* triplet state is raised and that of the second n,π* triplet is lowered leading to an increase of the decarboxylation rate constant (k_{dc}). Indeed in an Arrhenius-like description the observed ratio $k_{dc}^{CD}/k_{dc}^{free}$ implies a reduction of ~3.8 kJ mol^{-1} in the energy gap $T_2(n,\pi^*)$–$T_1(\pi,\pi^*)$ (see Scheme 13).[35]

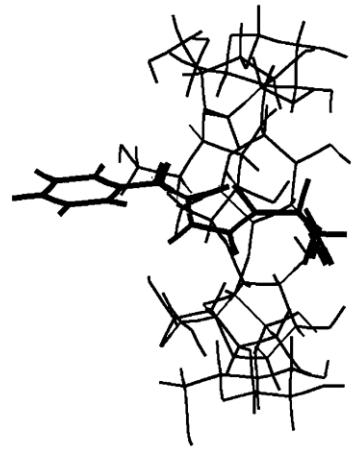
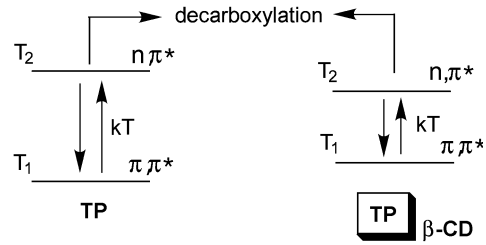
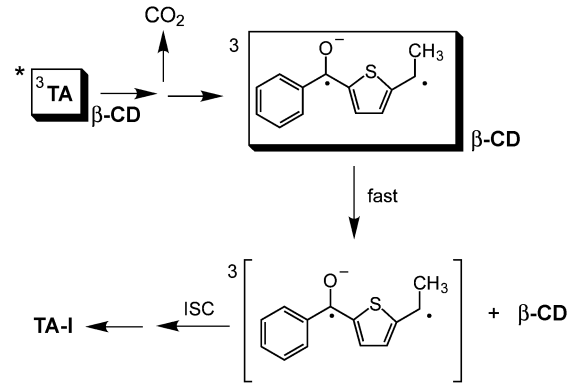


Fig. 13 Structure of the TA–β-CD complex obtained by combining conformational calculations with induced circular dichroism spectra. Details in ref. 35.



Scheme 13 Schematic energy ordering of the lowest excited triplet states in TA and TA–β-CD complex.

Contrary to the triplet, the biradical originating after decarboxylation has the same lifetime (τ = 1.4 μs) in the presence of CD as in the absence of the host. This fact points to a fast exit of the transient from the cavity (Scheme 14).[35] The structure of the complex, showing the incomplete insertion of TA in the β-CD interior, is consistent with such a fast dissociation.



Scheme 14 Proposed mechanism for the decay of the triplet biradical formed after TA–β-CD photodecarboxylation.

Suprofen. Like in TA, photodecarboxylation in SP is a temperature-dependent process.[34] The mechanism of SP photodecomposition is also basically similar, so that the reader can refer to the Scheme 12, by taking into account the different position of the isopropionic substituent (position 4 of the phenyl instead of the thiophene ring). The precursor SP triplet is produced with almost unitary efficiency but, contrary to what is observed in TA, its lifetime is strongly affected by the ground state concentration. Actually, τ_T ranges from 1–2 μs at 10^{-4} M to *ca.*40 μs at infinite dilution. This behavior is reflected in the dependence of the photodegradation quantum yield on the drug

concentration (see earlier) and is rationalized by the occurrence of the reaction

$$^3SP + SP \rightarrow 2SP \quad (3)$$

Incorporation of SP in the β-CD cavity has profound effects on the drug photobehavior. As shown in Fig. 14, the intensity, but

