

Application of Fluorescence with Polarized Light to Evaluate the Orientation of Dyes Adsorbed in Layered Materials

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A new method of fluorescence polarization is applied to evaluate the angle of the preferential orientation of Rhodamine 6G (R6G) dye adsorbed in supported thin films of Laponite (Lap) clay. The method is based in the determination of the fluorescence dichroic ratio, obtained from the recorded fluorescence spectra with the detection polarizer horizontally and vertically oriented, as a function of the twisted angle of the film around its vertical axis, keeping the excitation polarizer in a fixed direction. The validity of the method is checked by comparing the experimental results obtained with both vertically and horizontally polarized excitations to that previously provided by absorption spectroscopy with linearly polarized light. A preferential orientation angle with respect to the normal to the clay layer of 62° is derived for R6G monomers adsorbed in Lap films.

KEY WORDS: Dye adsorption; clay thin; anisotropy; fluorescent polarization .

INTRODUCTION

The incorporation of fluorescence organic dyes in rigid ordered inorganic materials is of great technological interest in the design of new hybrid host–guest systems with specific photofunctional responses [1–4]. These hybrid materials can provide interesting properties as non-linear optic phenomena, with interesting applications, for instance, as frequency doublers, memory devices, dichroic filters and so on [5–7].

In this sense, ordered layered host materials can supply two-dimensional arrangements for fluorescent molecules. There are a huge amount of inorganic layered materials (double hydroxides, zirconium phosphate, transition metal oxides, clays. . .) which are good candidates for the accommodation of fluorophores in their nanoscale interlayer space by a cation exchange mechanism. In this sense, clay minerals stand out due to their high adsorption capacity and low cost. These laminar minerals possess

a neat negative charge layers due to isomorphous substitutions of their structural ions in the tetrahedral (Si^{4+}) and/or octahedral (Al^{3+} , Mg^{2+}) sheets (see Fig. 1) by others with a lower oxidation state, which is compensated by exchangeable inorganic cations. Smectite-type clays, with a moderate negative charge, offer an optimum adsorption ability [8]. In this work Laponite (Lap) clay is employed, a synthetic smectite with a very small particle size (< 30 nm) to reduce the scattering and obtain stable aqueous suspensions and high purity and low content of iron (quencher of fluorescence of many aromatic compounds). Transparent and organized supported thin films of Lap were obtained by the spin-coating technique with a parallel distribution of their layer along the substrate [9].

Rhodamine 6G (R6G, Fig. 1), probably the most-used laser dye, has been successfully adsorbed in films of Laponite (Lap) lamellar-clay mineral. The intercalation is carried out by immersing the clay films in adequate dye solutions, and the dye loading is controlled by the immersing time, thickness of the film and concentration of the dye solution. Depending on the loading of the dye, new absorption and fluorescence bands of R6G have been observed [10, 11]. In this study, a very diluted dye concentration in the Lap films is used to ensure the lack

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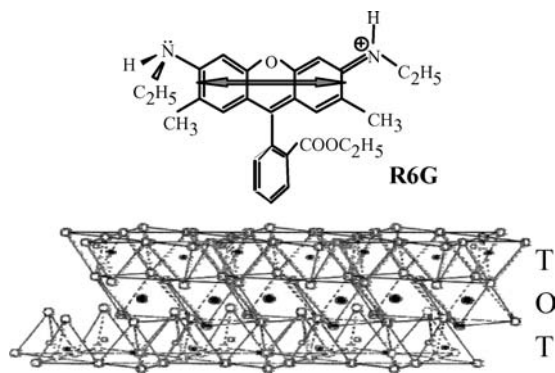


Fig. 1. Molecular structure of Rhodamine 6G and layer structure of smectite-type clays.

of aggregates and only the adsorption of R6G monomers in the interlayer space of the clay.

