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Albery Model Applied to Fluorescence Decay of Alloxazines Adsorbed into Cellulose

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Lumichrome (7,8-dimethylalloxazine = 7,8-dimethyl-benzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) is a representative of a class of nitrogen heterocycles related to lumazines and the biologically important flavins. Because of a number of active centres at N(10), N(5), N(3) and N(1), and at both carbonyl oxygens at C(2) and C(4), one can expect several different types of interaction between cellulose and alloxazines. Naturally occurring cellulose is a 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 [1]. The adsorption of organic probes into cellulose may be induced, if the adsorbate is dissolved in a good solvent for cellulose [2–4]. Such good solvents have strong interactions with the glycosidic chain segments, resulting in swelling of the polymer. In the present work, lumichrome was adsorbed into 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.

The subject of this communication is relatively straightforward – to characterise kinetic of fluorescence decay of alloxazines absorbed into cellulose, by using the Albery model, if necessary in the multimodal mode. The Albery model in conjunction with fluorescence lifetime distribution is hoped to be a method of choice to describe fluorescence kinetics in heterogeneous systems. The structures of all the compounds studied in this paper with their atom numbering are shown in Figure 1.

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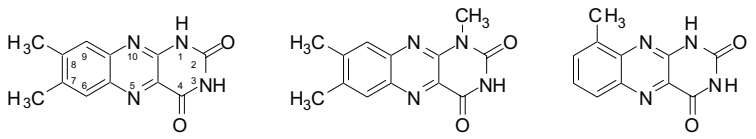

Lch 1MLch 9MAll

Figure 1. Structure of compounds studied; lumichrome, Lch, and its atom numbering, 1-methyllumichrome, 1MLch, and 9-methylalloxazine, 9MAll.

Microcrystalline cellulose (powder, 20 micron) and methanol 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. Alloxazines were 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 remaining traces of the solvent were removed by placing the sample in a vacuum oven at 40°C for about 4 h. The resulting loading was typically of 0.3 mg/g. The alloxazine derivatives were synthesized and purified, as described previously [5,6].

Fluorescence measurements were done at 45° angle, using front face emission scheme. Fluorescence decay curves were obtained at 340 nm excitation. Time-resolved fluorescence measurements were performed on a system described in detail in [7]. In brief, the frequency-doubled output of a mode-locked, synchronously-pumped, cavity-dumped argon-ion/R6G dye laser system served as the excitation source. The single-photon timing detection system employed a Hamamatsu microchannel plate photomultiplier detector and fast amplifier with an instrument response function of 80 ps fwhm. Fluorescence decays were analysed with the PTI TimeMaster Pro analysis package, using a lifetime distribution algorithm, based on the exponential series method [8]. The method uses a sum of up to 200 exponential functions with fixed and logarithmically spaced lifetimes. The preexponential factors are recovered by using the least-squares minimisation procedure.

Temporal fluorescence profiles measured for heterogeneous systems very often produce complicated fluorescence decays. A traditional starting method of dealing with these data is to fit the decay curves with single-, two-, or three-exponential functions. One of the examples from our own studies is the fluorescence decay of alloxazine and its monomethyl substituted derivatives adsorbed into microcrystalline cellulose, see Figure 8 from [9]. The results demonstrate that none of our fluorescence decays may be satisfactorily described by such simple decay functions. Recently, we applied with better results the Albery model to the analysis of the fluorescence decay of dimethyl-substituted alloxazines and 1-methyllumichrome adsorbed into microcrystalline cellulose [10]. A desire to understand photochemical processes in heterogeneous environments has led to a considerable scientific interest in studies of photophysics and photochemistry of organic molecules adsorbed on different substrates by time-resolved methods. For example, it has been demonstrated, that sometimes complex decays that arise from a lifetime distribution, may in fact be fitted with

a much simpler discrete 2- or 3-exponential decay model, fulfilling equally well the goodness-of-fit criteria [11]. Nevertheless, the usage of the lifetime distribution techniques provides a less biased approach to the fitting problem, without imposing any pre-conceived kinetic mechanism on the system. Due to the micro-heterogeneous nature of alloxazines adsorbed into cellulose, a distribution of fluorescence lifetimes instead of discrete lifetimes has been obtained. The nature of such distribution has been discussed in our previous papers covering photophysics of alloxazines on cellulose [9,10,12–14]. In brief, fluorescence decay data allowed to identify three emitting species for every molecule studied, excluding 1-methyllumichrome that lacks the capacity to rearrange into an iso-alloxazinic form. These results demonstrated the importance of introduction of methyl group into position 1 for alloxazines. A typical fitting result showing fluorescence lifetime distribution of alloxazines on cellulose is presented in Figure 2. The lifetime distribution obtained for 1-methyllumichrome fluorescence decay has a maximum at about 1.0 ns. The lifetime distribution of lumichrome also has a single maximum at 0.94 ns, however, a shoulder is apparent on the curve, suggesting the presence of other components. The most complicated lifetime distribution has been recorded for mono methyl substituted alloxazines, *e.g.* 9-methylalloxazine shown in Figure 2. In case of 9-methylalloxazine, three maxima in the lifetime distribution are present at *ca.* 0.45 ns, 1.35 ns and 5.0 ns.

