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Improving the fluorescence polarization method to evaluate the orientation of fluorescent systems adsorbed in ordered layered materials

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ABSTRACT

The fluorescence polarization method, recently developed for the evaluation of the preferential orientation of fluorescent dyes adsorbed in layered materials [F. López Arbeloa, V. Martínez Martínez, J. Photochem. Photobiol. A: Chem. 181 (2006) 44], is readapted to improve its application. Fluorescence polarization was previously obtained by recording the emission intensity for two orthogonal orientations of the emission polarizer (i.e., the horizontal and vertical polarized light) after excitation with vertical or horizontal polarized light. In the method proposed in this work, samples are excited with unpolarized light, reducing the polarization effect of the excitation light scattering at those emission wavelengths close to the excitation wavelength. Moreover, the present method decreases the effect of the orientation of other non-fluorescent species present in the system, which are active in the excitation process. Consequently, the new method is more simple, precise and sensitive. It is applied to evaluate the orientation of rhodamine 6G dye adsorbed in ordered laponite clay films with low and moderated dye loadings.

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1. Introduction

The incorporation of laser dyes in solid host materials is being extensively applied for the development of new tunable dye lasers in the solid state and for the design of new photoelectronic devices [2–8]. Both organic and inorganic systems have been used as host materials. Organic host systems, such as polymer matrices, have the advantage of good compatibility and solubility with organic laser dyes, whereas inorganic networks provide more rigid environments and enhance the photo and the thermostability of the dyes. In this sense, important efforts are now focussed on the incorporation of fluorescent dyes into hybrid organic/inorganic host materials, in which the benefits of both materials are reunified [9–11].

On the other hand, nanostructured host frameworks can provide a confined space for the intercalation of fluorescent dyes, inducing a preferential orientation of the guest molecules. A great variety of host materials with mono (nanotubes and one-dimensional (1D) multichannel systems), bi (layered materials, such as clays and double-layered hydroxides) and tridimensional (3D intercrossing channels, such as zeolites and zeolite-type

materials) frameworks can be found for this purpose [7,9,12–15]. A macroscopic organization of these nanostructured frameworks with a preferential orientation of the guest dyes provides a macroscopic orientation of the fluorescent molecules with an anisotropic photoresponse (both in absorption and emission) with respect to the plane of the linearly polarized light. These anisotropic systems have interesting technological applications in the design of new non-linear optics such as second harmonic generation, optical guides and so on [7,10,16].

It has been proven that the spin coating is a good method to elaborate supported films of clay particles with a parallel disposition of the clay layers [17]. A posterior intercalation of cationic dyes in these ordered clay films induces a spontaneous preferential orientation of the dye with respect to the normal of the films (or out-of-plane of the layers), leading to a macroscopic arrangement of oriented dye molecules in one direction. These systems present interesting anisotropy photoresponses. Indeed absorption spectroscopy with linearly polarized light has been largely used for the determination of the orientation angle of the dye adsorbed into the interlayer space of ordered clay systems [18–20]. Recently, our group has developed a new method to evaluate this orientation angle based on the polarization of the fluorescent emission of these systems [1,15,21,22].

This new method consists in the determination of the fluorescent dichroic ratio, D_{HV} , defined as the ratio between the fluorescence intensity for two orthogonal orientations of the

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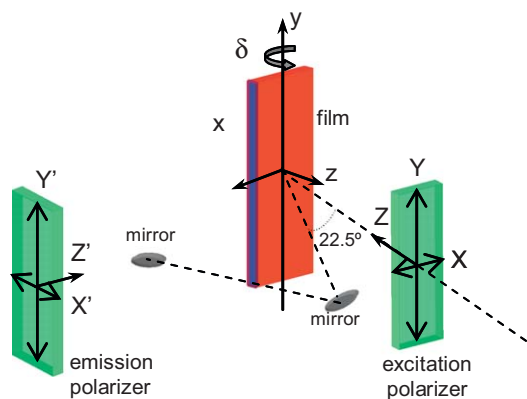


Fig. 1. Experimental setup to record fluorescence polarization of solid sample films with front-face configuration as a function of the twisted angle δ of the film around its vertical axis.