A preferential orientation of the R6G molecules adsorbed in organized Lap films will lead to a photoresponse of the dye/clay system to the linearly-polarized light. Indeed, R6G/Lap films reveal an anisotropic behavior with respect to the normal to the substrate [12]. In the present contribution a new fluorescent method is applied to evaluate the preferential orientation of R6G molecules adsorbed on Lap films [13]. The new method is based on the recording of the fluorescence spectra with the emission polarizer oriented in both horizontal and vertical directions as a function of the twisted δ angle between the normal of the film and the incident beam, maintaining the polarization direction of the excitation constant (horizontal or vertical orientations). From the evolution of the fluorescence dichroic ratio with the twisting angle δ of the sample, the tilted ψ angle between the transition moment of the dye and the normal to the Lap layer can be evaluated.

EXPERIMENTAL

Rhodamine 6G (R6G, Laser Grade) was supplied by Kodak and was used as received. The sodium form of Laponite B (Lap) was purchased by 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.

Supported thin films of Lap on glass substrates were obtained by the spin-coating technique from a 2% in weight aqueous suspension of the clay stirring during 2 days. Spinning conditions were 2500 rpm during 60 s. The thickness of the films was 450 nm at the centre of the film and 250 nm at the edge of the substrate, after

accumulation of two spinning procedures on the same substrate. These thicknesses were evaluated by Atomic Force Microscopy (AFM), recorded by the depression of the cantilever passing through the artificial groove performed in the Lap films [9].

The incorporation of R6G molecules in Lap films was performed by immersing the clay film into a solution of the dye in a water/ethanol mixture with 0.8 molar fraction in water. The intercalation of the dye in the interlayer space of Lap was checked by X-ray diffraction [9]. The dye loading was controlled by the concentration of the dye solution and the immersion time. A Lap film with a very low loading of R6G molecules was obtained after immersion in a 10^{-6} M solution of R6G during 5 min. After washing with ethanol and drying, the relative dye/clay concentration, expressed as the % of R6G molecules interchanged over the total cations exchange capacity of Lap (CEC, 76.3 meq/100 g [14]), for this sample was estimated around 0.1%CEC [10]. The sample was kept 2 days in the dark in order to get a good distribution of adsorbed molecules through the clay surface.

The linearly polarized fluorescence spectra of this sample were recorded after excitation at 495 nm with polarized light in a SPEX Fluorolog 3–22 fluorimeter using Glan-Thompson polarizers in the excitation and detection channels. The fluorescence spectra were registered for two orthogonal orientations of the emission polarizer (horizontal and vertical directions) keeping the excitation polarizer fixed. For comparison, two different fluorescence polarization measurements were performed: for horizontally polarized excitation light and for vertically polarized light. To correct the instrumental response to the plane of the polarized light, the isotropic G factor was experimentally obtained by recording the fluorescence spectra of an isotropic system in identical instrumental conditions: for instance, by means of a 10^{-5} M solution of R6G in ethanol using a 1 mm optical pathway cell.

RESULTS AND DISCUSSION

The spin-coating procedure is an adequate technique to elaborate supported films with an organized and parallel distribution of Lap layers through the plane of the substrate [9]. The AFM images provide a homogeneous surface morphology of thin Lap films obtained by this technique. Moreover, the clear d_{001} XRD peak observed for the pure Lap films confirms the good arrangement of the clay layers in parallel planes. The immersion of the clay films into R6G liquid solutions allows the intercalation of the dye molecules in the interlayer space of the Lap films [9]. Increasing the dye content (e.g., increasing

the immersion time and/or the dye concentration), the aggregation of R6G in Lap films is favored, and monomers, dimers and higher order aggregates of R6G in the interlayer space of Lap films have been characterized by UV/Vis absorption and fluorescence spectroscopy with unpolarized light [10, 11]. For the very low dye loading 0.1%CEC film used in the present work, R6G molecules are adsorbed as individual units in the interlayer space of Lap films.