The formalism of dispersive kinetics can be of assistance in better understanding the photophysics of heterogeneous systems; among existing approaches, the Albery model has been particularly successful [15,16]. In a heterogeneous system, the observed decay profile is a sum of contributions from different subsets of a microscopic species, each subset existing in a slightly different environment. The model assumes that the free energy of activation, $\Delta G^* = \Delta\overline{G}^* - \gamma x RT$, is distributed normally around the mean value $\Delta\overline{G}^*$. Parameter γ describes the distribution width. When $\gamma = 0$, there is no dispersion and the decay will correspond to the classical homogeneous kinetics, described by the single-exponential function. The assumed distribution of the free energy of activation leads to a log-normal distribution of the decay rate constants. This distribution may be characterized by an average rate constant, $\bar{k}$. The dispersion in the first-order rate constant for $-\infty \leq x \leq \infty$ then becomes

$$\ln(k) = \ln(\bar{k}) + \gamma x \tag{1}$$

Making the integration over the normal distribution, $\exp(-x^2)$, and additionally assuming that the luminescence intensity is proportional to the concentration of the excited probe molecules, we obtain the kinetic equation in the following form, after Albery and Thomas [15,17–19]:

$$\frac{I_t}{I_0} = \frac{\int_{-\infty}^{+\infty} \exp(-x^2)\exp[-\bar{k}t\exp(\gamma x)]dx}{\int_{-\infty}^{+\infty} \exp(-x^2)dx} \qquad \text{where} \quad \int_{-\infty}^{+\infty} \exp(-x^2)dx = \pi^{1/2} \tag{2}$$

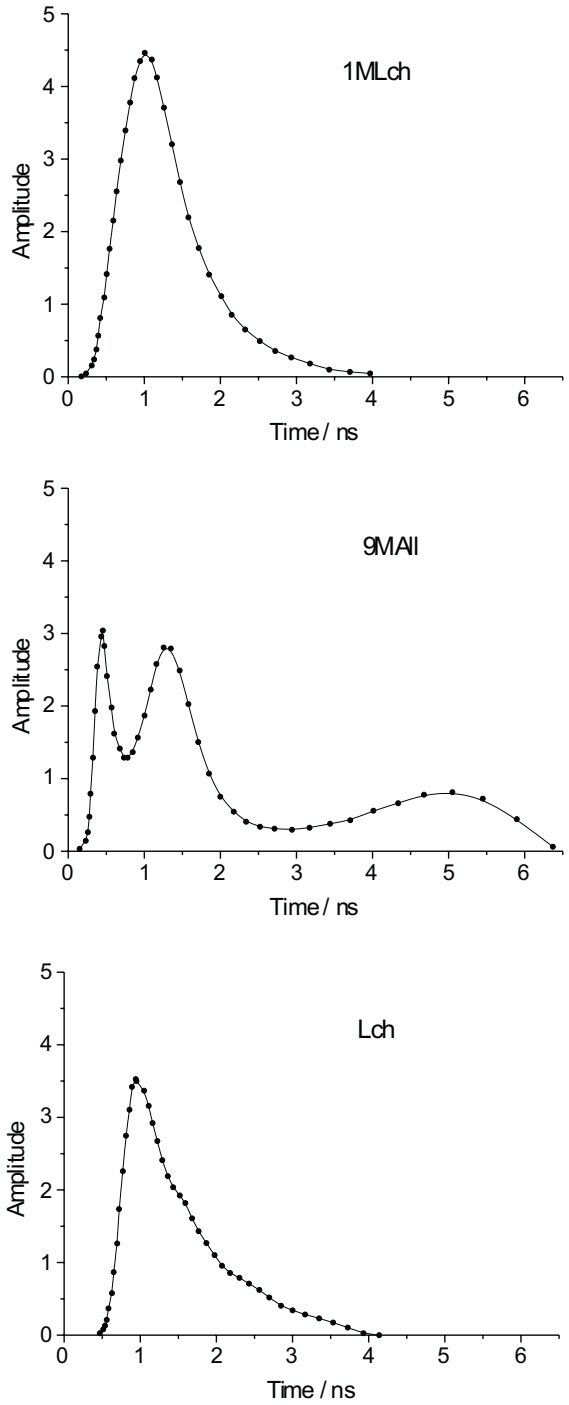

Figure 2. Fluorescence lifetime distribution from top to bottom, for 1-methyllumichrome, 1MLch, 9-methylalloxazine, 9MAll, and lumichrome, Lch. The fluorescence decays were measured using 340 nm excitation. The emission was collected at 462 nm.