emission polarizer. Experimentally (Fig. 1), the fluorescence spectra of ordered dye/clay films (registered in the front-face configuration, in which the emission detection channel was oriented 22.5° with respect to the excitation beam) were recorded with the emission polarization horizontally (H) and vertically (V) oriented, after excitation with horizontal or vertical polarized light, i.e., $D_{HV} = I_{HH}/I_{HV} = I_{VH}/I_{VV}$ where the first and second subindexes refer to the orientation of the excitation and emission polarizers, respectively. The dichroic ratio was obtained as a function of the twisted angle δ of the film around its vertical axis (δ is the angle between the excitation beam and the normal to the film, forming an angle with respect to the emission detection direction of $\delta+22.5^\circ$). Indeed, a linear relationship was established between D_{HV} and $\cos^2(\delta+22.5)$ [1,15,21,22]

$$D_{HV} = \frac{I_{VH}}{I_{VV}} = \frac{I_{HH}}{I_{HV}} = 2 \cot^2 \psi + (1 - 2 \cot^2 \psi) \cos^2(\delta + 22.5) \quad (1)$$

from which the preferential orientation of the transition dipole moment of the fluorescent dye with respect to the film normal, ψ , can be evaluated. The recorded dichroic ratio has to be corrected from the instrumental response to the linearly polarized light by means of a G factor evaluated from the fluorescence signal of an isotropy system (i.e., a liquid solution of a fluorescent dye in a low-viscous solvent) registered in identical experimental conditions.

The validity of Eq. (1) was checked for the adsorption of Rhodamine 6G (R6G) in Laponite B (Lap) clay films after excitation with both horizontally and vertically polarized light [21,22]. Excitation with H and V polarizations provide identical angles. However, it was observed an important influence of the polarization of the scattering light on the D_{HV} value at those wavelengths close to the excitation. Of course, the influence of light scattering polarization is important for parallel excitation and emission polarizers, I_{HH} and I_{VV} , rather than for orthogonal excitation and emission polarizers, I_{HV} and I_{VH} . This means that light scattering increases the D_{HV} value for horizontally polarized excitation, whereas the opposite behavior is observed for vertically polarized light.

In order to reduce the effect of the light scattering and to improve the methodology to evaluate the orientation of fluorescent dyes adsorbed in ordered clay films, the present work is focused on the determination of the dichroic ratio by recording the fluorescence spectra of R6G/Lap films with H - and V -emission polarizations after excitation with unpolarized light. In this sense, the method would become more simple (excitation polarization is not required), more precise (the effect of the polarized light

scattering is minimize) and more sensitive (the recorded fluorescence intensity should be increased, because the increase in the excitation intensity).

2. Experimental

Rhodamine 6G (R6G, Laser Grade) was supplied by Kodak and was used as received. The sodium form of Laponite B (Lap) was purchased from Laporte and was used without further treatment. This synthetic clay is characterized by its high purity and the very small particle size, around 30 nm, providing very stable aqueous suspensions and transparent supported films.

The elaboration of thin films of Lap was described previously [17]. Briefly, some drops of a stable 2% w/w aqueous suspension of the clay (stirred during two days) were dropped on glass substrates. After spinning at 2500 rpm during 60 s and accumulating two spinning procedures at the same substrate, Lap films with a thickness of 450 nm at the center of the film were obtained [17]. The intercalation of R6G molecules in Lap films was performed by immersing the clay film into a solution of the dye in a water(0.8)/ethanol(0.2) molar fraction mixture. The dye loading was controlled by the concentration of the dye solution and the immersion time. Two different R6G/Lap films with low and moderate dye loadings of 0.1 (immersion in 10^{-5} M R6G solution during 5 min) and 5.7% (8 h immersion in 10^{-5} M solution) of the total cation exchange capacity (CEC) of the clay were obtained. After washing with ethanol and drying, the films were kept two days in the dark to get a good distribution of adsorbed molecules through the interlayer space of the clay galleries.

