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Searching for fluorescent nanocrystals in aqueous solutions of 7-methoxycoumarin

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ABSTRACT

The unexpected formation of nanocrystals in dilute dye solutions was revealed using the hydrophobic dye, 7-methoxycoumarin, the spectroscopic behavior of which has been frequently studied in water; comparison was made with the water-soluble, sulfonated analogue. A solution of the dye at 10^{-5} M concentration range was found to be a suspension of nanocrystals; at concentrations of 1×10^{-4} M, > 70% dye was in the form of nanocrystals. Surprisingly, the presence of these nanocrystals was difficult to detect by routine absorbance and fluorescence spectroscopy and was mainly noticeable by measurement of fluorescence quantum yield, as the nanocrystals were poorly emissive compared to dissolved dye molecules; electron microscopy and dynamic light scattering gave clear evidence for the presence of nanocrystals. This article illustrates the fact that the formation of nanocrystals, although abundant, may easily go unnoticed.

1. Introduction

7-Methoxycoumarin (**1**, Fig. 1), also called herniarin, is a natural umbelliferone derivative found in some species of herbs and flowers, such as chamomilla [1]. The colorant shows antimicrobial activity against various bacteria and fungi [2–5], and is also an antihelmintic, anti-inflammatory and antidermatophytic agent [6,7]. In addition to its use in traditional pharmacopoeia, the dye's photophysical behavior is unusual compared to that of other coumarin derivatives [8,9].

From a spectroscopic viewpoint, 7-methoxycoumarin is a simple system, in which the methoxy group is the weak electron donor and the carbonyl group the acceptor. Coumarin derivatives built on this model generally exhibit collapse of their fluorescence efficiency with increasing solvent polarity and proticity. Curiously, 7-methoxycoumarin behaves in an opposite manner insofar as, the dye displays intense fluorescence in water, but is weakly fluorescent in organic solvents [10–13]. This phenomenon, which is easily seen in passing from ethanol to water (Fig. 2a), has been studied for a long time and is attributed to a change in the nature of the lowest excited singlet state (n-π^* vs $\pi-\pi^*$) that accrues from the ability of the solvent to form H-bonds. Indeed, some aromatic carbonyl compounds often have low-lying n-π^* and $\pi-\pi^*$ states [14]. As with all organic molecules, fluorescence arises from the lowest singlet state; in apolar solvents, the ^{1}(n-π^*) state is situated just below the ^{1}($\pi-\pi^*$) state and it is generally observed that ^{1}(n-π^*) states are poorly fluorescent. In contrast, in polar solvents, an inversion of the energy levels takes place and the energy of the singlet ($\pi-\pi^*$) state is lower than that of the (n-π^*) state. Since ^{1}($\pi-\pi^*$) states deactivate radiatively with good efficiency, considerable fluorescence enhancement is observed in polar/protic media [10,12,15]. This mechanism is well acknowledged and 7-alkoxycoumarins are archetypal examples of this special class of polarity probes [8,16].

However, in hindsight, the behavior of 7-methoxycoumarin is strongly reminiscent of that of dyes which exhibit the recently reported phenomenon of "aggregation-induced emission enhancement" (AIEE) (see for example [17–23]). These dyes have the unique parcularity of being poorly fluorescent in solvents in which they are only soluble and highly fluorescent in solvents in which they tend to aggregate. Generally, the design principle of these molecules is the connection of aromatic groups by rotatable single bonds, so that enhanced emission in the solid state has mainly been attributed to molecular planarization and restricted molecular motions, although other mechanisms, such as prevention of exciton diffusion and *J*-aggregate formation, have also been evoked.

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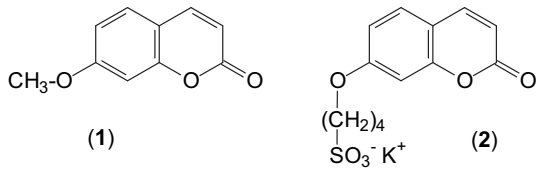

Fig. 1. Chemical structure of 7-methoxycoumarin (**1**) and 7-ω-sulfo-*n*-butoxy-coumarin potassium salt (**2**).

Two additional observations allow the formation of fluorescent aggregates to be suspected. Firstly, 7-methoxycoumarin has been used in the literature at quite high concentrations (between 10^{-4} M and 3×10^{-5} M). The compound is soluble in organic solvents but is only sparingly soluble in water (its water-solubility has been reported to be 2×10^{-3} M)[10] and, when viewed under a fluorescence microscope the powder is comprised of microcrystals that strongly emit in the violet–blue region (Fig. 2b). This means that 7-methoxycoumarin is fluorescent in the solid state and emission originating from micro- or nanocrystals in suspension must be considered.

Consequently, it seemed interesting to search for the presence of fluorescent micro- or nanoparticles in aqueous solutions of 7-methoxycoumarin, at concentrations usually employed for spectroscopic work. To do so, absorption and fluorescence spectroscopy was used, as well as electron microscopy and light scattering techniques. A comparison was made with a sulfonated coumarin derivative (**2**), which can be expected to be more soluble in water than its methoxylated analogue **1**. In this way, it will be decided whether or not the photophysical behavior of 7-methoxycoumarin must be revisited.

