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ORIGINAL PAPER

Fluorescent Microcrystals Obtained from Coumarin 6 Using the Reprecipitation Method

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Abstract The reprecipitation method was applied to Coumarin 6. A concentrated solution of the dye in acetone was mixed at room temperature with a large volume of water, and microcrystals rapidly appeared in the medium. Their size and shape were visualized by fluorescence microscopy, as well as by transmission electron microscopy. The absorption and fluorescence properties were studied on the suspensions and compared with those of the dissolved dye. This study is aimed at clarifying the influence of the reprecipitation process upon the fluorescence properties of the dye in the solid state.

Keywords Coumarin 6 · Fluorescence · Reprecipitation · Organic microcrystal

Introduction

There is no doubt that fluorescent organic nano- and microcrystals will play an increasing role in future technologies. They have recently made the proof of their utility as chemical and biological sensors [1–5], as well as photocatalysts [6], and they are also expected to lead to functional materials for optics [7–10] and optoelectronics [11]. However, their development is still a challenge for chemists. Their preparation is difficult to control while a major requirement for practical use is to obtain particles with precise shape and size. Moreover, it is generally thought that their optical properties are difficult to predict because they depend not only on the chemical structure of the constituting molecules, but also on the numerous intermolecular interactions that take place in the solid state [12].

Because of the thermal fragility of organic compounds, only a few methods can be used to prepare nano- and microparticles. Sublimation is convenient for a small number of compounds, but for the most part of them it is better to work at low temperature. Growing crystals in a confined medium such as a sol–gel matrix allows their size to be kept in the nanoscale range [5, 7], but the entrapped crystals thus obtained are not readily suitable for all applications. There is another method, simple and effective, to prepare organic nano- and micro-crystals [13], and other organized structures. It is the so-called 'reprecipitation method', which is based on a solvent exchange process usually run at room temperature. The organic compound is dissolved in a hydrophilic organic solvent, and this concentrated solution is poured into a large volume of water, which acts as a non-solvent. Consequently, the organic compound precipitates. According to its self-organization properties, different structures ranging from aggregates to crystals can be obtained as a suspension in aqueous environment. The free-standing particles can subsequently be dried or put in the form of thin layers for various applications. However, despite the interest of the reprecipitation method, there is still a lack of data to know which type of particles may be expected from a given organic compound and which optical properties will be associated to these particles.

For several years, we have been working on nitrobenzoxadiazole (NBD) dyes, which appeared to be particularly interesting because they lead to different types of microcrystals according to the experimental conditions used [14–17].

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Recently, we also studied the behavior of three dyes belonging to the 3-(2′-benzimidazolyl)-7-diethylaminocoumarin series. Interestingly, two of these dyes gave fluorescent nanofibers by the reprecipitation method, a behaviour rather unexpected for small molecules without any lipophilic moiety [18]. In the present work, we would like to complete this study by examining the behavior of a closely related dye, namely Coumarin 6, which bears a benzothiazolyl substituent in the 3-position (Fig. 1). This compound is strongly fluorescent in solution where it emits in the green and it is thus widely used as a laser dye [19] and for various applications related to its optical properties [20–23]. It is also fluorescent in the solid state, the commercial compound emitting a bright orange light in its package flask. According to its hydrophobic structure, the dye can be expected to precipitate in water. Finally, it tends to crystallize easily and its crystal structure has been published [24]. All these characteristics lead us to think that this dye could give nano- or microcrystals by the reprecipitation method and that these particles could exhibit interesting optical properties, maybe different from those of the dissolved dye.

In the following work, the reprecipitation method was applied to Coumarin 6 and the particles formed were visualized using fluorescence and electron microscopy. The absorption and fluorescence properties were studied on the suspension. The spectra were compared to those obtained for the very dilute dye, to see in which extent the formation of particles can affect the fluorescence properties.

Experimental

Materials

Laser grade 3-(2′-benzothiazolyl)-7-*N*,*N*-diethylaminocoumarin (Coumarin 6) was purchased from Aldrich and used as received. Analytical grade acetone (Prolabo) and high-pressure demineralized water (resistivity 16 MΩ cm, pH 5.5) prepared with a Milli-Q apparatus (Millipore) were used as solvents.