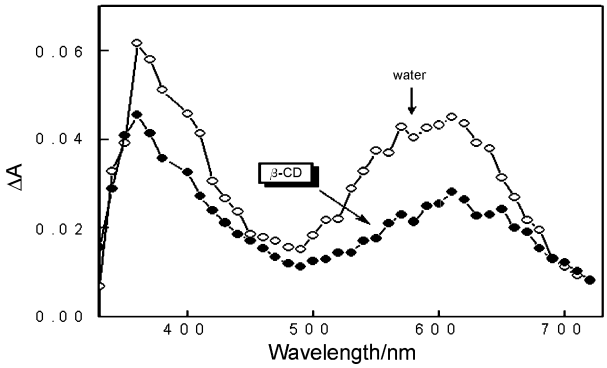


Fig. 14 Transient absorption spectra observed in a 3.5×10^{-4} M Ar-saturated neutral aqueous solution of SP 0.2 μs after a 20 ns laser pulse (355 nm) (○) in the absence and (●) in the presence of 10^{-2} M β-CD. Data from ref. 34.

not the profile, of the absorption band of the lowest excited triplet (the precursor state) decreases from aqueous to CD environment of *ca.* 30%, in agreement with a decrease in the ISC quantum yield.[34,35] Besides, the triplet lifetime becomes almost independent on the ground state concentration (Fig. 15),

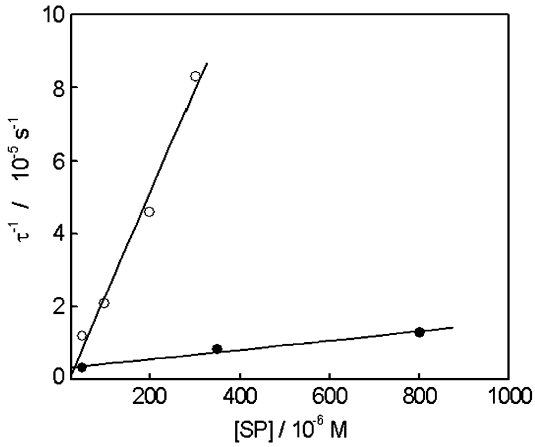


Fig. 15 Dependence of the triplet lifetime of SP on the drug ground state concentration (○) in the absence and (●) in the presence of 10^{-2} M β-CD. Data from ref. 34.

the bimolecular self-quenching constant dropping by more than one order of magnitude down (from 1.9×10^9 M^{-1} s^{-1} in the absence to 0.1×10^9 M^{-1} s^{-1} in the presence of β-CD).

The almost complete insertion of the aromatic skeleton in the cavity, as shown in Fig. 16,[35] explains the efficient protection of

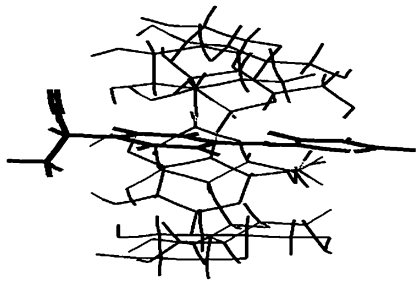
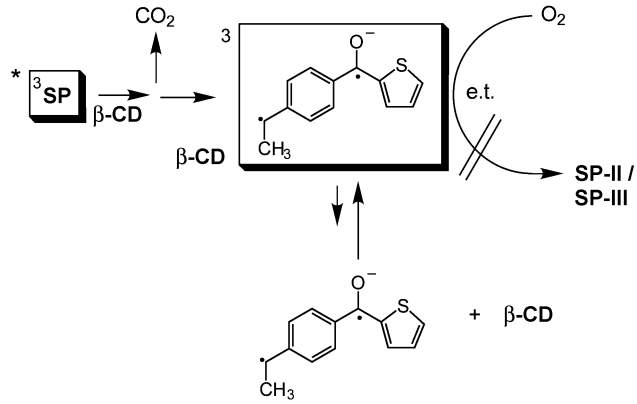


Fig. 16 Structure of the SP–β-CD complex obtained by combining conformational calculations with induced circular dichroism spectra. Details in ref. 35.