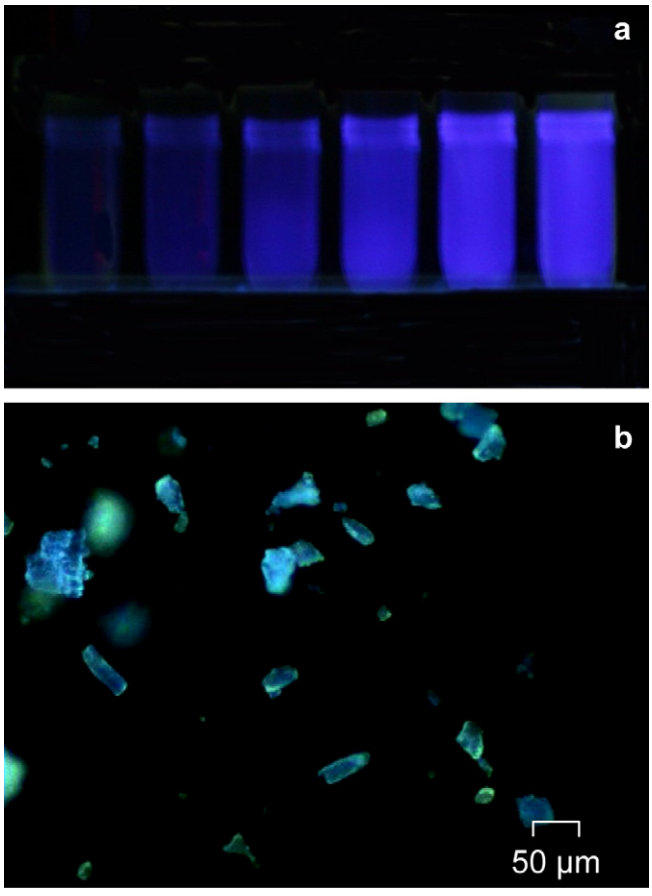

Fig. 2. (a) Image of test tubes containing 7-methoxycoumarin (**1**) at a concentration of 3.0×10^{-5} M in ethanol and in different water/ethanol mixtures upon illumination with a UV lamp ($\lambda_{ex} = 365$ nm). From left to right, proportion of water in ethanol (v/v): 0%, 19.7%, 39.4%, 59.1%, 78.8% and 98.5%. Samples were prepared using protocol 2. (b) Microcrystals of 7-methoxycoumarin as supplied by the manufacturer, visualized by fluorescence microscopy ($\lambda_{ex} = 350–380$ nm).

2. Experimental

2.1. Materials

Methanol (SDS), absolute ethanol (Prolabo) and high-pressure demineralized water (resistivity 16 MΩ cm) prepared with a Milli-Q apparatus (Millipore) were used as solvents. Fluorescence grade 7-methoxycoumarin was purchased from Fluka and used as received. 7-Hydroxycoumarin and 1,4-butane-sultone were from Aldrich.

2.2. Preparation of the potassium salt of 7-ω-sulfo-n-butoxy-coumarin

The compound was prepared according to the method described [24]. Potassium hydroxide (0.70 g) and 2 g (1.23×10^{-2} mol) 7-hydroxycoumarin were dissolved in 25 mL methanol. Butane-sultone (1.87 g, 1.37×10^{-2} mol) was then added and the ensuing mixture was refluxed for 2.5 h. After cooling, the yellow precipitate was filtered, recrystallized four times from methanol and dried under vacuum at 50 °C, giving a white powder with a 10% yield. The compound gave a single spot by TLC. ^{1}H NMR (300 MHz, D_2O): δ = 1.77 (m, 4H, $2 \times CH_2$), 2.84 (t, 2H, $J = 7.5$ Hz, CH_2–S), 3.94 (t, $J = 5.9$ Hz, 2H, CH_2–O), 6.09 (d, $J = 9.6$ Hz, 1H, H_3), 6.64 (d, $J = 2.4$ Hz, 1H, H_8), 6.74 (dd, $J = 2.4$ and 8.7 Hz, 1H, H_6), 7.32 (d, $J = 8.7$ Hz, 1H, H_5), 7.71 (d, $J = 9.3$ Hz, 1H, H_4). MS: 297.4 (M^-).

2.3. Preparation of samples

2.3.1. Protocol 1

Powdered coumarin derivatives were placed in an anactinic flask containing water, ethanol, or a previously prepared mixture of both solvents (1:4, 2:3, 3:2 and 4:1). The solution was gently stirred for 10 min, filtered and diluted with the same solvent system when it was necessary to adjust absorbance.

2.3.2. Protocol 2

For most experiments, a stock solution of 7-methoxycoumarin at 2×10^{-3} M in ethanol was prepared. A small aliquot (30 µL) of the dye solution was mixed with a large volume (1.97 mL) of water, or a previously prepared water/ethanol mixture (dilution in pure ethanol was also made for comparison purposes); the final dye concentration was 3.0×10^{-5} M. The solutions were kept in the dark at room temperature under gentle magnetic stirring for at least 1 h before spectroscopic measurement or microscopic examination. When higher concentrations were necessary, the same procedure was followed, starting with a more concentrated stock solution of **1** in ethanol.

2.4. Apparatus and methods

The mass spectrum was recorded at the "Service Commun de Spectrométrie de masse de l'Université Paul Sabatier de Toulouse" with an API 365 spectrometer using the electrospray ionization technique, negative mode. The ^{1}H NMR spectra were recorded on a Bruker AC300 spectrometer operating at 300.13 MHz.

Spectroscopic measurements were conducted at 20 °C in a temperature-controlled cell. UV/Vis absorption spectra were recorded on a Hewlett–Packard 8452A diode array spectrophotometer. For the determination of the molar extinction coefficient,

cells of different optical pathway (10 cm, 5 cm, 1 cm, 0.1 cm) were used. Corrected steady state fluorescence spectra in solution were recorded with a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. The fluorescence quantum yields in solution (Φ) were determined 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 [25]. Quinine sulfate dehydrate in 1.0 N aqueous sulfuric acid ($\Phi = 0.546$) was taken as the standard, with excitation at 332 nm [25]. Photoluminescence quantum yields in the solid state have been measured using a Jobin-Yvon Fluorolog-3 fluorometer equipped with an F-3018 integrating sphere and a UV–vis detector R2658. Samples were mounted as pure solids between two glass cover slips and inserted in the integrating sphere using the solid sample holder. The quantum yields were determined using the method developed by de Mello et al. [26] Excitation was performed at two different wavelengths (340 and 348 nm for **1**, 330 and 340 nm for **2**). For each sample, three measurements were performed, corresponding to different experimental conditions: (a) the integrating sphere is empty; (b) the sample is placed inside the sphere, but the xenon lamp beam is directed onto the sphere wall; (c) the lamp beam is directed onto the sample. The absolute quantum yield value was calculated using the expression:

$$\Phi = \frac{P_c - (1 - A)P_b}{AL_a}$$

where $A = 1 - L_c/L_b$, L is the area under the lamp excitation profile, P is the area under the curve of the emitted light and subscripts refer to the three types of measurements. In each case, the area under the lamp profile is proportional to the amount of unabsorbed light. To record the signals L_i, a neutral density filter (0.5%) was used to attenuate the very high intensity of the lamp profile and its effect was taken into account in the calculation of Φ. Before integrating each signal, correction functions accounting for the whole spectrometer assembly and the integrating sphere were applied. In addition, blank spectra were recorded using glass cover slips and subtracted from P_b and P_c.

The size and shape of the microcrystals were observed with a Zeiss Axioskop fluorescence microscope equipped with a camera. To prepare the samples, a drop of the suspensions was placed between two slides of glass. The excitation wavelength was 350–380 nm, and the emission wavelength was set at around 400 nm, using suitable filters. Size characterizations were performed at 25 °C using the Nanosizer Nano ZS (Malvern Instruments). The refractive index and viscosity values of water were used as parameter inputs. Malvern Dispersion Technology Software was used for data acquisition and analysis, applying the general purpose algorithm (Non-Linear Least Square) for calculating size distributions. Transmission electron microscopy was performed at the "Service Commun de Microscopie Electronique de l'Université Paul Sabatier", using a JEOL JEM 1011 microscope equipped with a SIS Megaview III camera. To prepare the samples, a droplet of the 7-methoxycoumarin aqueous suspension (dye concentration 3.0×10^{-5} M, ethanol 1.5% v/v) was taken and put on a carbon grid. 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 24 h at least under vacuum at 50 °C.

3. Results

The fluorescence enhancement effect in which we are interested can be easily visualized by increasing the proportion of water in a water/ethanol mixture (Fig. 2a). Two distinct protocols were used in this work for sample preparation (see Experimental section). The first one is the direct dissolution of the dye in various media, ranging from 100% water to 100% ethanol. The second protocol consists in dissolving the dye in ethanol and then pouring a small volume of this concentrated ethanol solution into a large volume of water or water/ethanol mixture. This last method is derived from the reprecipitation method, which is a solvent-exchange process often used to prepare aqueous suspensions of nano- and microparticles from hydrophobic dyes [27–30].

3.1. UV–Vis absorption properties of solutions

The samples were first observed by UV–vis absorption spectroscopy. It is well recognized that the formation of aggregates often leads to a variation of the absorption spectrum, which may shift to high wavelengths (*J*-aggregates) [31] or short wavelengths (*H*-aggregates) according to the molecular arrangement in the aggregate. Sometimes, only an enlargement of the absorption spectrum is noted. In any cases, the formation of aggregates is accompanied by a decrease of the molar extinction coefficient – although the coefficient may increase at some wavelengths.

In the present case, when the samples were prepared by the reprecipitation method, the spectra of **1** in ethanol (Fig. 3) and in water/ethanol mixtures containing 19.7%, 39.4%, 59.1%, 78.8% and 98.5% water were very similar for the same dye concentration (2×10^{-3} M). The absorption maximum was situated at 322 nm in ethanol and in the sample with the highest content of water, but a slight red shift of 2 nm was observed in all intermediate mixtures. It must be noted that no evolution of the absorption spectrum was observed within 3 h for the sample containing 98.5% water and 1.5% ethanol. Filtration of this sample with Millipore filters lead to no significant absorbance variation. Moreover, no absorption difference was detected between the top and bottom of the tubes after centrifugation during 1 h at 5000 t/min.

When methoxycoumarin **1** was directly dissolved in various water/ethanol mixtures (1:4, 2:3, 3:2 and 4:1 v/v), the absorption spectra were similar to those obtained by the reprecipitation method.

To our knowledge, the effect of concentration in water has not been reported yet. This effect was investigated here. 7-Methoxycoumarin was dissolved directly in water at a concentration of 2×10^{-3} M, by alternating gentle heating and sonication. The solubilization process was tedious, but the solution finally appeared clear to the naked eye and no precipitate was observed. It must be

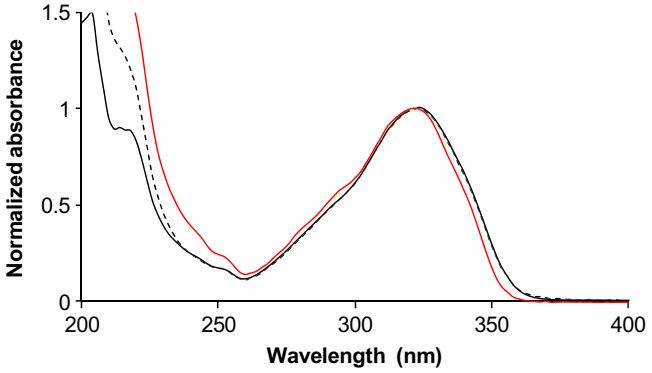

Fig. 3. Normalized absorption spectra of **1** at 1×10^{-3} M (black, plain line) and 2×10^{-6} M (black, dotted line) directly dissolved in water, and 2×10^{-5} M in ethanol (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

noticed that this concentration corresponds to the solubility limit of the dye that can be found in the literature [10]. Then, this concentrated solution was diluted with water and the samples were stirred for equilibration during 5 min before spectroscopic measurement. The variation of the molar extinction coefficient ε at 322 nm is reported in Fig. 4. It can be seen that the ε value increased with decreasing dye concentration, indicating that the dye was not fully dissolved in the most concentrated solution. Interestingly, the variation was sharp between 1×10^{-4} M and 2×10^{-6} M. This suggests the progressive dissolution of aggregates in this concentration range. The average ε value found for the most diluted solution was 14 600 M^{-1} cm^{-1}, in good accordance with the reported values that range between 14 100 and 15 400 M^{-1} cm^{-1} in different organic solvents [32]. It is noteworthy that the normalized absorption spectra of solutions at 1×10^{-3} M and 2×10^{-6} M in water were superimposable in the 260–360 nm region, and very close to the spectrum of the dye in ethanol (Fig. 3). Consequently, the presence of aggregates induced no change in the shape of the absorption spectrum.