The preferential orientation of R6G molecules adsorbed in macroscopically organized Lap layers (such as the films elaborated by the spin-coating technique) provides a photoactive anisotropic system able to respond to the plane of the linearly polarized light. Indeed, the application of absorption spectroscopy with linearly polarized light to the 0.1%CEC R6G/Lap film has demonstrated the anisotropy response along the normal to the film [12]. This is a direct consequence of the preferential orientation of the R6G monomer units with respect to the perpendicular of the Lap layers. The clay plane itself is an isotropic system because there is not a preferential orientation in this plane, and the 0.1%CEC R6G/Lap sample does not show any anisotropy in the film plane [12].

Now the fluorescence spectroscopy with linearly polarized light is applied to demonstrate the anisotropic response of the 0.1%CEC R6G/Lap film, and to evaluate the preferential orientation of R6G monomers with respect to the normal to the Lap layers [13]. The fluorescence technique presents certain advantages with respect to absorption spectroscopy, such as the higher sensibility and selectivity of the former technique. Apart from the possibility to chose two working wavelengths, fluorescence polarization allows the use of two different polarizers (excitation and emission), which allows multiple combinations.

The experimental setup used in this work is illustrated in Fig. 2. The fluorescence spectra were recorded in the front-face configuration, by detecting the fluorescence intensity of the sample at 22.5° (emission Z' -axis) with respect to the excitation beam (excitation Z -axis). The emission polarizer was oriented in both the horizontal (H , along the emission X' -axis) and the vertical (V , along the Y' -axis) direction, and the corresponding fluorescence spectra were recorded as a function of the twisting angle δ of the sample along its vertical y -axis. This implies the modification of the relative orientation of the film normal with respect to the excitation and detection channels. The δ angle, defined as the angle between the normal to the film and the excitation beam, was changed from -20° (normal to the film oriented close to the detection channel) to 50° (72.5° between the normal to film and the detection channel), Fig. 2. The fluorescence polarization was recorded after excitation with linearly polarized light,

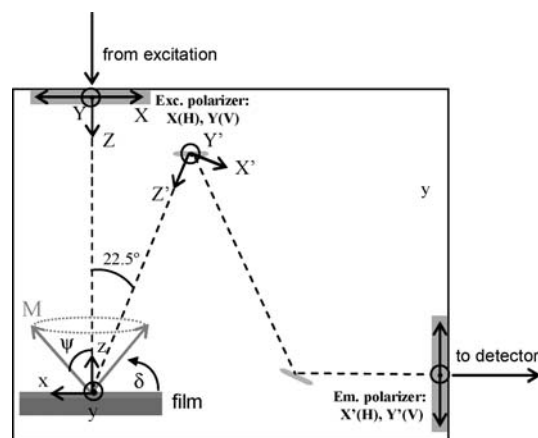


Fig. 2. Top view of the experimental setup used in the present work. Excitation axis: XYZ ; detection axis: $X'Y'Z'$; and sample axis: xyz . The emission intensity was recorded in the front-face configuration at 22.5° with respect to the excitation beam and the sample was twisted a δ angle around its y -axis.

by orienting the excitation polarizer to the horizontal (H , along the excitation X -axis) and the vertical (V , along the Y -axis). Therefore, four different fluorescence polarization setups were performed: I_{HH} , I_{HV} , I_{VH} , and I_{VV} , where the first and second subindexes mean the orientation of the excitation and emission polarizers, respectively.

Figure 3 shows the fluorescence spectra of 0.1%CEC R6G/Lap films with the excitation and/or emission polarizers in the H and V directions (I_{HH} , I_{HV} , I_{VH} , and I_{VV}) as a function of the twisting δ angle of the films. These spectra were corrected for the instrumental response to the polarized light and the changes in the optical pathway of the excitation and the detected emission beams with the twisting motion by means of emission spectra for an isotropic system (a liquid solution of R6G in a 1 mm pathway cell) recorded in identical conditions (I_{HH}^{iso} , I_{HV}^{iso} , I_{VH}^{iso} , and I_{VV}^{iso}). These corrections were performed considering the ratio between the fluorescence spectrum of the isotropic system recorded at any twisting angle of the sample δ and any orientation (H and V) of the emission polarizer over that recorded at $\delta = 0^\circ$ and with the emission polarizer parallelly oriented with respect to that of the excitation polarized, i.e., $I_{UV}^{iso}(\delta)/I_{UV}^{iso}(0)$ with $U = H$ or V and $W = H$ and V .