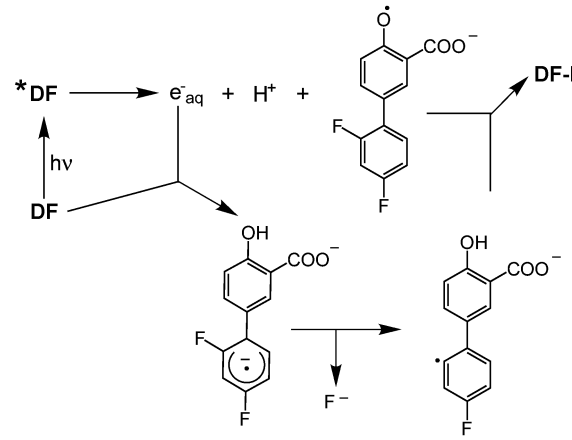
the SP excited state from bimolecular interactions and rationalizes the strong increase of the photodecarboxylation quantum yield. Indeed the ratio $k_{dc}^{CD}/k_{dc}^{free}$ corresponds to a reduction in the energy gap between T_2 and T_1 of *ca.* 4.6 kJ mol^{-1},[35] a higher value than that found for TA. The deeper penetration of SP in the CD cavity also explains the lack of significant amounts of oxidation photoproducts in the steady-state photolysis of the SP–CD complex (see earlier). Indeed, although the long lifetime of the complexed SP triplet prevents direct detection of the SP decarboxylated triplet biradical, it is conceivable that this species will not promptly exit from the cavity and, entrapped in the CD cage, will interact less efficiently with oxygen (Scheme 15).[35]



Scheme 15 Proposed scenario for the triplet biradical formed after SP–β-CD photodecarboxylation.

The examples of TA and SP clearly illustrate how, despite a very close structural analogy in these molecules, the time evolution of the intermediates photogenerated in the CD cavity exhibits differences intimately connected to the structural peculiarities of the respective ground state associates.

Diflunisal. An uncommon defluorination reaction characterizes the DF photodecomposition in aqueous media. As summarized in Scheme 16 the primary photochemical process is



Scheme 16 Mechanistic pathways for DF photodegradation in neutral aqueous solution.

photoionization. Electron photoejection takes place *via* mixed one and two-photon pathways mediated by the excited singlet state. A phenoxyl radical is generated. Defluorination takes place *via* the radical anion, formed by trapping of the hydrated electrons by the DF ground state. A σ-aryl radical is produced after loss of the fluoride anion.[44] Formation of the main stable photoproduct DF-I in the steady-state photolysis (see earlier) is fully consistent with the occurrence of a cross-combination reaction between the phenoxyl and the σ radical centers.

Inclusion of DF in the β-CD cavity does not affect the primary photochemical events. Both efficiency and relative

weight of the mono and two-photon photoionization pathways are similar to those observed in the absence of β-CD. This is in good agreement with both singlet lifetime and structure of the DF–β-CD complex. Actually, since the former is only slightly different from that of the free guest, the probability of absorption of a second photon during the laser excitation does not change significantly and, consequently, the biphotonic photoionization yield is not affected. The quantum yield of the one-photon photoionization process also does not change. Indeed the hydroxy group of DF, likely experiencing an aqueous environment, is still able to act as both H-donor and H-acceptor, making possible the formation of H-linked structures to two water molecules, that are known to control one photon ejection in phenol derivatives.[45]

The lack of CD effects in the primary photoprocesses appears to be in contrast with the almost total suppression of the formation of the product DF-I and the higher photostability of the drug in the presence of β-CD. A satisfactory rationalization of these results, requires that the two important parameters of mobility and reactivity of the phenoxy and σ-aryl radicals, involved in the cross-combination reaction, are considered.

