PERKIN 2

Study of the effect of β-cyclodextrin on the photophysics of alloxazines† in the solid state

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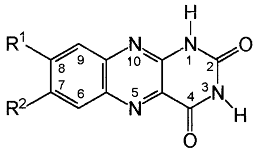
The influence of β-cyclodextrin (β-CD) on the photophysics of alloxazines† (lumichrome, Lch; 7-methylalloxazine, 7MA11; and 8-methylalloxazine, 8MA11) has been studied in the solid state by ground-state absorption and steady-state fluorescence methods. We support the existence of inclusion complexes between Lch and β-CD in water. The presence of similar complexes for 7MA11 and 8MA11 has also been found. Complexes of alloxazines and β-CD are also present in the solid state. The formation of hydrogen bonds in solid inclusion complexes between alloxazines and β-CD have been postulated in order to explain the luminescence behaviour of these solid complexes. New spectroscopic evidence has been found in the solid state of doubly hydrogen-bonded lumichrome dimers capable of executing double proton transfer in the excited state.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides of six to eight α-D-glucose units (α-CD, six; β-CD, seven; γ-CD, eight) that possess internal hydrophobic cavities able to include a great variety of guest molecules from aqueous solutions.[1–3] The oligosaccharide ring forms a torus with the primary hydroxy groups of the glucose residues on the narrow end of the torus and the secondary ones on the wider end. All the hydroxy groups are directed towards the outside of the cavity but they can also form hydrogen bonds with the included guest and/or also with external molecules.

The photochemistry and photophysics of molecules can be modified by inclusion in cyclodextrins.[4–6] These changes may be due to several effects: specific interactions between the guest and the cyclodextrins,[7] specific photoreactions between the guest and the cyclodextrins,[8,9] sensitivity of guest photoprocesses to the environment polarity,[9] and constraints imposed by the cavity on the conformations of the guest molecules and on the translational and rotational motions of the photoinduced intermediates.[10] The changes of the photophysics and photochemistry of the guest molecules included in CDs are especially important in solid complexes.[4,5] However, few studies[9–13] — mainly using the technique of diffuse reflectance laser flash photolysis developed by Wilkinson and coworkers[14,15] — have been done on the photophysical properties of these solid complexes. There are also a few reports of room temperature phosphorescence studies of several organic molecules included in solid mixtures of α- and β-CD and NaCl.[16]

Recently,[17] the formation of a β-CD inclusion complex of 7,8-dimethylalloxazine (lumichrome) in aqueous solution has been reported. Lumichrome, one of the products of decomposition of the biologically important flavins, and other alloxazines have been found in biological materials.[18,19] Alloxazines unsubstituted at N^1 undergo excited state proton transfer from N^1 to N^{10} to produce the excited isoalloxazinic tautomer **2**, which has a differentiated emission, in the presence of compounds having proton donor and acceptor functions and able to form appropriate hydrogen bonds with alloxazine molecules (*i.e.* some carboxylic acids, bases and water).[20–26]



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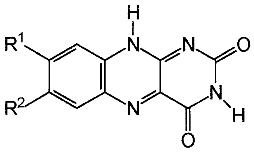

2

Lch $R^1 = R^2 = CH_3$
7MAll $R^1 = H, R^2 = CH_3$
8MAll $R^1 = CH_3, R^2 = H$

Lfv $R^1 = R^2 = R^3 = CH_3$

In this paper we present studies on the effect of β-CD on the photophysical properties of alloxazines using absorption and steady-state fluorescence methods. For this study we chose as a model compounds three alloxazines: lumichrome (Lch), 7-methylalloxazine (7MA11) and 8-methylalloxazine (8MA11). Results obtained for alloxazine–β-CD complexes in the solid state were compared with results derived from measurements made for alloxazine–β-CD mechanical mixtures and alloxazines adsorbed on cellulose (which can be considered as a noncyclic analogue of CD) as well as on KBr used as a solid support.

Experimental

β-CD, cellulose (powder, 20 micron) and KBr supplied by Aldrich were used as received. Although it is known that some commercial samples of cyclodextrins show some impurity luminescence,[7] no emission of β-CD was detected under our experimental conditions. Three alloxazines examined in this study were synthesized as described elsewhere.[21] Distilled water was used for preparing solutions.

β-CD solid complexes were prepared by dissolving a certain amount of the alloxazines in 0.011 M β-CD aqueous solutions under magnetic stirring at 70 °C for 4 h. Water evaporation was then carried out *in vacuo* with a rotary evaporator and the solid obtained was dried under vacuum at 40 °C for 4 h. Sample loading was usually between 0.5 and 2 mg of the substrate per g of β-CD. For some experiments lower (0.1 $mg\ g^{-1}$) or higher (12.7 $mg\ g^{-1}$) sample loadings were used.