Fluorescence spectra of the films were recorded after excitation at 495 nm (except in those specified cases) in a SPEX Fluorolog 3-22 fluorimeter, using the front-face configuration in which the emission intensity was recorded at 22.5° with respect to the excitation beam. Linearly polarized emission spectra were recorded using a Glan-Thompson polarizer in the detection channel, without previous polarization of the excitation light. The emission polarizer was oriented in the horizontal and vertical directions, and the fluorescence dichroic ratio D_{HV} was determined from the recording fluorescence intensities I_H and I_V , respectively. The fluorescence spectra were registered for different orientations of the films with respect to the excitation beam by means of a rotating solid sample holder. The angle between the normal to the films and the excitation beam, δ , was changed from -20° to 45° (avoiding the specular reflexion of the excitation beam to the emission detector at around -10°). The instrumental response to the plane of the polarized light was experimentally obtained by recording the fluorescence spectra of an isotropic system in identical instrumental conditions, a diluted solution of R6G in a low-viscous solvent.

3. Results

Fig. 2 shows the fluorescence spectra of R6G in Lap with a very low dye loading of 0.1% CEC recorded with the emission polarizer oriented at the H and V directions as a function of the orientation of the film with respect to the excitation beam (δ angle). These spectra have been corrected for the sensitivity of the emission channel for H - and V -polarized light by registering the H - and V -signal of an isotropy system recorded with identical experimental conditions (a 10^{-5} M solution of R6G in ethanol in a 1 mm optical pathway cell and at the same orientation angles). The different evolution observed in I_V and I_H with the δ angle confirms the anisotropy behavior in the fluorescent emission of R6G in Lap

films (similar to those obtained after excitation with linear polarized light [21,22]).

This anisotropy photoresponse suggests a preferential orientation of R6G monomers with respect to the normal to the film. This anisotropic behavior implies both a macroscopic ordered stacking of clay layers in a parallel arrangement in the supported plate and a preferential inclination out-of-plane of the dye molecules adsorbed in the interlayer space of clay particles. Indeed, an isotropic photoresponse was previously reported in the absorption spectra of this sample in the plane of the supported film, indicating a perfect stacking of the clay layers parallel to the film and a random distribution of the dye molecules in the plane of the clay layers [15,20].

The fluorescence dichroic ratio $D_{HV} = I_H/I_V$ of this 0.1% CEC R6G/Lap film (Fig. 3(A)) does not depend on the emission wavelength. This result confirms the emission from a unique R6G species or the emission of several R6G species with identical orientation with respect to the clay layers. Previous studies proved the adsorption of individual R6G molecules (monomers) into the Lap galleries for this very diluted dye R6G/Lap film [20,21]. The important decrease/increase in the D_{HV} values close to the excitation wavelength have been reported for this sample after excitation with *H*- and *V*-polarized light [1,22], owing to the intrinsic polarization of the excitation light scattering. Consequently, excitation with unpolarized light has at least two important advantages with respect to polarized excitation: an increase in the excitation and, as consequence, in the detected emission intensity (improving the sensibility of this fluorescent method) and a reduction in the polarization effect of the excitation light scattering.

According with Eq. (1) and as is illustrated in Fig. 3(B), the D_{HV} values obtained in the present work with unpolarized excitation show a good linear relationship (correlation coefficient, $r = 0.998$) with the twisted angle δ of the film through the $\cos^2(\delta+22.5)$ parameter. This relationship is obtained at the maximal emission wavelength of R6G monomers in Lap films, at 550 nm, although similar linear relations can be drawn for other emission wavelengths. From the corresponding slope, the preferential orientation angle ψ of the R6G monomers with respect to the layer normal can be evaluated. The obtained ψ value for this 0.1% CEC R6G/Lap film ($\psi = 64^\circ$, Table 1) is similar to that obtained from fluorescence polarization with *H*- and *V*-polarized excitation and from absorption with polarized light (ψ values obtained from these techniques are included in Table 1 for comparison).

Consequently, it can be concluded that the emission polarization after excitation with unpolarized light is an adequate method to evaluate the orientation of dye fluorescence molecules adsorbed in oriented clay films. Even, it is a recommended method with respect to polarized excitation, because of the increase in the sensibility and reduction in the effect of the excitation polarization due to light scattering.