In the β-CD-drug system the phenoxyl radical, although generated as efficiently as its partner, the hydrated electron, is present in the aqueous phase at a sensibly lower concentration, as shown in Fig. 17. This was rationalized as due to trapping of

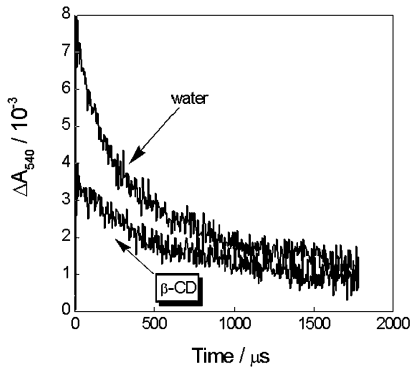


Fig. 17 Kinetic traces for the decay of the phenoxy radical monitored at 540 nm formed upon 266 nm laser excitation of optically matched solutions of DF and DF–β-CD complex. Data from ref. 26.

a significant fraction of it in the CD cavity where fast regeneration of the starting compound occurs by H-abstraction (Scheme 17). Indeed the phenoxy radical, because of its more hydrophobic nature, is expected to spend more time than DF in the CD environment and react by abstracting one of the fourteen available hydrogen atoms bonded to CD secondary carbons.

The kinetics of the hydrated electron decay in the presence of CD is consistent with an electron trapping process by the ground state DF, whose rate is not affected by the exit dynamic of the DF molecule from the cavity. Therefore the electron capture occurs as efficiently as in the absence of CD and leads to the generation of the radical anion in the solvent bulk. This transient, because of its extremely polar nature, is expected to remain in the aqueous phase where, by loss of fluoride, it converts to the σ-aryl radical.

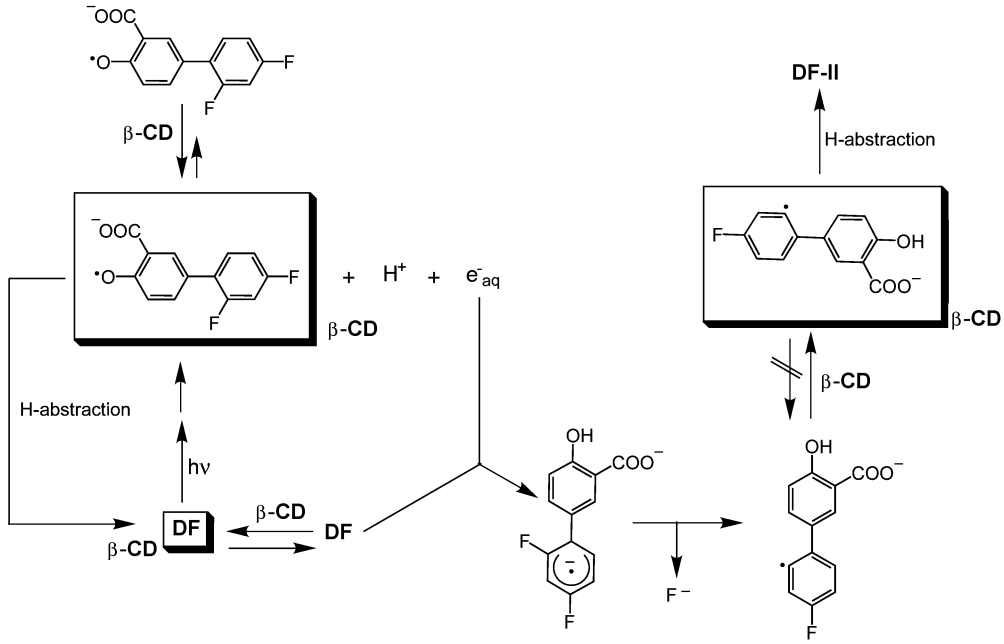
As depicted in Scheme 17, this species can efficiently enter into the cavity, whereby prompt H-abstraction from the CD converts to the monofluorinated photoproduct DF-II. Such a reaction pathway makes, of course, the σ-aryl radical unavailable for the cross-termination reaction with the residual phenoxyl radical in the bulk solvent and justifies the total lack of the cross-combination product DF-I.[26]

The behavior of DF shows how the CD microenvironment may control the reactivity of radical species generated either inside or outside the cavity and provide a rationale for the photochemical steady state outcome, *i.e.* the increased photostability of the inclusion complex.