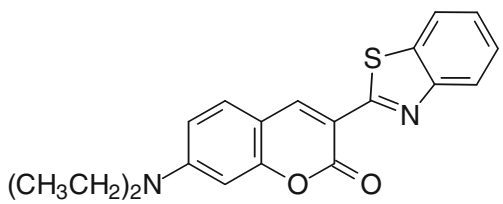

Fig. 1 Chemical structure of Coumarin 6

Apparatus

Spectroscopic measurements were conducted at 25 °C in a temperature-controlled cell. UV/vis absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Corrected steady state fluorescence spectra were recorded with a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. The fluorescence quantum yields of the suspensions (Φ) were estimated using the classical formula: $\Phi_x = (A_s \times F_x \times n_x^2 \times \Phi_s)/(A_x \times F_s \times n_s^2)$ where A is the absorbance at the excitation wavelength, F the area under the fluorescence curve and n the refraction index. Subscripts s and x refer to the standard and to the sample of unknown quantum yield, respectively. The absorbance of the samples at the excitation wavelength was below 0.05. Coumarin 6 in ethanol (Φ=0.78) was taken as the standard [19]. Fluorescence decay was measured with the stroboscopic technique utilizing a Strobe Master fluorescence lifetime spectrophotometer from PTI. The excitation source was a flash lamp filled with a mixture of nitrogen and helium (30/70). Data were collected over 200 channels with a time-base of 0.1 ns per channel. Excitation was performed at 337 nm, and emission was at 560 nm. Analysis of fluorescence decay was performed using the multiexponential method software from PTI. The size and shape of the microparticles were observed with a Zeiss Axioskop fluorescence microscope equipped with a standard camera. The objective had a lens of ×100 magnification, the eyepiece had a lens of ×2.5 magnification. The excitation wavelength was 430–450 nm, and the emission wavelength was set at around 500–530 nm, using suitable filters. Electron microscopy was performed at the Service Commun de Microscopie Electronique de l'Université Paul Sabatier. A JEOL JEM 1011 microscope equipped with a SIS Megaview III camera was used for transmission electron microscopy. To prepare the samples, a droplet of coumarin aqueous suspension was deposited on a carbon grid after reprecipitation was complete. The excess liquid was drawn off with paper and the sample was revealed with ammonium molybdate (2%, pH 5) as a contrasting agent and allowed to dry for 3 h under vacuum at 60 °C. For the powder X-ray diffraction analysis, the dye particles were prepared by the reprecipitation method, filtered on a microfilter and dried under vacuum during many days before use. The diffraction patterns were collected in transmission mode, on capillaries samples, on a θ–θ XPert Pro Panalytical diffractometer, with λ (Cu Kα1, Kα2) 1.54059, 1.54439 Å. The extraction of peak positions for indexing was performed with the fitting program, available in the PC software package Highscore+ supplied by Panalytical.

Results

Dye reprecipitation

Reprecipitation was carried out as follows. First, a concentrated solution of Coumarin 6 at 1×10^{-3} M in acetone was prepared. Then, 40 µL of this solution were transferred into a cell containing 1.96 mL of water. The dye concentration in the mixture was then 2.0×10^{-5} M, the proportion of acetone in water being 2% *v*/*v*. The mixture was kept at room temperature under constant stirring. Immediately after mixing, the solution was bright yellow, but it rapidly lost its colour, turning pale yellow and cloudy, while a deposit formed on the magnetic stirrer. The reprecipitation process was monitored by UV/vis absorption spectroscopy (Fig. 2). The initial spectrum showed an intense band centered around 450 nm. During the reprecipitation process, absorbance was decreased at this wavelength, while the spectrum was progressively widened and some new bands appeared in the red. The deviation of the baseline indicates the formation of fine particles in suspension. No more evolution of the absorption spectrum was observed after 4 min, suggesting that the reprecipitation process was complete. It can be noted that the intensity of the final absorption spectrum may differ from one experiment to the other, because it depends very much on experimental conditions.

Observations by fluorescence and electron microscopy

Once the reprecipitation process was terminated, a drop of the aqueous mixture was deposited between two glass slides and placed under a fluorescence microscope. Small rectangular objects were observed, which showed a trend for agglomeration (Fig. 3). They measured around 1 µm long and emitted yellow light.