Since cellulose readily adsorbs a considerable amount of water the microcrystalline cellulose was dried in a vacuum oven

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† IUPAC name for alloxazine = benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione.

Table 1 Emission maxima (λ/nm) of lumichrome, LcH; 7-methylalloxazine, 7MAII; and 8-methylalloxazine, 8MAII, in different media (λ_{exc} 355 nm)

Medium	Lch	7MAII	8MAII
Water	472, 525[a]	472	449, 505[a]
β-CD in water	457	463	448
β-CD solid complex	434,[a] 483	439,[a] 485	431,[a] 487
β-CD mechanical mixture	465, 525	458, 500–600[b]	443
Solid crystals	475,[c] 530	—	—
KBr mechanical mixture	470,[a] 528	—	—
Cellulose[d]	452	458	440

[a] Shoulder. [b] Broad band. [c] Very weak band. [d] Obtained by dissolving the substrate in acetonitrile with cellulose and then evaporating the solvent.

at 70 °C for at least 24 h prior to use. Alloxazines were dissolved in acetonitrile and a known amount of this solution was added to the previously dried cellulose. The resulting suspension was stirred periodically and then allowed to evaporate slowly. After several hours any residual solvent was removed by placing the sample in a heated vacuum oven.

β-CD–alloxazine, β-CD–lumiflavin and KBr–alloxazine mechanical mixtures of similar concentrations to those employed for the solid complexes were obtained by mixing them in an agate mortar. These mechanical mixtures were dried under vacuum at 40 °C for 4 h. UV–VIS absorption spectra were recorded on a HP8453 diode array spectrophotometer. Ground state diffuse reflectance absorption spectra were recorded on a PU8800 UV–VIS spectrophotometer equipped with an integrating sphere. Steady-state fluorescence spectra were recorded on a Fluoromax spectrofluorimeter (λ_{exc} 355 nm). All the measurements were performed at room temp. with air-equilibrated samples.

Results

In water solution

The emission spectra of lumichrome and 8-methylalloxazine in water (Fig. 1) show broad bands with maxima at 472 and 449 nm, respectively, each with a shoulder on the red side of the emission band at *ca.* 520 and 500 nm, respectively. These shoulders could be attributed to the emission of isoalloxazinic tautomers.[21] 7-Methylalloxazine shows only a broad band with the maximum at 471 nm (Fig. 1).

Addition of β-CD to the aqueous solutions of lumichrome and 7-methylalloxazine causes a blue shift of their emission maximum (Table 1 and Fig. 1) and in the cases of lumichrome and 8-methylalloxazine the disappearance of the shoulders at 520 and 500 nm respectively.

In the solid state

The diffuse reflectance absorption spectra and fluorescence emission spectra of Lch, 7MA11, 8MA11–β-CD solid complexes; of Lch, 7MA11, 8MA11–β-CD mechanical mixtures; and of Lch, 7MA11 and 8MA11 adsorbed on cellulose were recorded.

Absorption spectra of all studied compounds in the solid state show two absorption bands in the region 300–500 nm. Spectra of Lch are shown in Fig. 2 as a typical example. The absorption spectra of 8-methylalloxazine and 7-methylalloxazine in β-CD mechanical mixtures and on cellulose are similar to the spectra of these compounds in solution.[20,21] The absorption spectra of 7MA11 and 8MA11 β-CD complexes show an increase of absorptivity of the long wavelength band and the appearance of shoulders at 400 and 450 nm, respectively. The absorption spectrum of the lumichrome–β-CD complex also shows a relative increase of absorption intensity of the long wavelength band compared to the corresponding spectrum recorded when cellulose was used as a solid support. However, absorption spectra of lumichrome–β-CD and also lumichrome–KBr mechanical mixtures differ markedly from the spectrum recorded on cellulose (see Fig. 2). In these last spectra a red shift is observed and there is a change of the shape of the long wavelength absorption band.