Fig. 4 shows the corrected *H*-polarized fluorescent spectra of the 5.7% CEC R6G/Lap film as a function of the twisted angle δ of the sample. Clearly, two different evolutions of I_H with the twisted angle δ can be observed: at shorter wavelengths (around 550 nm) the fluorescence intensity decreases with the twisted angle, whereas the opposite behavior is detected for longer emission wavelengths (around 590 nm). These results suggest the emission from at least two different fluorescent species: the monomer of R6G with emission wavelengths around 550 nm, and a J-type

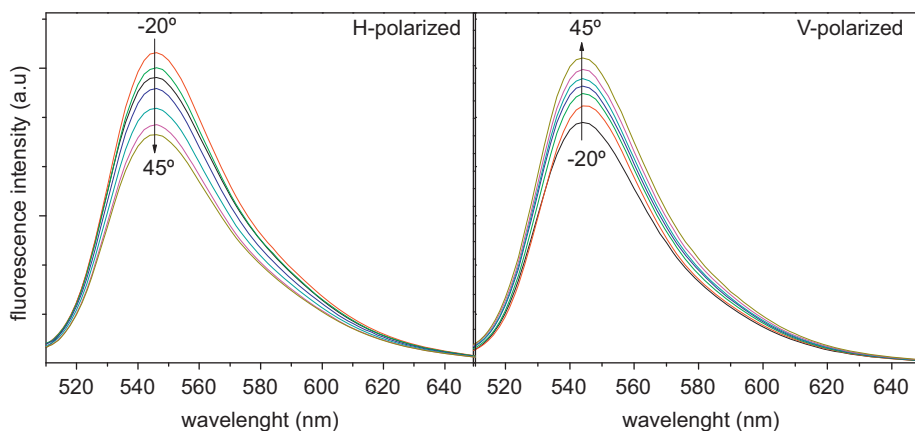


Fig. 2. Evolution of the *H*- and *V*-polarized fluorescence spectra of R6G in the 0.1% CEC R6G/Lap sample with the twisted angle δ of the film around its vertical axis.

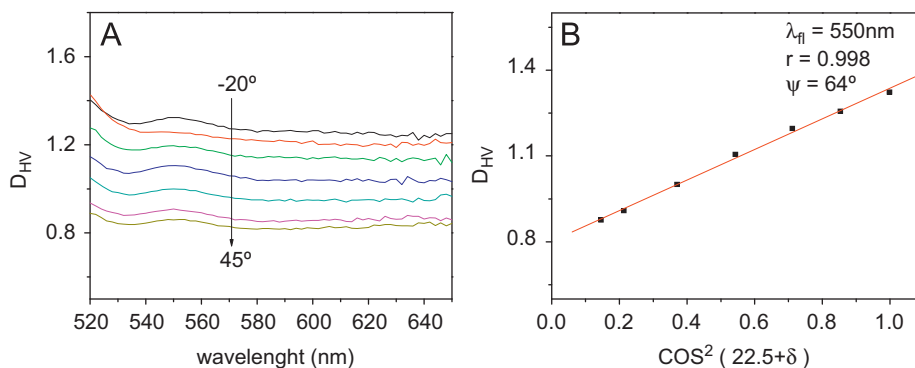


Fig. 3. Evolution of the fluorescence dichroic ratio D_{HV} of the 0.1% CEC R6G/Lap film with the emission wavelength for different twisted angle δ of the film (A) and linear relationship of D_{HV} with the twisted angle δ analyzed at the monomer emission peak (B).

Table 1

Orientation angle ψ with respect to the clay normal of several R6G species in Lap films observed for very diluted (0.1% CEC) and moderate (5.7% CEC) dye loadings evaluated by different methods based on polarized spectroscopy.

Method	ψ (monomer) ($\lambda_{ab} = 530$ nm) ($\lambda_{fl} = 550$ nm)		ψ (H-dimer) ($\lambda_{ab} = 495$ nm) non-fluorescent 5.7% CEC	ψ (J-dimer) ($\lambda_{ab} = 545$ nm) ($\lambda_{fl} = 590$ nm)
	0.1% CEC	5.7% CEC		5.7% CEC
Absorption polarization ^a	62°	–	48°	61°
Fluorescence polarization with <i>H</i> - or <i>V</i> -polarized excitation ^b	62°	61°	–	49° ($\lambda_{exc} = 495$ nm) 60° ($\lambda_{exc} = 570$ nm)
Fluorescence polarization with unpolarized excitation ^c	64°	59°	–	57° ($\lambda_{exc} = 495$ nm) 63° ($\lambda_{exc} = 570$ nm)

^a Ref. [20].