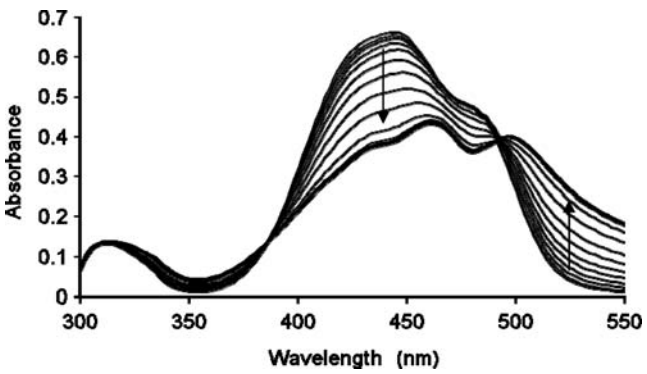

Fig. 2 Evolution of the UV/vis absorption sepctrum of Coumarin 6 (2×10^{-5} M) in water containing 2% *v*/*v* acetone. One measurement every 20 s

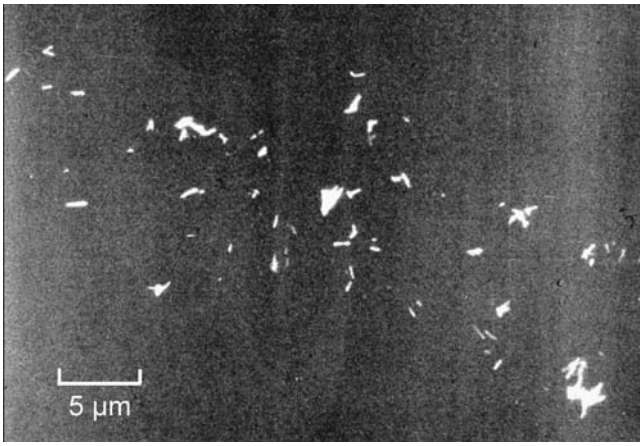

Fig. 3 Fluorescence microscopy image of a suspension of Coumarin 6 reprecipitated in water. Dye concentration: 2×10^{-5} M

Transmission electron microscopy confirmed the presence of rectangular structures and allowed their size to be measured more precisely (1.7 µm×100 nm for the longest particles; Fig. 4). The particles generated an electron diffraction spectrum, which shows that they are microcrystals.

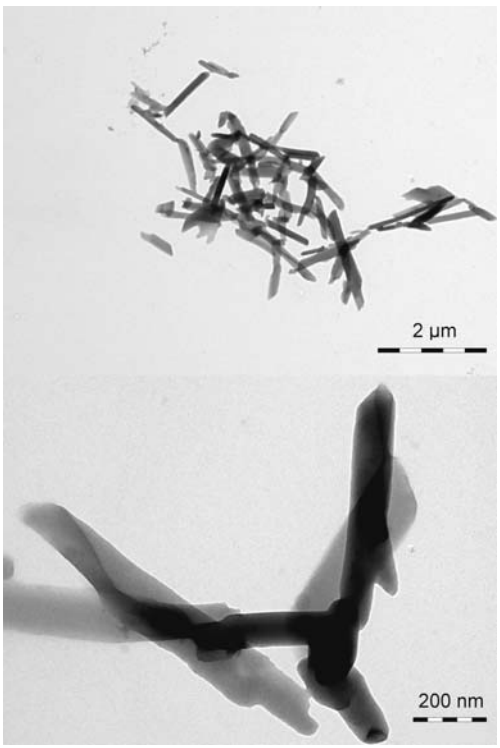

Fig. 4 Observation by transmission electron microscopy. Dye concentration: 2×10^{-5} M