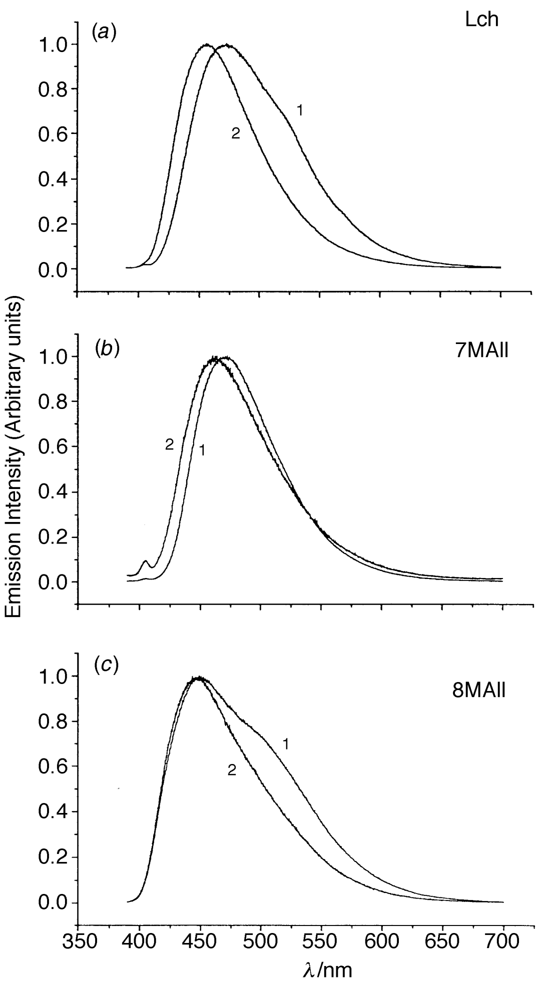


Fig. 1 Normalised fluorescence emission spectra of lumichrome (Lch), 7-methylalloxazine (7MA11) and 8-methylalloxazine (8MA11); in water (1) and in water with β-CD (0.011 M) (2). The excitation wavelength was 355 nm.

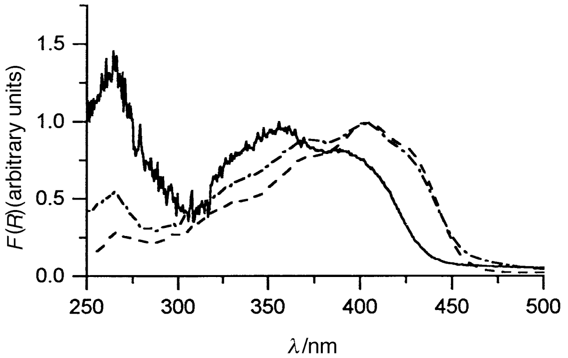


Fig. 2 Ground state absorption spectra of lumichrome on cellulose (——); in a β-CD mechanical mixture in the solid state (- - -); and, in a KBr mechanical mixture in the solid state (-••••-). Ground state diffuse reflectance absorption spectra in the solid state are plotted as F(R), the Kubelka–Munk remission function.[14,33]

Emission spectra of all studied alloxazines on cellulose exhibit one emission band with maxima at *ca.* 455 nm (Fig. 3). The emission spectra of all β-CD solid complexes exhibit a band with a maximum centred between 483 and 487 nm and a shoulder on the blue side of the emission band at *ca.* 430–440 nm. Emission spectra recorded for alloxazine–β-CD mechanical mixtures show two emission bands in the case of lumi-