Dye **2** readily dissolved in water. Its absorption spectrum was very close to that of **1**. The maximum wavelength was 324 nm, the weak red shift being attributed to the electron donor effect of the butyl group. No variation of the molar extinction coefficient value was observed when passing from 2×10^{-3} M to 2×10^{-6} M.

3.2. Fluorescence properties of solutions

The fluorescence properties of 7-methoxycoumarin in various media have already been well studied. For instance, studies on solutions of **1** (around 10^{-4} M) in different organic solvents, water/solvent mixtures and water have shown that the fluorescence spectrum is a single band that has been attributed to the monomeric excited singlet state. The emission maximum is slightly shifted to long wavelengths when the solvent polarity is increased [10,13,15,32,33] passing from 378 nm in benzene to 392–393 nm in water [10,12]. The position of the bands determined experimentally is in good agreement with predicted theoretical values [34]. This behavior is exactly what can be expected from a coumarin derivative in which the methoxy group acts as a weak electron donor and the carbonyl group as the electron acceptor, thus inducing a moderate intramolecular charge transfer that is slightly increased in the excited state [15,33].

It has been early reported that 7-methoxycoumarin is very weakly fluorescent in organic solvents such as benzene, acetone, dichloromethane, acetonitrile, and methanol [10–13]. For instance, the fluorescence quantum yield in methanol has been given between 0.02 and 0.054 [11–13]. In contrast, the quantum yield values in water vary between 0.16 and 0.52 [11–13]. The authors who have used 7-methoxycoumarin in more complex systems have also reported a drastic decrease of fluorescence intensity when the dye passes from an aqueous environment to that offered by micellar systems [10] and cyclodextrin cavities [35]. This is very well in line with the fact that fluorescence is much more intense in water than in organic systems. However, it is interesting to notice that the values of the fluorescence quantum yields in water reported by various authors show a strong discrepancy, which underlines a difficulty in the measurement. Besides, no obvious correlation has been found by Muthuramu et al. between the relative fluorescence intensities measured in various solvents and water/solvent mixtures and the macroscopic properties of the medium such as the dielectric constant or other polarity parameters [10].

In the present work, it would have been interesting to monitor the fluorescence quantum yield at different dye concentrations. Unfortunately, reliable fluorescence measurements can only be performed at very low optical density, hence at low dye concentration, to prevent inner filter effects. For highly absorbing samples, special devices must be used and corrections must be applied [36]. Consequently, it was chosen to work at the same dye concentration in different media. The evolution of the emission spectrum of **1** was firstly studied after direct dissolution of the dye in ethanol, water and several intermediate mixtures of these two solvents. The emission maximum shifted from 382 to 392 nm when passing from ethanol to water (Fig. 5a). Meanwhile, the quantum yield was

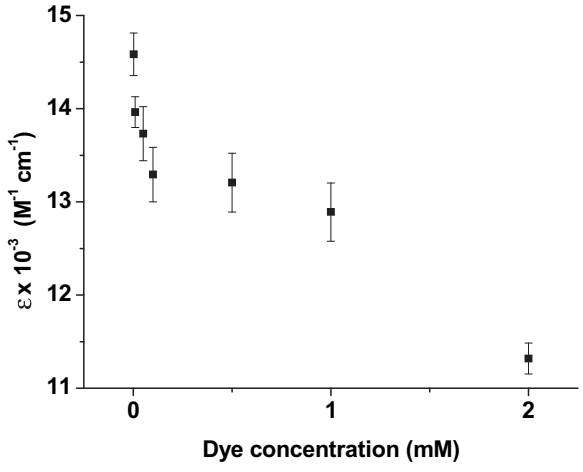

Fig. 4. Variation of the molar extinction coefficient ε at 322 nm versus dye concentration for **1** directly dissolved in water (average of three series of measurements).

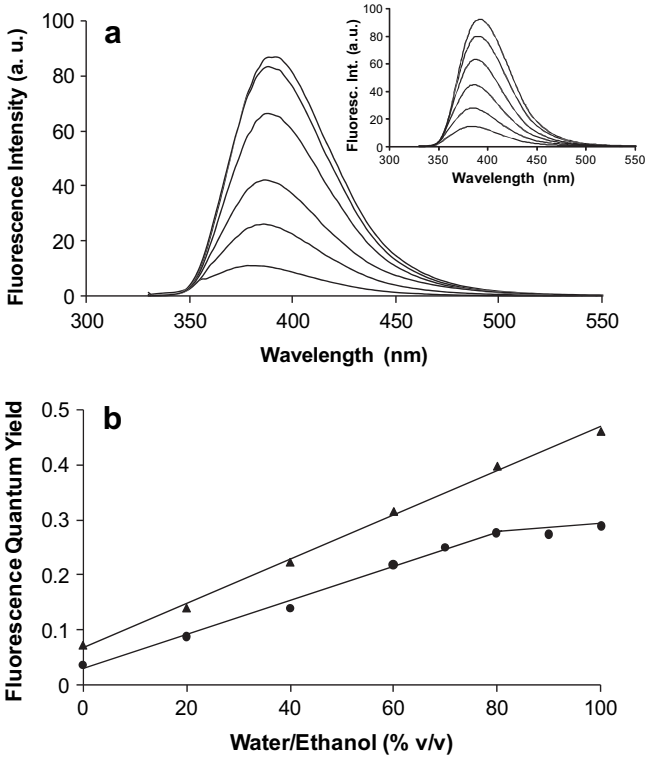

Fig. 5. a) Evolution of the emission spectrum of **1** and **2** (inset) in different media. From bottom to top: water/ethanol 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0. The fluorescence intensity at the maximum wavelength is proportional to the quantum yield. Dye concentration: 3×10^{-5} M. Direct dissolution. $\lambda_{ex} = 324$ nm b) Variation of the fluorescence quantum yield of **1** (circles) and **2** (triangles) when passing from ethanol to water.