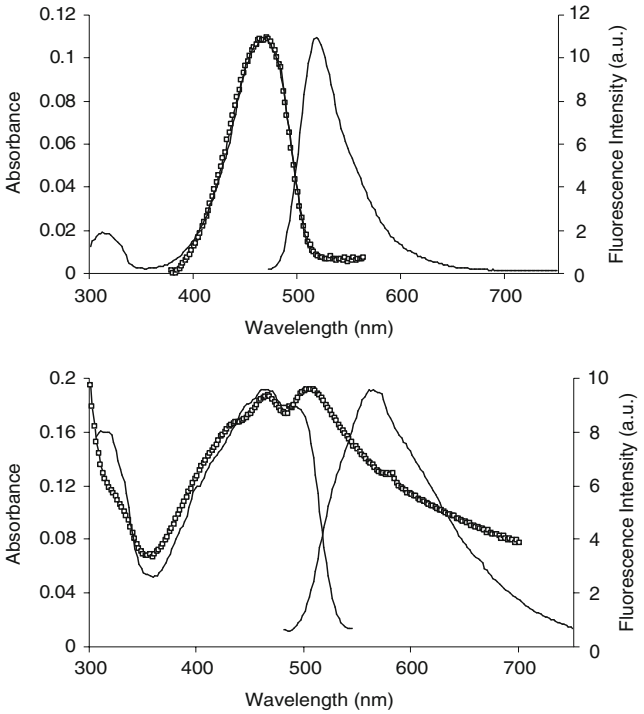

Fig. 5 UV/Vis absorption spectrum (*squares*), excitation and emission spectra (*plain lines*). *Top*: highly dilute Coumarin 6 (2×10^{-7} M, λ_{ex}=466 nm, λ_{em}=520 nm); *Bottom*: microcrystal suspension after the reprecipitation process (total dye concentration: 2×10^{-5} M, λ_{ex}= 466 nm; λ_{em}=564 nm). Both samples are in water/acetone 98:2. The fluorescence spectra are normalized with respect to the absorption spectrum. The spectrum of the highly dilute dye was recorded with a 10 cm optical pathlength cell

Crystallinity

Additional information about the crystallinity of our microparticles was searched for. To do so, microcrystals prepared by the reprecipitation method were filtered and dried, and an X-ray powder diffraction analysis was performed. The diffraction pattern was very close to that of the commercial dye, and both were in line with the pattern predicted by the X-ray structure [24]. This indicates that the molecular arrangement is similar, whatever the crystals are grown in an organic solvent or prepared by the reprecipitation method.

Spectroscopic characteristics of the microcrystal suspensions and comparison with the highly dilute dye

The spectroscopic and photophysical behavior of Coumarin 6 has been known for a long time [25–28]. This dye is a classical donor–acceptor system, in which the diethylamino group in the 7-position plays the role of electron donor and the carbonyl of the lactone group acts as the electron-withdrawing group. The dye is moderately polar in the ground state and its polar character is reinforced in the excited state. The absorption spectrum displays an intense charge transfer band at long wavelengths and fluorescence mirrors the absorption features.

The absorption and fluorescence spectra were recorded for the dye suspension in the native water/acetone (98:2) mixture, at the end of the reprecipitation process. For the sake of comparison, the spectra were also recorded on a sample of highly dilute dye (2×10^{-7} M) in the same medium. They are shown in Fig. 5 and their characteristics are gathered in Table 1. The difference between the two series of spectra is very striking. The absorption spectrum of the highly dilute dye shows only one band peaking at 466 nm, exactly superimposable with the excitation spectrum. The emission spectrum shows a rather narrow unresolved band, with a maximum at 518 nm. In contrast, the absorption spectrum of the dye microcrystals is very wide with two maxima at 464 and 502 nm. Absorption is still significant above 700 nm, which can be partly due to light scattering. On the excitation spectrum, the two main bands can also be seen, but no signal was detected above 530 nm. The emission spectrum was much wider than that of the dilute dye, and red-shifted by 46 nm. Moreover, for both the dilute dye and the microcrystal suspension, no variation in the position of the emission spectrum was noticed when varying the excitation wavelength from 420 to 500 nm, and conversely, the excitation spectrum did not depend on emission wavelength (λ_{em} passing from 500 to 650 nm). This reveals the presence of only one type of fluorophore in the medium. The fluorescence quantum yield of the highly dilute dye was 0.23. This value is rather low, if compared to the quantum yield of dissolved Coumarin 6 in organic solvent (0.78 in ethanol, for example [19]). This can be due to a special solvent effect, although the dye is known to be fairly insensitive to the presence of water up to 50% in organic solvent [29]. Most likely, this low quantum yield can be assigned to the fact that the highly dilute solution contains small aggregates, because the dye is very sparingly soluble in aqueous medium. The fluorescence quantum yield of the

Table 1 Spectroscopic characteristics of the microcrystal suspension (dye concentration: 2×10^{-5} M) and of the highly dilute solution (2×10^{-7} M) of Coumarin 6 in a water/acetone 98:2 mixture

	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	Φ_f	τ (ns)
Microcrystal suspension	438 (sh), 464, 502	464, 492	564	0.010±0.004	0.70±0.10
Highly dilute dye	466	468	518	0.23±0.04	0.91±0.10

