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Journal of Photochemistry and Photobiology A: Chemistry 156 (2003) 267–271

Journal of Photochemistry and Photobiology A:Chemistry

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Photophysics of lumichrome on cellulose

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Received 24 July 2002; received in revised form 7 November 2002; accepted 7 November 2002

Abstract

Lumichrome samples were prepared by depositing lumichrome on cellulose from methanol solutions. Diffuse reflectance absorption bands at 354 and 388 nm (shoulder) and the fluorescence band at 460 nm similar to those observed in polar solvents were attributed to a $\pi \rightarrow \pi^*$ transition. The emission decay has a complex kinetics similar to that found in alloxazines, suggesting at least three emitting species with the respective lifetimes of about 0.95, 1.4 and 2.5 ns. Diffuse reflectance laser flash photolysis experiments provided the decay kinetics of the triplet state with the lifetime on the microsecond time scale and of the longer-lived semi-reduced radical absorbing in the 500–600 nm range with 0.24 ms lifetime.

Keywords: Photophysics; Lumichrome; Cellulose

1. Introduction

Our recent focus has been on the spectroscopy and photophysics of organic dyes adsorbed onto different kinds of solids. Owing to that interest, we have undertaken the research project aimed at characterising the spectral and photophysical properties of lumichrome adsorbed onto microcrystalline cellulose.

Lumichrome (7,8-dimethylalloxazine, see Fig. 1) is a representative of a class of nitrogen heterocycles related to lumazines and the biologically important flavins. Recently, interest in the photochemical studies of lumichrome has become more intense, because of some of the possible applications. For example, it has been proposed that lumichrome may play an important role in the photodegradation of polyamidehydroxyurethane polymers in aqueous solution [1], and that singlet oxygen may be involved in the process. It has been shown that the efficient polymerisation of 2-hydroxyethyl-methacrylate may be photoinitiated by lumichrome in presence of triethanolamine [2]. Another interesting application is an optical transistor device with a thin film of lumichrome on conductive SnO_2 glass [3]. A further point of interest is the possibility of using alloxazines to sensitise the photo-oxidation of substituted phenols in water [4,5].

Apart from our own reports [6–9], the available information about alloxazines in the solid phase, lumichrome in particular, is very scarce. To our best knowledge, there are only a few published reports on spectral and photophysical properties of lumichrome on solids. Recently, in an interesting report on the dynamics of photon-induced degradation and fluorescence of riboflavin, an analogue of lumiflavin and 3-methyllumiflavin, in riboflavin microparticles, riboflavin was found to easily convert into lumichrome [10]. The analysis of photo-induced conversion of riboflavin into lumichrome and of the subsequent lumichrome fluorescence enabled the authors to calculate the fluorescence lifetime of lumichrome as 0.5 ns. Based on the FT-IR results, Lee and co-workers [11,12] studied lumichrome on the colloidal silver surface. In the solid state, the importance of the role of hydrogen bonds in the crystal packing of some alloxazines has been recognised, for instance, in 9-methylalloxazine monohydrate [13]. In a short report, the existence of dimers was suggested in Nujol mulls of alloxazine crystals [14].

The present investigation was carried out with an aim of giving a more systematic insight into the photophysics of lumichrome adsorbed onto microcrystalline cellulose. Diffuse

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doi:10.1016/S1010-6030(02)00427-6

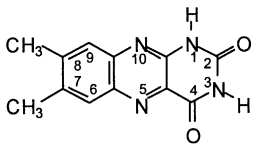

Fig. 1. Structure of lumichrome.

reflectance, emission and transient diffuse reflectance spectra, decay kinetics of lumichrome embedded in cellulose have provided information concerning the excited singlet and triplet states and transient intermediates formed from lumichrome excited in the near UV.

2. Experimental

Cellulose powder, 20 μm, lumichrome and methanol, all from Aldrich, were used as received. Microcrystalline cellulose, which readily adsorbs water, was dried in a vacuum oven at 70 °C for at least 24 h prior to use. Lumichrome was dissolved in methanol and a known amount of this solution was added to a known mass of dry cellulose suspended in the solvent. The resulting suspension was stirred periodically and left to slowly evaporate. After several hours, the final traces of solvent were removed in a vacuum oven at 40 °C for about 4 h. The loadings used were typically of 0.3 mg/g.