3.3 Photogenerated reactive oxygen species

Interaction of the drug triplet with oxygen is at the basis of the generation of singlet oxygen by energy transfer and/or superoxide anion by electron transfer. These intermediates are very reactive and highly relevant to the photosensitising effects of the drugs. It is therefore of interest to examine how the inclusion in a CD cavity affects such processes with NSAIDs.

Shielding of the drug excited state by the cavity walls should protect it from the interaction with molecular oxygen and decrease the efficiency of production of both reactive species. As regards singlet oxygen, there have been quantitative studies, showing that the influence of the CD inclusion on the yield of production is difficult to predict. Indeed the rate constant for quenching of the SP triplet state by oxygen is decreased from *ca.* 10^9 M^{-1} s^{-1} in the free molecule to 2.4×10^8 M^{-1} s^{-1} in the β-CD inclusion complex. However this fact does not lead to a comparable decrease in the quantum yield of singlet oxygen



Scheme 17 Mechanisms for DF–β-CD photodegradation.

production, which only decreases from 0.25 in the free molecule to 0.18 in the β-CD complex. This is due to the substantial lengthening of the lifetime of the SP triplet in the CD cavity, determined by the slowing down of the self-quenching process (see above), which leaves the fraction of triplets quenched by oxygen and generating singlet oxygen almost unchanged .[34]

Other NSAIDs like KP and TM do not produce singlet oxygen in an aqueous buffer, due to the short lifetime of their reactive triplet.[21,22] However with KP, singlet oxygen is generated in the system upon accumulation and photoexcitation of the decarboxylated photoproducts. In the presence of β-CD such effects are depressed due to the inclusion in the cavity of these more hydrophobic benzophenone derivatives, which in this case appear effectively prevented from interaction with molecular oxygen.[30]

No quantitative information is available on the effect of CD inclusion on the production of superoxide anion by electron transfer. In the case of the triplet carbanion of TM, the rate constant for oxygen quenching decreases by a factor 3 by CD inclusion, but again the lifetime of the intermediate is longer by a factor of 5, allowing to reasonably figure that the relevant superoxide anion quantum yield should not be substantially depressed.[21]

4 Concluding remarks

The emission of drugs in CD media has a powerful diagnostic potential for drug–CD binding and may help to determine the association constants at very low drug concentrations, which is very useful in the case of poorly soluble systems. It is worth pointing out that the association constants obtained by emission are relevant to the drug excited state equilibria whereas those derived from UV-Vis absorption, induced circular dichroism, NMR pertain to the drug ground state equilibria. In principle the former could be different and lead to a different partitioning of free and bound molecules during the lifetime of the excited state. However this usually does not occur, since the time constants for entry/exit of guests from the CD cavity are slower by one or more orders of magnitude than the fluorescence lifetimes (usually in the nanosecond domain).[46] This possibility should only be considered in the case of microsecond or longer lived excited states.

The study of the emission properties of drug–CD complexes may be useful to determine the drug chemical form relevant to a particular biological environment, identify the photosensitizing species, gain insight into the role of conformational features, steric constraints and polarity factors in the excited state deactivation.

The features of the photoreactivity of drugs in CD cavities clearly show that inclusion does not necessarily bring about an increase of the photostability. This is due to the manifold action of the changed environment, differently affecting the intrinsic reactivity of the photoactive excited states and the fate of the intermediates. The participation of the CD as co-reagent often comes into play. An increased efficiency in the photodegradation in the presence of CD may be directly determined by a variation in the energetic layout of the excited states. Alternatively, the photoreactivity may decrease, as far as the reaction occurring in the aqueous medium is concerned, but a new photochemical channel may be open in the microenvironment of CD, due to the lower polarity and availability of abstractable hydrogens. When neither the intrinsic reactivity of the photoactive state is changed nor additional photoreactions take place one can actually observe an increased photostability, because of less efficient formation or decreased photoreactivity of the active state.

The outcome in the steady state photolysis sometimes does not rely on the primary photoprocesses, but reflects affinity and reactivity of secondary radical species toward the CD microenvironment. Radical–radical processes may be strongly depressed by the interaction of the partners with the CD and result in an increased photostability of the system. On the other hand, formation of adducts to CD, may come into play by recombination of radical fragments prevented from separation.