λ_{abs} maximum absorption wavelength, λ_{ex} maximum excitation wavelength, λ_{em} maximum emission wavelength, Φ_f fluorescence quantum yield with excitation at 466 nm for the dilute dye and 458 for the microcrystals, τ fluorescence lifetime, *sh* shoulder

microcrystal suspension was measured using the same procedure (see experimental part) to give an estimate of the emission efficiency for the sake of comparison. It was found to be around 0.010. It is therefore much lower than that of the dilute dye, the decrease being now attributed to intermolecular interactions that take place in the solid state and favour non-radiative deactivation pathways. It can be noted that this value is lower than that of the quantum yields measured previously for microcrystals and nanofibers of benzimidazolyl coumarins, which ranged between 0.094 and 0.046 [18].

The fluorescence lifetime of the dilute dye was in the nanosecond range (that is markedly lower than in an organic solvent [25]) and the decay was found to be monoexponential, which is in line with the presence of only one type of fluorophore in the medium. The lifetime of the microcrystal suspension was even shorter, below 0.7 ns. It must be noted that no precision can be obtained with our apparatus below one nanosecond and the presence of two short lifetimes could not be distinguished.

Discussion

According to our experimental procedure, reprecipitation takes place in aqueous environment. It is thus advisable to wonder what is the state of protonation of the dye in this medium. Surprisingly, the acid–base properties of Coumarin 6 have only been studied quite recently [29–31]. It was shown that the center of protonation for the monocation occurs exclusively at the ring nitrogen, the pK_a value being around 1.6 in a water/methanol 50:50 mixture [29]. In slightly different aqueous media, the pK_a value fall in the range 0.92–2 [30, 31], which is consistent with the low basicity of the benzothiazolyl group. Consequently, the dye is essentially in its neutral form in our working conditions at pH 5.5. This is in line with the position of the absorption and emission spectra recorded here, while protonation should lead to a marked red-shift of both spectra [29–31].

Another important question is that of molecular conformation, because it influences the self-association properties. Actually, different conformations can be expected for Coumarin 6 because rotation is permitted between the coumarin motif and the benzothiazolyl group. However, X-ray diffraction analysis (XRD) has shown that these two ring systems are almost planar in the solid state [24]. This conformation probably favours molecular stacking in the crystal. Molecules are arranged centrosymmetrically in a triclinic $P1^{-}$ space group. We can think on the basis of our XRPD pattern that this molecular arrangement also takes place in our microcrystals. The crystals that have been used in the literature for XRD analysis are described as orange needles (1.00×0.20×0.20 mm) [24]. This crystal shape is reminiscent of that of our microcrystals, although the latter are slightly more elongated. This means that the reprecipitation method allowed the crystal characteristics of the dye to be retained, while reducing drastically the crystal size. It must be noted that our attempts to prepare suspensions of microcrystals by extensive sonication of commercial Coumarin 6 in a water/acetone 98:2 mixture were totally unsuccessful. No absorption spectrum could be recorded, and the fluorescence spectra were extremely noisy. The reprecipitation method is therefore very efficient to obtain a suspension of free-standing crystals of very small size.

Regarding now the spectroscopic properties of the microcrystals of Coumarin 6, it is interesting to compare our results with those reported by Corrent et al. [29]. When studying concentrated solutions of Coumarin 6 in dichloromethane, these authors have observed dual fluorescence at 500 and 620 nm, and they have attributed the long-wavelength emission band to the formation of head-to-tail or J-aggregates. Besides, they have reported that the solid dye emits at 630 nm. In the present work, no emission was detected for the microcrystal suspension in this wavelength range. This shows that microcrystals have their own spectroscopic behaviour, which can be partly influenced by the surrounding presence of water and maybe by the presence of water molecules within the crystals.

Conclusion

This work supports the conclusions that we have previously established for a related compound, namely Coumarin 7 that bears a benzimidazolyl group in the 3-position [18]. The dye microcrystals generated by the reprecipitation method have all the characteristics of the crystals grown in organic solvents, except that their size is markedly reduced. It would be interesting to see whether introducing tiny modifications in the chemical structure of Coumarin 6 leads to the formation of other types of particles, for example nanofibers, as it was the case for Coumarin 7. Work is presently underway to obtain crystals with a length below 100 nm, in view of applications in the fields of OLEDs and biomedical research.

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