Fluorescence decay curves of all samples were obtained at 340-nm excitation. Time-resolved fluorescence was recorded using a time-correlated single-photon-counting system which has been described in detail in [15]. Time-resolved diffuse reflectance laser flash photolysis experiments were performed using an LKS50 instrument from Applied Photophysics by exciting the samples at 354.7 nm in a quartz cell. The ground state diffuse reflectance absorption spectra were recorded on a Perkin-Elmer LambdaBio 40 spectrophotometer equipped with an integrating sphere. Steady-state fluorescence spectra were recorded on a Spex Fluoromax.

Fluorescence decays were analysed with a PTI TimeMaster Pro analysis package using the lifetime distribution algorithm based on the Exponential Series Method [16]. The package uses a sum of up to 200 exponential functions with fixed logarithmically spaced lifetimes, with the pre-exponential factors recovered by the least-squares minimisation procedure.

Unless otherwise indicated, the samples had been purged with nitrogen. All measurements were performed at room temperature.

3. Results and discussion

Cellulose is one of the most abundant organic materials on Earth, which has led to a large amount of research into its applications and properties. Physicochemical properties of cellulose make it an interesting support for organic dyes. Naturally occurring cellulose is the polymer of 1–4 linked β-D-glucose, in a variety of arrangements. Both intra- and intermolecular hydrogen bonds are formed, involving the OH groups at C(2), C(3) and C(6) positions [17]. The adsorption of organic probes onto cellulose may be induced if the adsorbate is dissolved in a good solvent for cellulose [18–20]. Such good solvents have strong interactions with the glycosidic chain segments resulting in swelling of the polymer. In the present work, lumichrome was adsorbed onto cellulose from methanolic solutions. In methanol the cellulose-to-cellulose hydrogen bonds are replaced by cellulose-to-solvent bonds. Lumichrome can then penetrate into the sub-microscopic pores of the cellulose, staying entrapped between the cellulose chains after solvent removal.

In order to characterise quantitatively the molecules adsorbed on solid opaque samples by diffuse reflectance measurements, it is necessary to establish a relationship between the reflectance and the concentration of the ground state molecules adsorbed onto the solid matrix. A simple and successful approach describing the interaction of light with a diffusing sample was proposed by Kubelka and Munk in 1931 [21]. In this model, the remission function $F(R)$ for an ideal diffuse scatterer which is optically thick at the wavelength of choice and for a homogeneous distribution of absorbers throughout the sample is given by the Kubelka–Munk function, i.e.

$$F(R) = \frac{(1-R)^2}{2R} = \frac{K}{S} \quad (1)$$

where R represents the observed diffuse reflectance from the surface of the sample, K and S being absorption and scattering coefficients, respectively, both in $(\text{distance})^{-1}$ units [21,22].

The ground state diffuse reflectance absorption and emission spectra of lumichrome on cellulose have been recorded. Solutions with different lumichrome concentrations were used for sample preparation, enabling to compare different lumichrome loadings on cellulose. At higher loadings, over 0.7 mg/(g cellulose), the non-linearly of $F(R)$ in function of loading suggests some aggregate formation, although no significant effect on the absorption and emission spectral shapes could be observed. Thus, all the measurements reported were done at lower sample loadings, usually at 0.3 mg/g, where $F(R)$ remains a linear function of loading. Fig. 2 shows the remission function $F(R)$, for lumichrome adsorbed on cellulose from methanol, at 0.3 mg/g loading. The lumichrome absorption is masked by the intrinsic absorption of microcrystalline cellulose below about 300 nm. In the long-wavelength region, the diffuse reflectance absorption spectrum of lumichrome shows a well-resolved maximum at about 354 nm and a shoulder corresponding to a band at 388 nm, as obtained by spectral decomposition into Gaussian bands.

Fluorescence spectroscopy of dyes adsorbed onto solids is virtually identical to that in liquids, apart from the need

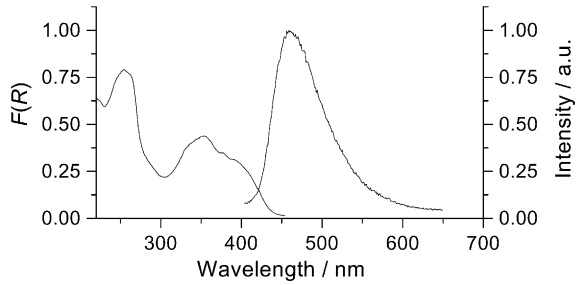

Fig. 2. Ground state diffuse reflectance absorption spectrum of lumichrome on cellulose at a loading of 0.3 mg/g, plotted using the Kubelka–Munk remission function, together with the normalised fluorescence emission spectra.