The modes of the interaction of photoexcited drugs with molecular oxygen in CD media is also diversified. One cannot affirm, in general, that singlet oxygen or superoxide anion yields are decreased, or that the formation of photoproducts resulting from interaction of intermediates with molecular oxygen is inhibited.

The photobehavior of CD–drug inclusion complexes show that the use of CDs as drug carriers cannot be proposed as a general strategy to increase drug photostability and decrease production of reactive oxygen species, in order to minimize photoinduced toxic effects on biological matter. Because no generalizations are possible, each system must be investigated in detail with the aim of elucidating the effect of the CD on the primary and secondary reactions following the light absorption.

Rather, it appears that the CD medium controls the photochemical pathways in the drug and may help to disclose peculiar photoreactivity, activated by interaction with apolar, hydrophobic and H-donating environments, thus confirming its value as model for hydrophobic protein and nucleic acid pockets and lipophilic layers of membranes.

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6 References

1 J. Szejtly, *Chem. Rev.*, 1998, **98**, 1743.
2 V. T. D′Souza and K. B. Lipkowitz, *Chem. Rev.*, 1998, **98**, 1741.
3 P. Bortolus and S. Monti, *Adv. Photochem.*, 1996, **21**, 1.
4 K. Uekama, F. Hirayama and T. Irie, *Chem. Rev.*, 1998, **98**, 2045.
5 J. A. Arancibia, M. A. Boldrini and G. M. Escandar, *Talanta*, 2000, **52**, 261.
6 J. A. Arancibia and G. M. Escandar, *Analyst*, 1999, **124**, 1833.
7 F. Bosca, M. L. Marin and M. A. Miranda, *Photochem. Photobiol.*, 2001, **74**, 637.
8 K. Uekama, T. Irie and F. Hirayama, *Chem. Lett.*, 1978, 1109.
9 T. H. Hoshino, K. Ishida, T. Irie, F. Hirayama and M. Yamasaki, *J. Incl. Phen.*, 1988, **6**, 415.
10 G. De Guidi, G. Condorelli, S. Giuffrida, G. Puglisi and G. Giammona, *J. Incl. Phenom. Mol. Recogn. Chem.*, 1993, **15**, 43.
11 M. Partyka, B. H. Au and C. H. Evans, *J. Photochem. Photobiol. A-Chem.*, 2001, **140**, 67.
12 D. Diaz, C. M. E. Llanos and M. J. B. Bernad, *Drug Dev. Ind. Pharm.*, 1999, **25**, 107.
13 E. Junquera and E. Aicart, *Int. J. Pharm.*, 1999, **176**, 169.
14 I. Oh, M. Y. Lee, Y. B. Lee, S. C. Shin and I. Park, *Int. J. Pharm.*, 1998, **175**, 215.
15 M. D. G. Seye, C. Prot, A. Adenier, J. J. Aaron and N. Motohashi, *New J. Chem.*, 2001, **25**, 1290.
16 Y. H. Kim, D. W. Cho, S. G. Kang, M. Yoon and D. Kim, *J. Lumin.*, 1994, **59**, 209.
17 J. A. Martins, M. M. Sena, R. J. Poppi and F. B. T. Pessine, *Appl. Spectrosc.*, 1999, **53**, 510.
18 S. M. Andrade and S. M. B. Costa, *Phys.Chem. Chem. Phys.*, 1999, **1**, 4213.
19 S. M. Andrade, S. M. B. Costa and R. Pansu, *Photochem. Photobiol.*, 2000, **71**, 405.
20 R. S. Tsai, P. A. Carrupt, N. Eltayar, Y. Giroud, P. Andrade, B. Testa, F. Bree and J. P. Tillement, *Helv. Chim. Acta*, 1993, **76**, 842.
21 S. Sortino, J. C. Scaiano, G. De Guidi and S. Monti, *Photochem. Photobiol.*, 1999, **70**, 549.
22 S. Sortino and J. C. Scaiano, *Photochem. Photobiol.*, 1999, **69**, 167.