^b Refs. [1,20,22].

^c This work.

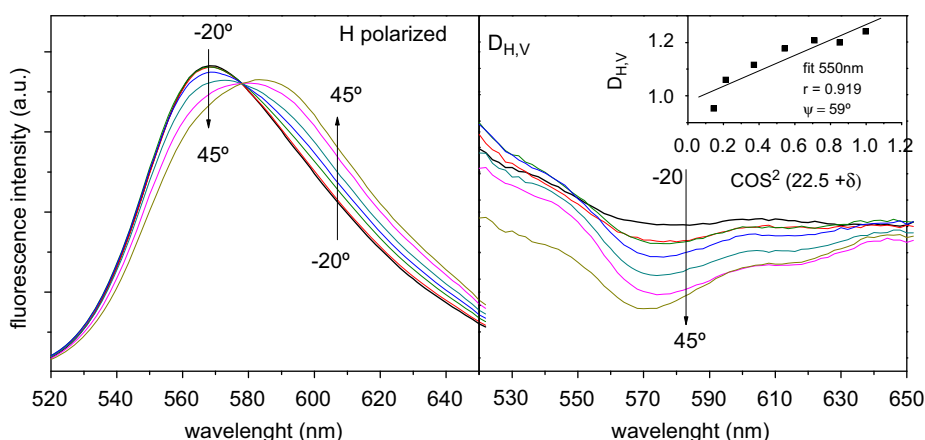


Fig. 4. (A) Left: evolution of the *H*-polarized fluorescence spectra of the 5.7% CEC R6G/Lap film with the twisted angle δ . (B) Right: evolution of the fluorescence dichroic ratio D_{HV} with the emission wavelength for different twisted angle δ (inset: linear relationship of D_{HV} vs. the twisted angle analyzed at the monomer emission band).

aggregate (a dimer, previously characterized for this 5.7% CEC sample [21]), with an emission maximum centered at around 595 nm.

It was previously proven that an increase in the dye loading induces the aggregation of R6G molecules adsorbed in Lap films [23,24]. Both *H*-type and *J*-type aggregates are characterized by absorption spectroscopy by new spectral bands at higher (470–500 nm) and lower (545 nm) energies, respectively, with respect to the monomer absorption band (530 nm). Moreover, *J*-type aggregates are fluorescent, and they are also characterized by emission bands at lower energies (590–620 nm) than that of the monomer emission band (550 nm).

As a consequence of the fluorescent emission of different R6G species present in this 5.7% CEC sample, the dichroic ratio is dependent upon the emission wavelength (Fig. 4(B)). The analysis of the linear relationship D_{HV} vs. $\cos^2(\delta+22.5)$ at the monomer emission (around 550 nm), inset Fig. 4(B), provides an inclination angle of $\psi = 59^\circ$, similar to that obtained for other methods (absorption polarization and emission polarization with polarized excitation light), Table 1.

These results confirm previous results in the sense that the orientation of adsorbed R6G monomers in Lap films is around 62° with respect to the layer normal, independent of the dye content.

On the other hand, the corresponding D_{HV} vs. $\cos^2(\delta+22.5)$ linear relationship of this moderate 5.7% CEC sample analyzed at 630 nm (far away from the monomer fluorescence band) (Fig. 5) provides an inclination angle of 57° for the fluorescent *J*-dimer characterized for this sample after excitation at 495 nm. This inclination angle is slightly lower than that obtained for the