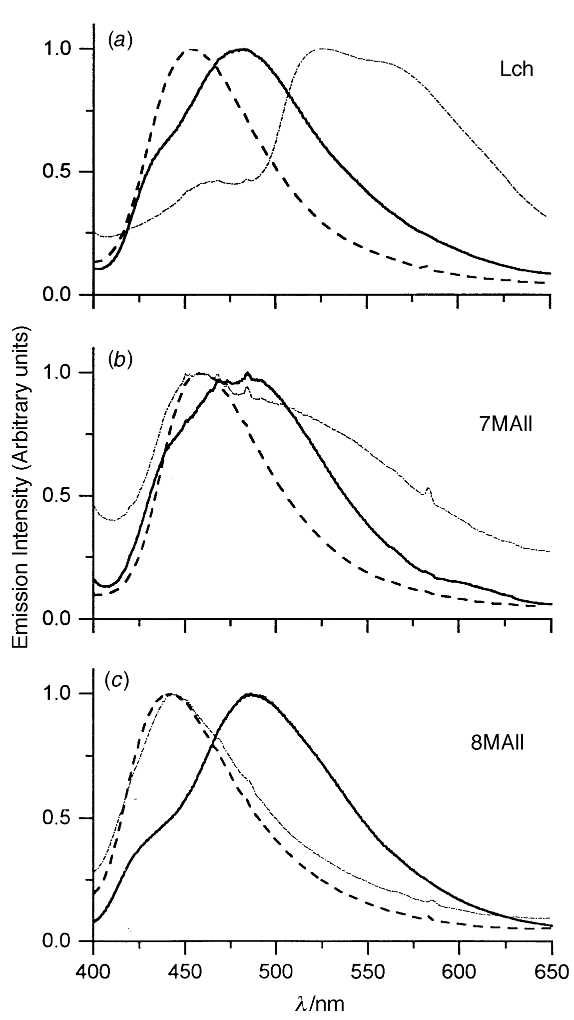


Fig. 3 Normalised fluorescence emission spectra of lumichrome (Lch), 7-methylalloxazine (7MA11) and 8-methylalloxazine (8MA11) in alloxazine–β-CD complexes in the solid state (——), in alloxazine–β-CD mechanical mixtures (-····-) and adsorbed on cellulose (- - -). The excitation wavelength was λ_{exc} 355 nm

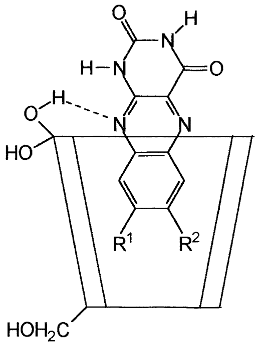
chrome and 7-methylalloxazine and one emission band for 8-methylalloxazine (Fig. 3).

Discussion

Recently, it was shown[17] that lumichrome forms inclusion complexes with β-CD in water. The main evidence for the formation of such inclusion complexes was a blue shift of the emission band of lumichrome in the presence of β-CD compared with its emission in water. A blue shift of the emission maximum of lumichrome in the presence of β-CD is expected for inclusion complexes due to the less polar microenvironment of the β-CD compared to aqueous media since the fluorescence emission spectra of alloxazines are known to be highly sensitive to the solvent polarity.[21] Similar blue shifts of the emission maxima to those observed for lumichrome were recorded by us also for 7MA11 and 8MA11. These changes clearly demonstrate that 7MA11 and 8MA11 also form inclusion complexes with β-CD in water. Additionally the disappearance in the presence of β-CD of the long wavelength shoulders in the emission spectra of Lch and 8MA11 (which can be ascribed to emission from isoalloxazinic forms) supports this conclusion.

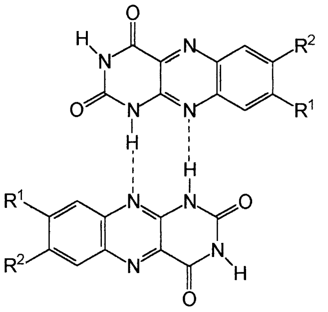
We assume that because complexes between alloxazines and β-CD exist in water they should also exist in the solid state (see Experimental). Changes in absorption spectra of solid alloxazine–β-CD complexes as compared to the spectra on cellulose and for mechanical mixtures with β-CD indicate that there are specific interactions between alloxazines and β-CD in the ground state in such solid inclusion complexes.

The emission spectra of alloxazine–β-CD complexes (see Fig. 3) show that probably there are two different emitting species in such solid samples. Although it is not easy to determine the position of emission bands from the observed shoulders, the position of the shoulders suggest that the emission band with lower intensity has a maximum rather close to the position of the emission maxima of alloxazines on cellulose (this is confirmed when the spectrum is resolved into component Gaussian curves). The position of the apparent maxima of the most intense emission bands shifts to longer wavelength, at *ca.* 485 nm for all three compounds. Lasser and Feitelson[27] reported a very weak fluorescence with a maximum at 490 nm for lumiflavin (isoalloxazine structure compound, **2**) which was assigned by them to the protonated singlet state of this compound. We propose that this long wavelength emission in alloxazine–β-CD complexes derives from alloxazines hydrogen-bonded to β-CD. It is possible that the alloxazine molecule is included in the β-CD cavity in such an arrangement (Scheme 1) that the N^{10} and the hydroxylic groups of the β-CD are placed close enough to form a hydrogen bond in the ground state. If the hydrogen bond already exists in the ground state, it will probably become stronger in the singlet excited state because upon excitation the electron density at N^{10} increases.[25] An excited singlet state arising from such a hydrogen bonded complex is suggested as being responsible for the emission with maxima at *ca.* 485 nm for all three cases.