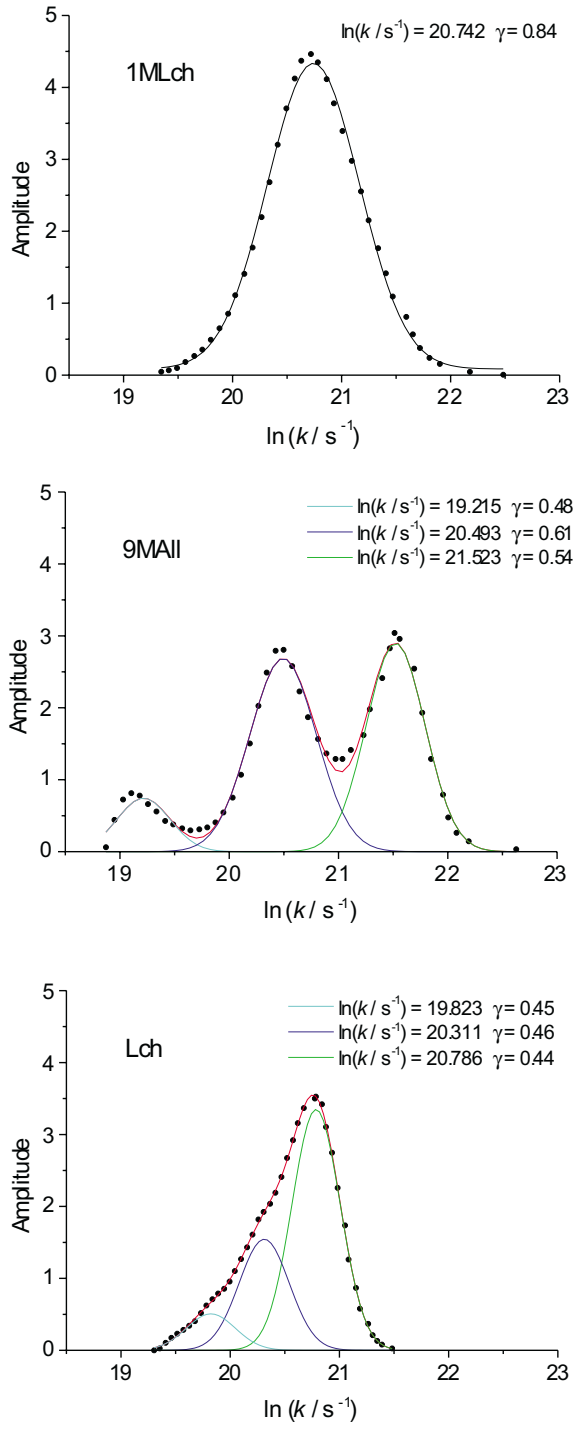

Figure 3. Albery model applied to fluorescence lifetime distribution from top to bottom, for 1-methyl-lumichrome, 1MLch, 9-methylalloxazine, 9MAll, and lumichrome, Lch.

Here I_t and I_0 are the luminescence intensity after the excitation pulse at times t and $t = 0$, respectively. An analysis of the decay data, using this model, provides information about the mean, $\bar{k}$, and the width, γ, of the log-normal distribution of the rate constants. At $\gamma = 0$, the model reproduces the classical well-defined single-exponential decay.

A simple procedure for integrating the numerators in (2) can be obtained after a substitution: $x = \ln(\lambda)$ for $x < 0$ and $x = -\ln(\lambda)$ for $x > 0$, [15]. As described by Albery [15] and Thomas [18,19] the extended Simpson's rule applied to (2) produces

$$\frac{I(t)}{I(0)} = \frac{0.2}{3\pi^{1/2}}[2[g(0.1) + g(0.3) + g(0.5) + g(0.7) + g(0.9)] + g(0.2) + g(0.4) +$$

$$+ g(0.6) + g(0.8) + \exp(-\bar{k}t)] \qquad (3)$$

where

$$g(\lambda) = \lambda^{-1}\exp(-[\ln(\lambda)]^2)[\exp(-\bar{k}t\lambda^{\gamma}) + \exp(-\bar{k}t\lambda^{-\gamma})]$$

An error should be noted in a previous review, that presents (3) [18].

Figure 3 shows the results of applying the Albery model to fluorescence decay of alloxazines adsorbed into microcrystalline cellulose. The data are the same as presented in Figure 2. Note that abscissa is expressed as $\ln(k/s^{-1})$. The analysis shows, that the fluorescence lifetime distribution of 1-methyllumichrome has a single mode with the maximum at $\ln(k/s^{-1}) = 20.7$. The three-modal distribution for 9-methylalloxazine has been confirmed. However, decomposition of the fluorescence lifetime distribution of lumichrome into Gaussian bands demonstrates, that similar to other alloxazines the lumichrome fluorescence may also be adequately described by a three-modal distribution.

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