increased from 0.037 in ethanol to 0.292 in water. Interestingly, the quantum yield leveled off when the proportion of water in ethanol reached 80% (Fig. 5b). A similar quantum yield was obtained using the reprecipitation method for the sample that contained the highest proportion of water, and this value was not affected by the ultrafiltration of the samples with a 200 μm porosity filter. Then, a comparison was made with the sulfonated analogue **2**. The emission and excitation spectra were located at almost the same position than those of compound **1**, with a very slight red shift of 2 nm attributable to the electron donor effect of the butyl group (Fig. 5a, inset). But, remarkably, the quantum yield showed in this case a regular increase with increasing water concentration (Fig. 5b).

The presence of two emitting species in the different solutions of **1** was looked for by recording the emission spectra at different excitation wavelengths and, conversely, recording the excitation spectrum for different emission wavelengths. Insignificant differences were observed, so only one fluorescent species was detected.

3.3. Fluorescence properties of the dyes in the solid state

The fluorescence properties of the dyes in the solid state were investigated on the microcrystalline powders directly purchased from the manufacturer for **1** and issued from synthesis for **2**. The emission spectra of **1** and **2** were single bands that peaked respectively at 405 and 385 nm (Fig. 6). The excitation spectra of **1** and **2** were reminiscent of those of the dissolved dyes, with a maximum at 347 and 348 nm, respectively. The photoluminescence quantum yields were measured and found to be 0.008 and 0.015 for **1** and **2**, respectively.

3.4. Observation by transmission electron microscopy (TEM)

Two samples of **1** (3.0×10^{-5} M) prepared by the reprecipitation method and containing the maximum proportion of water (water/ethanol 98.5:1.5 v/v) in the final mixture were observed by transmission electron microscopy (TEM). A drop of each sample was deposited on a grid, dried and stained by a contrasting agent. The first sample was freshly prepared and had been stirred during 4 h in the dark after mixing of the dye solution with water, before being observed. TEM revealed the presence of numerous particles, with a spherical or slightly elongated shape, most of them measuring about a hundred nanometers (Fig. 7a). With some of these particles, a clear electron diffraction spectrum was obtained, indicating their crystalline nature. With others, the diffraction spectrum was more difficult to get. This can be attributed either to the small size of the particles, or to the fact that the particles are aggregates and the crystallization process is not achieved. The second sample was left to age in the dark at 4 °C during 4 days. It showed the same type of particles than the previous one, together with bigger particles, measuring between 500 and 900 nm (Fig. 7b), and generating beautiful electron diffraction spectrum. Aqueous "solutions" of **1** at 3.0×10^{-5} M are therefore genuine suspensions of nano- or microcrystals.

3.5. Observation by fluorescence microscopy

The samples were also observed by fluorescence microscopy, with excitation in the ultra-violet. The freshly prepared samples showed a lot of very bright little points. In the four-day old sample, some big particles were also easily distinguished, strongly emitting in the blue (Fig. 8). This shows that the nano/microcrystals present in the suspension may contribute to fluorescence emission.

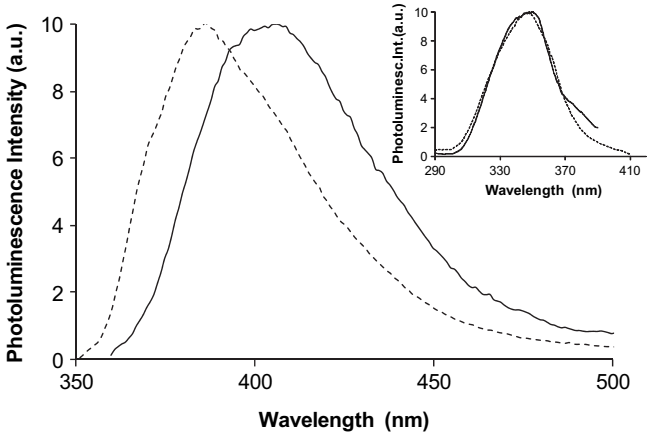

Fig. 6. Normalized photoluminescence emission spectra of the coumarin derivatives **1** (plain line) and **2** (dotted line) in the solid state. $\lambda_{ex} = 348$ and 340 nm, respectively. Inset: Normalized excitation spectrum of **1** ($\lambda_{em} = 400$ nm) and **2** ($\lambda_{em} = 420$ nm). All spectra are corrected.