23 M. Valero, S. M. B. Costa, J. R. Ascenso, M. M. Velazquez and L. J. Rodriguez, *J. Incl. Phenom. Macrocycl. Chem.*, 1999, **35**, 663.
24 L. J. Martinez and J. C. Scaiano, *Photochem. Photobiol.*, 1998, **68**, 646.
25 M. Valero, S. M. B. Costa and M. A. Santos, *J. Photochem. Photobiol. A-Chem.*, 2000, **132**, 67.
26 S. Sortino, S. Giuffrida, S. Fazio and S. Monti, *New J. Chem.*, 2001, **25**, 707.
27 S. Sortino, L. J. Martinez and G. Marconi, *New J. Chem.*, 2001, **25**, 975.
28 L. L. Costanzo, G. De Guidi, G. Condorelli, A. Cambria and M. Fama, *Photochem. Photobiol.*, 1989, **50**, 359.
29 F. Bosca, M. A. Miranda, G. Carganico and M. Mauleon, *Photochem. Photobiol.*, 1994, **60**, 96.
30 S. Monti, S. Sortino, G. De Guidi and G. Marconi, *New J. Chem.*, 1998, **22**, 1013.
31 S. Monti, S. Sortino, G. De Guidi and G. Marconi, *J. Chem. Soc., Faraday Trans.*, 1997, **93**, 2269.
32 G. De Guidi, R. Chillemi, L. L. Costanzo, S. Giuffrida, S. Sortino and G. Condorelli, *J. Photochem. Photobiol. B-Biol.*, 1994, **23**, 125.
33 J. V. Castell, M. J. Gomez-Lechon, C. Grassa, L. A. Martinez, M. A. Miranda and P. Tarrega, *Photochem. Photobiol.*, 1994, **59**, 35.
34 S. Sortino, G. De Guidi, G. Marconi and S. Monti, *Photochem. Photobiol.*, 1998, **67**, 603.
35 S. Monti, S. Encinas, A. Lahoz, G. Marconi, S. Sortino, J. Perez-Prieto and M. A. Miranda, *Helv. Chim. Acta*, 2001, **84**, 2452.
36 M. C. Jimenez, M. A. Miranda and R. Tormos, *J. Photochem. Photobiol. A-Chem.*, 1997, **104**, 119.
37 S. Giuffrida, G. De Guidi, S. Sortino, R. Chillemi, L. L. Costanzo and G. Condorelli, *J. Photochem. Photobiol. B-Biol.*, 1995, **29**, 125.
38 G. De Guidi, R. Chillemi, S. Giuffrida, G. Condorelli and M. Cambria Famà, *J. Photochem. Photobiol. B-Biol.*, 1991, **10**, 221.
39 L. J. Martinez and J. C. Scaiano, *J. Am. Chem. Soc.*, 1997, **119**, 11066.
40 C. D. Borsarelli, S. E. Braslavsky, S. Sortino, G. Marconi and S. Monti, *Photochem. Photobiol.*, 2000, **72**, 163.
41 G. Cosa, L. J. Martinez and J. C. Scaiano, *Phys. Chem. Chem. Phys*, 1999, **1**, 3533.
42 S. Encinas, M. A. Miranda, G. Marconi and S. Monti, *Photochem. Photobiol.*, 1998, **67**, 420.
43 S. Encinas, M. A. Miranda, G. Marconi and S. Monti, *Photochem. Photobiol.*, 1998, **68**, 633.
44 S. Sortino, J. C. Scaiano and G. Condorelli, *J. Phys. Chem. B*, 1999, **103**, 9279.
45 S. Monti, G. Kohler and G. Grabner, *J. Phys. Chem.*, 1993, **97**, 13011.
46 M. H. Kleinman and C. Bohne, in *Organic Photochemistry*, ed. V. Ramamurthy and K. S. Schanze, Marcel Dekker, New York, 1997.