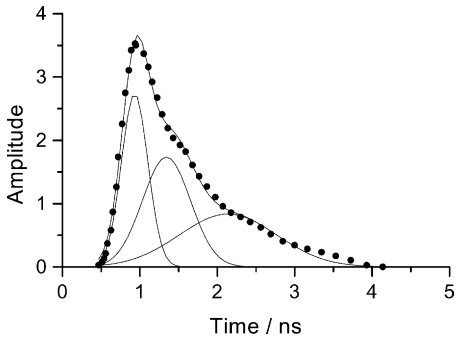

Fig. 3. Fluorescence lifetime distribution for lumichrome. The fluorescence decays were measured using 340 nm excitation. The emission was collected at 462 nm.

of the front surface geometry to be used. The fluorescence emission spectrum of lumichrome exhibits a single band with a maximum at about 460 nm. In cellulose—a polar medium that can form hydrogen bonds with lumichrome—red shifts in the absorption and emission spectra are observed relative to the corresponding spectra in less polar environments. By analogy to the well-documented behaviour of these compounds in solution, the absorption and emission bands of lumichrome on cellulose are attributed to allowed electronic-dipole $\pi \rightarrow \pi^*$ transitions [23].

Fluorescence decay kinetics was measured for the lumichrome on cellulose. As frequently happens in heterogeneous systems, our results demonstrate that none of the fluorescence decays may be satisfactorily described by a simple exponential decay function. To learn more about lumichrome adsorbed on cellulose, we applied exponential lifetime distribution analysis to the fluorescence decay kinetics. It has been demonstrated that complex decays arising from lifetime distributions may in fact be fitted with a much simpler discrete 2- or 3-exponential decay model fulfilling the same goodness-of-fit criteria [24]. Nonetheless, the use of the lifetime distribution technique provides a less biased approach to the fitting problem, without imposing a pre-conceived kinetic mechanism on the system.

Lumichrome is a multifunctional molecule with numerous proton-donor and proton-acceptor sites. It is reasonable to expect that for alloxazines on cellulose different hydrogen bonds can be formed between alloxazine at N(10), N(3), N(1), and N(5) and at both carbonyl groups at C(2) and C(4), and the cellulose chains. Due to the micro-heterogeneous nature of alloxazines adsorbed into cellulose, a distribution of fluorescence lifetimes instead of discrete lifetimes is to be expected. A typical result showing fluorescence lifetime distribution of lumichrome on cellulose is presented in Fig. 3. The lifetime distribution obtained for the lumichrome fluorescence decay has a maximum at about 1.0 ns and a shoulder, suggesting the presence of longer-lived components. Recently, we have reported three-modal distributions for the methyl-substituted alloxazines with the maxima in the range of 0.5–4.0 ns. Decomposition of the fluorescence lifetime distribution of lumichrome into Gaussian bands demonstrates that similar to other alloxazines the lumichrome fluorescence may be adequately described by a three-modal distribution with the corresponding maxima at 0.95, 1.4 and 2.4 ns.

To interpret these distributions, note that the effect of local pH values on the lumichrome photophysics can be important, as the lumichrome deprotonation may take place at either the N(1) or N(3) nitrogen. According to theoretical calculations, the charge densities on these two nitrogen atoms are almost identical, resulting in very similar expected values of the dissociation constants at the two positions [23,25]. This prediction is supported by the reported pK_a values for the lumichromes methyl-substituted at N(1) or N(3) position, the pK_a values for 1MLch and 3MLch being 8.65 and 8.50, respectively [23]. Therefore, it is reasonable to expect that the deprotonation of lumichrome will result in two different monoanions. Interestingly, in the singlet excited state, the pK_a^* values for deprotonation of alloxazines at N(1) are considerably lower, while for the deprotonation at N(3)—only slightly lower than those obtained for the ground state [23]. The apparent pK_a^* values for 1- and 3-methyl-lumichromes are in the range of 2.3–4.0 for the N(1) monoanions and 7.4–7.7 for the N(3) monoanions. It has been proposed that the dissociation at the N(1) nitrogen is accompanied by an electron rearrangement to give an *isoalloxazine-like* distribution of charges, with the fluorescence lifetime of 4.1 ns [25]. In contrast, the dissociation at the N(3) nitrogen gives a monoanion with the typical *alloxazine-like* structure and the lifetime of about 1.2 ns [25]. Note that lumichrome adsorbed on the colloidal silver surface exhibited characteristics of a resonant isoalloxazine ring system [11,12]. Based on the FT-IR results, Lee and co-workers [11,12] postulated for lumichrome on colloidal silver surface, deprotonation at N(3) upon adsorption, readily followed by concurrent deprotonation at the N(1) position and protonation at the N(10) position, causing in effect a rearrangement of the entire molecular electronic structure to yield an *isoalloxazine-like* structure. A similar mechanism might be expected for lumichrome adsorbed on cellulose.An alternative, although a less likely explanation, may be proposed after noting that