Scheme 1

The emission spectra of alloxazines in β-CD mechanical mixtures and on cellulose were markedly different except for the case of 8MA11 (see Fig. 3). The new emission band of lumichrome–β-CD mechanical mixture (1.1 mg g^{-1}) at longer wavelength can be assigned to the excited isoalloxazinic tautomer of lumichrome on the basis of the comparison with the emission of a mechanical mixture of β-CD and lumiflavin, Lfv (7,8,10-trimethylisoalloxazine, λ_{max} 526 nm) and literature data.[21] The fact that a similar spectrum was obtained for lumichrome when KBr was used as a solid support suggests that this new emission could be due to the existence of doubly hydrogen-bonded lumichrome dimers capable of executing double proton transfer in the excited state (Scheme 2). Such dimers have never been reported in solution.[28,29] However, in the solid state the importance of the role of hydrogen bonds in the crystal packing



Scheme 2

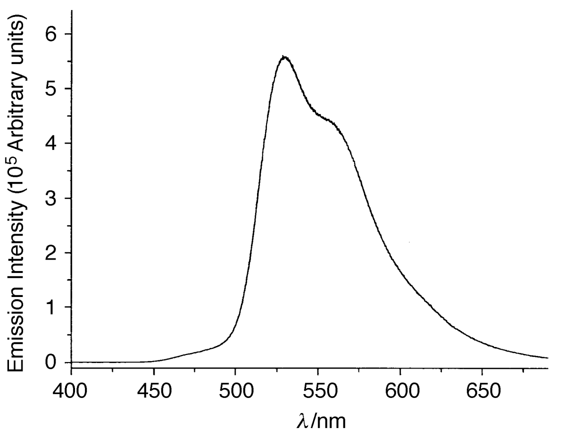


Fig. 4 Fluorescence emission spectrum of lumichrome crystals (λ_{exc} 355 nm)

of some alloxazines has been recognised (for instance, 9-methylalloxazine monohydrate[30]) and the existence of these dimers was suggested in Nujol mulls of alloxazine crystals.[29] Similar emission spectra of lumichrome–β-CD mechanical mixtures were obtained at lower (0.1 mg g^{-1}) and higher (12.7 mg g^{-1}) sample loadings. These results exclude the hypothesis of formation of dimers with increasing loading and suggest that these doubly hydrogen-bonded dimers are already present in the solid lumichrome crystals. This fact was confirmed by measuring the fluorescence of lumichrome crystals which show an intense isoalloxazinic emission (Fig. 4). The observed changes in the absorption spectra of lumichrome in mechanical mixtures and on KBr as compared to the spectra on cellulose may be attributed to the existence of such dimers. The broad emission (500–600 nm) of the 7-methylalloxazine–β-CD mechanical mixture probably is also due to such dimers. The existence of photoinduced double proton transfer in hydrogen bonded dimers in the solid state has been reported for other compounds such as 1-azacarbazole[31] and salicylic acid;[32] in the former case, the existence of dimers was proved on the basis of crystallographic data. Unfortunately, as far as alloxazines are concerned, crystallographic data are only available for 1,3,8,9-tetramethylalloxazine and for 9-methylalloxazine[30] for which the hydrogen bonded dimers were not observed. However, in the case of 1,3,8,9-tetramethylalloxazine the formation of such a dimer is impossible as the molecule lacks a hydrogen atom at N^1 and in the case of 9-methylalloxazine the formation of a dimer may be inhibited due to steric hindrance caused by the methyl group at C^9. Crystals of the alloxazines used in this work are being prepared for crystallographic examination in order to confirm or otherwise our hypothesis of the presence of double hydrogen bonded dimers in the solid state.

It is interesting to point out here that a solid sample of lumichrome adsorbed onto cellulose prepared by the same procedure used for the preparation of cyclodextrin complexes (see Experimental) shows a band with the maximum at 457 nm assignable to the alloxazinic emission. This result can be attributed to the destruction of the doubly hydrogen-bonded dimers when the solid is dissolved and to the fact that they are not reformed upon solid supports such as CDs or cellulose.

Conclusions

On the basis of the presented data we can note that 7MA11 and 8MA11 form real inclusion complexes with β-CD in water similar to those formed by Lch.[17] In addition, the formation of β-CD inclusion complexes in solution makes the process of phototautomerisation of alloxazines more difficult.

The results presented here show also how CDs can alter the photophysical properties of the guest molecules, especially in the solid state. We propose that the long wavelength emission of the alloxazine–β-CD complexes derive from inclusion complexes where the alloxazines are hydrogen-bonded to β-CD. Finally we describe an emission from lumichrome crystals which we suggest is due to the existence of doubly hydrogen-bonded lumichrome dimers capable of executing double proton transfer in the excited state.

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