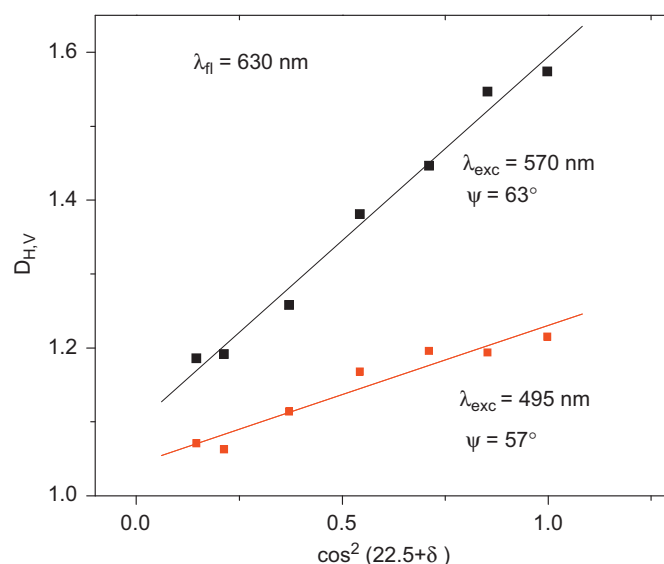


Fig. 5. Linear relationship of D_{HV} vs. the twisted angle δ for the 5.7% CEC R6G/Lap film, analyzed at the *J*-aggregate emission (630 nm), as a function of the excitation wavelength.

absorption band associated to this *J*-aggregate ($\psi = 61^\circ$ [20]) and from fluorescence polarization after direct excitation of the *J*-aggregates at 570 nm ($\psi = 63^\circ$), Table 1.

These results contrast with those obtained from fluorescence polarization after excitation with polarized light, where the reported ψ value strongly depends on the excitation wavelength: $\psi = 49^\circ$ and 60° after excitation at 495 and 570 nm, respectively, with polarized light [1,22], Table 1. These results were previously discussed on the basis of the excitation energy transfer between excited states: from the excited state directly populated by the excitation process to the emitting state [21,22].

In fact, at the excitation wavelength of 495 nm (at the monomer vibration shoulder), both monomers and H-type aggregates are the main absorbing species [21]. Therefore, the emission from J-type aggregates (at around 595 nm) should be observed after the energy transfer from monomer and H-type aggregates to the excited state of J-type aggregates. As a consequence of this energy transfer, the fluorescence polarization from the J-aggregate emission should be affected by the orientation of absorbing species at the excitation wavelength. Indeed, the inclination angle derived from the fluorescence dichroic ratio at 630 nm (J-aggregate emission) after polarized excitation at 495 nm ($\psi = 49^\circ$) reaches the orientation angle of the H-type aggregate ($\psi = 48^\circ$ obtained by absorption polarization) rather than that of the fluorescent J-aggregate ($\psi = 61^\circ$ from absorption polarization) [22], Table 1.

The energy transfer between excited H- and J-type aggregates is experimentally confirmed by the fact that the fluorescence dichroic ratio of the J-type aggregate depends on the excitation wavelength (Fig. 5). Thus, as is reflected in the data listed in Table 1, the inclination angle $\psi = 63^\circ$ derived for this aggregate after exclusively excitation of the J-aggregate (at 570 nm) reaches the values obtained from the absorption polarization ($\psi = 61^\circ$) and from fluorescence polarization with polarized excitation at the absorption band of the J-type aggregate ($\psi = 60^\circ$).

These results suggest the validity of the fluorescence polarization with unpolarized excitation light to evaluate the orientation of different fluorescent species present in ordered clay films. Moreover, since the fluorescence polarization is affected by the orientation of the absorbing species at the excitation wavelength, the difference in the ψ angle derived from unpolarized and polarized excitation light could be used to estimate relative orientation between the absorbing and emitting. Indeed, the rate constant for the energy transfer between the exciting and emitting states should depend on the relative orientation between both moieties [25].

4. Conclusions

Fluorescence polarization is an adequate tool to evaluate the preferential orientation of fluorescent molecules adsorbed in

oriented layered materials. The use of unpolarized vs. polarized excitation light is recommended, because the effect of the light scattering polarization is reduced and because the technique becomes more sensitive. Moreover, the effect of the excitation of non-fluorescence species with other inclination angles on the evaluated angle of fluorescent species is less prominent after excitation with unpolarized than with polarized excitation light

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