the lifetimes determined for all the alloxazines in protic solvents, such as water and alcohols, are the longest if compared to non-polar 1,2-dichloroethane or polar aprotic acetonitrile [7,26]. The fluorescence distribution may thus be assigned to lumichrome buried into the different cellulose microenvironments, resulting in different local pH values, local polarity and/or different possible hydrogen bond interactions. Especially interesting seems the possibility that some longer fluorescence lifetimes might originates from fractions of lumichrome molecules with strong hydrogen bond interaction between lumichrome at the N(10) and/or N(1)–H positions and cellulose, which can lead to an *isoalloxazine-like* structure with a longer fluorescence lifetime.

Laser flash photolysis has been used increasingly to study different transparent media, but it fails in opaque systems. Diffuse reflectance laser flash photolysis extends the advantages of laser flash photolysis to non-transmissive media and enables transient spectra and kinetics of photoinduced elementary reactions to be determined in heterogeneous environments. The techniques of diffuse reflectance laser flash photolysis have been developed by Wilkinson in Loughborough UK (see [22] and references therein). Since its invention 20 years ago, the diffuse reflectance laser flash photolysis has remained one of the most important tools to study the non-transmissive media. These techniques applied to lumichrome on cellulose were able to detect two transient species. The short-lived species has a lifetime of the order of microseconds and responds to oxygen, suggesting its assignment to the lowest lumichrome triplet excited state. No spectrum of this short-lived species has been recorded. The spectrum of the long-lived transient species generated by lumichrome adsorbed onto cellulose is shown in Fig. 4. Similar spectra have been recorded for other alloxazines and methyl-alloxazines adsorbed onto cellulose [8]. The long-lived species absorption has a maximum in the 500–600 nm range. The decay time associated to the long-lived species recorded at 550 nm is 0.24 ms. The transient spectrum of lumichrome is similar to that of the semi-reduced riboflavin radical formed in methanol [2]. The

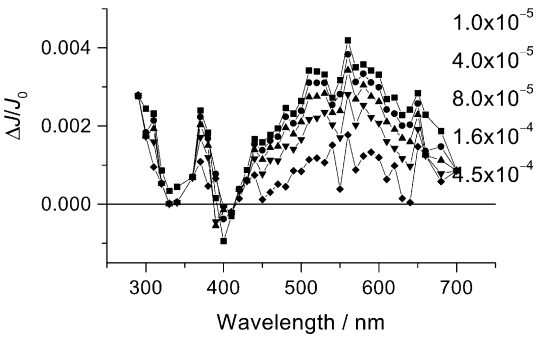

Fig. 4. Time-resolved diffuse reflectance laser flash photolysis spectra of lumichrome at a 0.3 mg/g loading on cellulose at room temperatures in deaerated samples. Excitation is at 355 nm. The delay times in seconds are indicated on the panel.

presence of the semi-reduced lumichrome radical absorption in the same spectral region has also been reported [2]. Kinetics and spectra of lumichrome are essentially similar to those of methyl-substituted alloxazines. The long-lived species may be tentatively identified as the semi-reduced radical, formed by hydrogen or proton transfer from the glucose unit to the carbonyl group as supported by the results published for riboflavin [2].

4. Conclusions

Lumichrome samples were prepared by depositing lumichrome on cellulose from methanol solutions. Diffuse reflectance absorption bands at 354 and 388 nm (shoulder) and the fluorescence band at 460 nm similar to those observed in polar solvents were attributed to a $\pi \rightarrow \pi^*$ transition. The emission decay is not exponential, similar to what was found in alloxazines [8,9], and was interpreted in terms of three distinct emitting species with nanosecond lifetimes. Diffuse reflectance laser flash photolysis experiments provided the kinetics of the triplet state with a lifetime of the order of microseconds and of the longer-lived semi-reduced radical with an absorbance maximum in the 500–600 nm range and 0.24 ms lifetime.

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