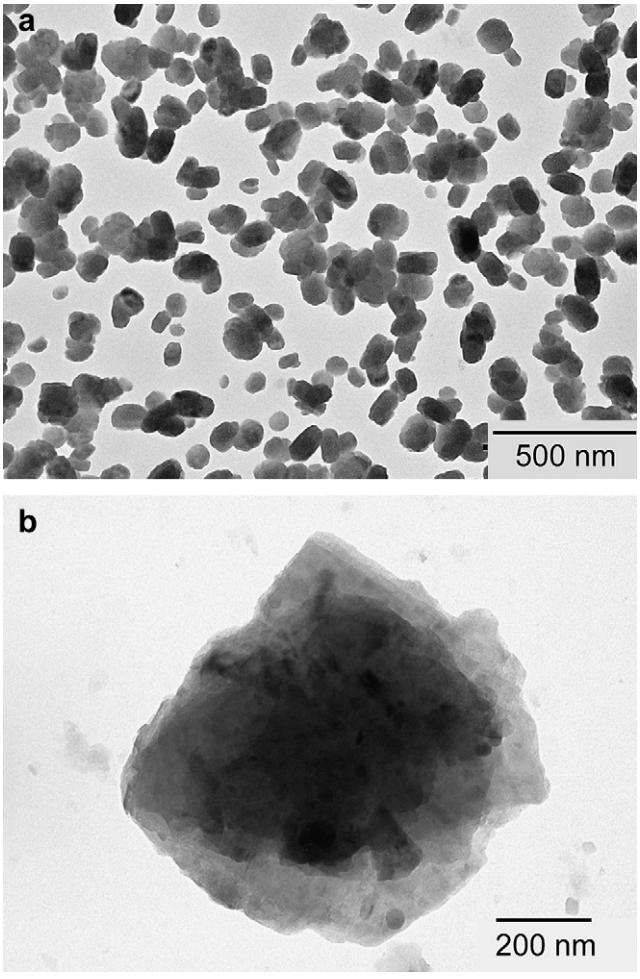

Fig. 7. Transmission electron microscopy (TEM) image of the aqueous suspensions of 7-methoxycoumarin (dye concentration 3.0×10^{-5} M in water containing 1.5% ethanol v/v). (a) freshly prepared sample; (b) 4-days old sample.

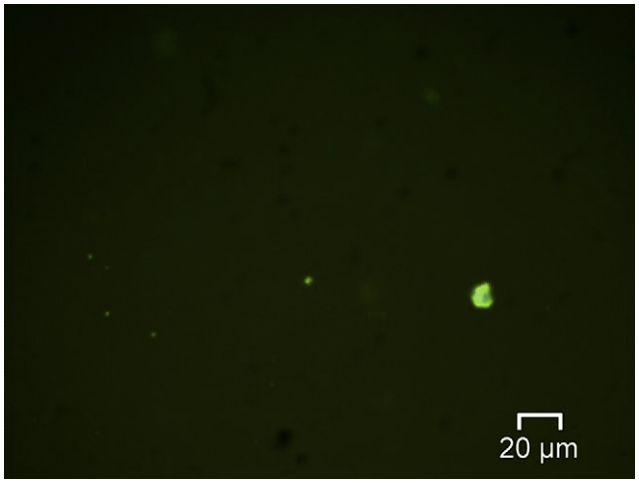

Fig. 8. Fluorescence microscopy image of the aqueous suspensions of a 4-days old sample of 7-methoxycoumarin (dye concentration 3.0×10^{-5} M in water containing 1.5% ethanol v/v).

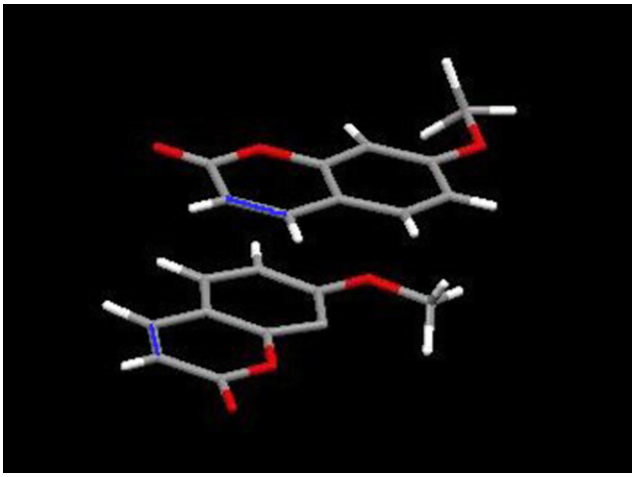

Fig. 9. Crystal unit of 7-methoxycoumarin. The angle formed between the bonds in blue is 65° (from Refs. [39,40]). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.6. Light scattering measurements

TEM observations are achieved with dried samples. To make sure that the nanoparticles are present in the solutions, light scattering experiments were undertaken. Two solutions of **1** at 3.0×10^{-4} M and a 3.0×10^{-5} M in water containing 1.5% ethanol were prepared by the reprecipitation method. Measurements were performed after 1h stirring in the dark at room temperature. In the concentrated solution, a single signal centered on 100 nm was obtained. In the solution at 3.0×10^{-5} M, the intensity of the scattered light was weaker than for the concentrated solution, but a population of particles centered around 250 nm was clearly distinguished. As expected, no clear signal was found for compound **2** at 3.0×10^{-4} M in water, thus confirming the solubility of this compound.

3.7. Crystal packing mode

The emission of fluorescence in the solid state is not common. As a matter of fact, the drastic collapse of emission efficiency generally observed in the solid state is due to intermolecular interactions that provide nonradiative decay routes. In particular, the frequently encountered side-by-side stacking of fluorophores that generates intermolecular coupling of electronic transition dipole moments leads to virtually non-fluorescent solids [18,37,38]. The origin of the fluorescence emission that is observed in the nano- and microcrystals of **1** must thus be researched in the packing mode.

The X-ray crystal structure of 7-methoxycoumarin has been reported by Ramamurthy, Venkatesan et al. who were interested in the dimerization of coumarins in the solid state [39,40]. The packing mode was found to be quite unusual. The dye crystallizes in the triclinic P-1 system. In the crystal unit, the molecules are displayed asymmetrically, so that the ethylenic bond of their pyranone cycle form an angle of 65° (Fig. 9). The transition dipole moments, which can be expected to be directed more or less along the long axis of the molecule, are thus crossed and this configuration is favorable to fluorescence emission [41]. It is interesting to note that the particular packing mode exhibited by 7-methoxycoumarin is not encountered when the methoxy group is replaced by a hydrogen atom, or a hydroxyl, methyl or acetyl group. It is thus related to the presence of the methoxy group placed in the 7-position. However, no generality has been observed for other methoxycoumarins. In fact, all four methoxycoumarin bearing the methoxy group in the 8, 7, 6 and 4 positions crystallize differently. The authors conclude that the interactions resulting from the methoxy group are too weak to really control the mode of packing.

4. Discussion

7-Methoxycoumarin is considered to be “sparingly” soluble in water. But, it appears here that the actual solubility of 7-methoxycoumarin in water is probably much lower than the commonly accepted value of 2×10^{-3} M [10]. The precise measurement of the solubility limit by usual absorption spectroscopy methods is difficult because the characteristics of the nanocrystals are quite close to those of the dissolved dye. However, our study shows that the compound is far from being dissolved at a concentration of 3×10^{-5} M. It can be deduced that a 1×10^{-4} M “solution” contains more than 70% dye as nanocrystals. This explains why authors who work at concentration in the 10^{-5} M range (and sometimes much higher) may be confronted with the undesired formation of nanocrystals, while such concentrations are usually perfect for performing spectroscopic work with the simplest members of the coumarin series. Evidence was given by TEM and light scattering for the formation of such nanocrystals. However, their presence was very difficult to detect: whatever the mode of preparation, the solutions looked clear to the naked eye and ultrafiltration was not enough to retain the particles that measured about 100 nm. Most of spectroscopic experiments that are routinely used to detect the presence of particles were unsuccessful, because the absorption and emission spectra of the dye in the solid state are situated close to those of the dissolved dye. The influence of the nanocrystals upon the fluorescence properties was quite weak. In aqueous medium, the weak emission of the nanocrystals is masked by the strong emission of the dissolved dye, so that no distortion of the emission spectrum was detected. The best way to measure the influence of nanocrystals in this medium was to make a comparison between **1** and the well-soluble sulfonated dye **2**. The fluorescence quantum yield of **2** showed a progressive increase when changing the solvent composition. This can be seen as the normal behavior of the fluorophore when passing from one medium to the other. In ethanol-rich media, the fluorescence quantum yield of **1** follows the same trend as that of **2**, but the two curves deviate when the water concentration is increased above

80%. The special behavior observed for **1** can be attributed to the formation of nanocrystals that are less emissive than the dissolved dye. It can be extrapolated that the fluorescence quantum yield of **1** in the absence of nanocrystals would be around 0.37.

It must be underlined that suspensions of 7-methoxycoumarin were not steady in the considered period of time. Nanocrystals formed very quickly and the "solutions" used for routine spectroscopic work actually contained these nanocrystals. However, with aging, the nanocrystals underwent classical Ostwald ripening and progressively became of microscopic size. Monitoring the evolution of this system with time would be interesting, although this is beyond the scope of the present article. It is also noteworthy that no obvious difference was found between the suspensions prepared by direct dissolution of **1** and those prepared using the reprecipitation method.

The presence of nanocrystals is probably easier to detect in organic media. This hypothesis allows observations reported by many authors to be better understood. For instance, as soon as in 1959, Wheelock has noticed that the fluorescence intensity of various coumarin derivatives in ethanol changes with concentration. In particular, he has reported that the fluorescence intensity of 7-methoxycoumarin in alcohol drastically increases when concentration passes from 5 to 300 mg per 100 mL [42]. Lysenko and Potapenko, also working in ethanol, have reported a similar effect [43]. They have also noted that the absorption spectrum depends on concentration, and that the fluorescence excitation spectrum is similar to the absorption spectrum only at high concentration. They have concluded that the dye forms dimers or larger aggregates in ethanol, and that these aggregates are able to emit fluorescence. Actually, the nanocrystals strongly contribute to the total fluorescence of the ethanol suspension because in this medium their photoluminescence quantum yield is close to that of the dissolved dye. It must be noticed that the photoluminescence quantum yield measured on our sample of microcrystals may be lower than that of the nanocrystals actually formed in the suspensions, because it has been shown recently that this value strongly depends on crystal defects and impurities, and thus on the preparation procedure [44].

The present work shows that there is no need to re-visit the behavior of 7-methoxycoumarin in water. Nanocrystals are actually formed, but their contribution to the total fluorescence emission is quite weak and the phenomenon observed is not due to an aggregation-induced emission enhancement effect (AIEE). Should these new results lead us to re-consider the works already published about spectroscopic applications of 7-methoxycoumarin? In our opinion, in the case where **1** was used for incorporation in cyclodextrins [35] or in micelles [10], the presence of nanocrystals does not question the validity of the experiments. It is obvious that 7-methoxycoumarin is incorporated in these media, and the dye localization and the binding constants reported are certainly correct. However, taking into account the nanocrystal dissolution step would be necessary if the aim was to study the equilibria involved and the kinetics of incorporation.

It is also interesting to underline that the present results only concern 7-methoxycoumarin. The case of other analogues, for example 4-methyl-7-methoxycoumarin [16] and its derivatives with longer alkyl chains, would deserve being considered separately. In fact, since these compounds can be expected to be more hydrophobic than **1**, they could form nanocrystals at much lower concentrations. These nanocrystals could be far more fluorescent than those of **1**, because very small chemical modifications are enough to change drastically the packing mode and thus the properties of the solid state. No AIEE phenomenon was detected here, but this does not mean that it does not take place for other members of the 7-alkylcoumarin series.

5. Conclusions

For a long time, spectroscopists have almost exclusively focused on the study of organic dyes dissolved in solution or dispersed in different media such as polymers. Only recently have they begun to get interested in the behavior of dyes in their solid state, because the latter are much more in demand than dissolved dyes for "real-world" applications. With this in mind, they bring more and more attention to the possible formation of particles in suspension and the unexpected consequences of their presence.

In the case of 7-methoxycoumarin, the formation of nanocrystals, although abundant, has gone unnoticed until now because these particles only slightly perturb the spectroscopic measurements. However, the problem of poor dissolution is quite well spread and may be at the origin of many spectacular artifacts, especially in the measurement of fluorescence quantum yields. One of the aims of the present article is to heighten awareness of this problem, which is far more frequent than commonly thought.

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References

[1] Ahmad A, Misra LN. International Journal of Pharmacognosy 1997;35:121–5.
[2] Franke R, Schilcher H, editors. Chamomile. Industrial profiles. Boca Raton: Taylor & Francis, CRC Press; 2005.
[3] Kayser O, Kolodziej H. Zeitschrift für Naturforschung C 1999;54:169–74.
[4] Montagner C, de Souza SM, Groposo C, Delle Monache F, Smania EFA, Smania Jr A. Zeitschrift für Naturforschung C 2008;63:21–8.
[5] Smyth T, Ramachandran VN, Smyth WF. International Journal of Antimicrobial Agents 2009;33:421–6.
[6] Silván AM, Abad MJ, Bermejo P, Sollhuber M, Villar A. Journal of Natural Products 1996;59:1183–5.
[7] Mares D, Romagnoli C, Bruni A. Plantes Médicinales et Phytothérapie 1993;26:91–100.
[8] Valeur B. Molecular fluorescence. Principles and applications. Weinheim: Wiley-VCH; 2002. p. 221–222.
[9] Wagner BD. Molecules 2009;14:210–37.
[10] Muthuramu K, Ramamurthy V. Journal of Photochemistry 1984;26:57–64.
[11] Heldt JR, Heldt J, Stoń M, Diehl HA. Spectrochimica Acta A 1995;51:1549–63.
[12] Wenska G, Paszyc S. Canadian Journal of Chemistry 1988;66:513–6.
[13] Azuma K, Susuki S, Uchiyama S, Kajiro T, Santa T, Imai K. Photochemical and Photobiological Sciences 2003;2:443–9.
[14] Mantulin WW, Song PS. Journal of the American Chemical Society 1973;95:5122–9.
[15] Aaron JJ, Buna M, Parkanyi C, Antonious MS, Tine A, Cissé L. Journal of Fluorescence 1995;5:337–47.
[16] Seixas de Melos JS, Becker RS, Maçanita AL. Journal of Physical Chemistry 1994;98:6054–8.
[17] Lim SJ, An BK, Jung SD, Chung MA, Park SY. Angewandte Chemie, International Edition in English 2004;43:6346–50.
[18] Oelkrug D, Trompert A, Gierschner J, Egelhaaf H-J, Hanack M, Hohloch M, et al. Journal of Physical Chemistry B 1998;102:1902–7.
[19] Xu J, Liu X, Lv J, Zhu M, Huang C, Zhou W, et al. Langmuir 2008;24:4231–7.
[20] Bhongale CJ, Chang CW, Lee CS, Diau EWG, Hsu CS. Journal of Physical Chemistry B 2005;109:13472–82.
[21] Dong S, Li Z, Qin J. Journal of Physical Chemistry B 2009;113:434–41.
[22] Ren Y, Lam JWY, Dong Y, Tang BZ, Wong KS. Journal of Physical Chemistry B 2005;109:1135–40.
[23] Chen J, Xu B, Ouyang X, Tang BZ, Cao Y. Journal of Physical Chemistry A 2004;108:7522–6.
[24] Passal F. Ger. Offen. 1969. DE 191611819691016.
[25] Demas JN, Crosby GA. Journal of Physical Chemistry 1971;75:991–1024.
[26] De Mello JC, Wittmann HF, Friend RH. Advanced Materials 1997;9:230–2.
[27] Nakanishi H, Oikawa H. In: Masuhara H, Nakanishi H, Sasaki K, editors. Single organic nanoparticles. , Berlin: Springer-Verlag; 2003. p. 17–31.
[28] Kasai H, Nalwa HS, Oikawa H, Okada S, Matsuda H, Minami N, et al. Japanese Journal of Applied Physics 1992;31:L1132–4.
[29] Bertorelle F, Lavabre D, Fery-Forgues S. Journal of the American Chemical Society 2003;125:6244–53.
[30] Abyan M, De Caro D, Fery-Forgues S. Langmuir 2009;25:1651–8.

[31] Kobayashi T, editor. J-Aggregates. Singapore: World Scientific; 1996.
[32] Nizomov N, Kholov AU, Ishchenko AA, Ishchenko VV, Khilya VP. Journal of Applied Spectroscopy 2007;74:626–34.
[33] Parkanyi C, Antonious MS, Aaron JJ, Buna M, Tine A, Cissé L. Spectroscopy Letters 1994;27:439–49.
[34] Jacquemin D, Perpète EA, Scalmani G, Frisch MJ, Assfeld X, Ciofini I, et al. Journal of Chemical Physics 2006;125:164324 (1–11).
[35] Wagner BD, Fitzpatrick SJ, McManus GJ. Journal of Inclusion Phenomena and Macrocyclic Chemistry 2003;47:187–92.
[36] Oelkrug D. Fluorescence spectroscopy in turbid media and tissues. In: Lakowicz JR, editor. Topics in fluorescence spectroscopy: probe design and chemical sensing. New York: Plenum Press; 1994. p. 223–53.
[37] Birks JB. Photophysics of aromatic molecules. London: Wiley; 1970.
[38] Silinsh EA. Organic molecular crystals. Berlin: Springer-Verlag; 1980.
[39] Gnanaguru K, Ramasubbu N, Venkatesan K, Ramamurthy V. Journal of Organic Chemistry 1985;50:2337–46.
[40] Bhadbhade MM, Murthy GS, Venkatesan K, Ramamurthy V. Chemical Physics Letters 1984;109:259–63.
[41] Xie Z, Yang B, Li F, Cheng G, Liu L, Yang G, et al. Journal of the American Chemical Society 2005;127:14152–3.
[42] Wheelock CE. Journal of the American Chemical Society 1959;81:1348–52.
[43] Lysenko EP, Potapenko AYa. Studia Biophysica 1988;124:115–22.
[44] Katoh R, Suzuki K, Furube A, Kotani M, Tokumaru K. Journal of Physical Chemistry C 2009;113:2961–5.