In general, the shape of the recorded emission spectra of the 0.1%CEC R6G/Lap film (Fig. 3) does not change with the twisting angle δ , suggesting the emission from a R6G species, the monomer in this diluted dye/clay sample. However, the fluorescence intensity changes with the twisting angle δ , and these changes depend on the configuration of the excitation and emission polarizers. For a

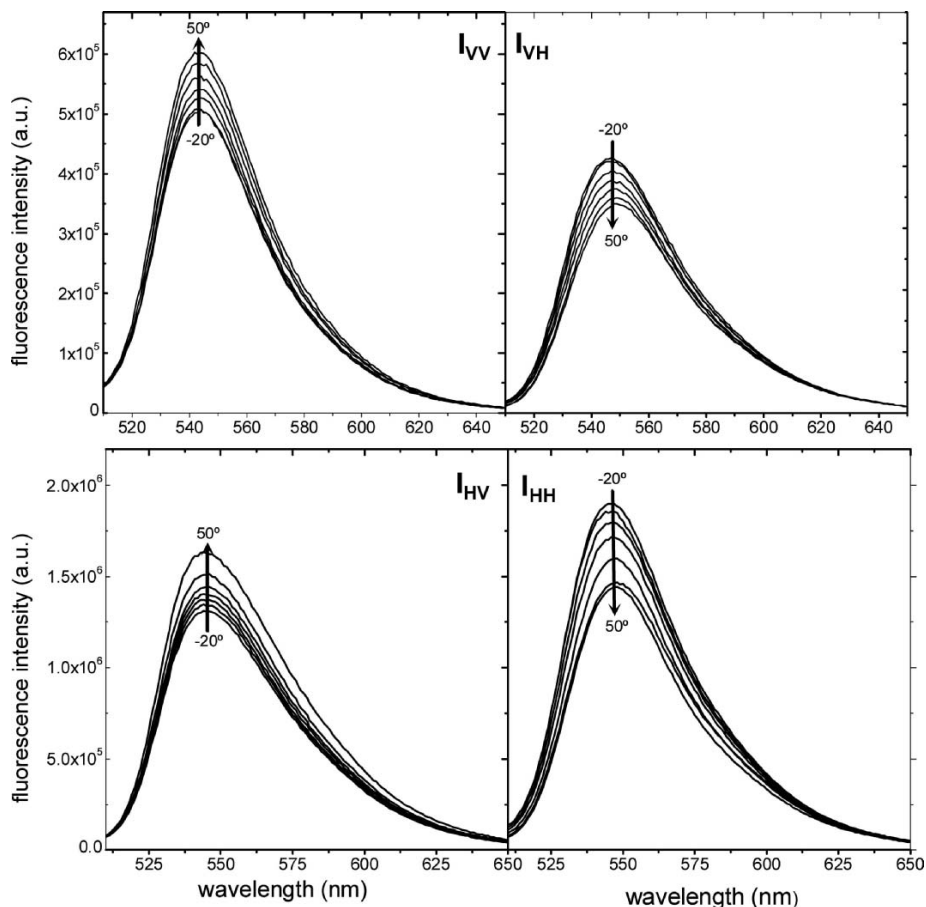


Fig. 3. Fluorescence spectra (after excitation at 495 nm) of 0.1%CEC R6G/Lap film with the emission polarizer vertically (*left*) and horizontally (*right*) oriented with horizontal (*bottom*) and vertical (*top*) excitation. The spectra were corrected for the response of the instrument to the polarized light by means of the fluorescence spectra recorded for a isotropic system.

fixed excitation polarization, an opposite tendency in the evolution of the fluorescence intensity with the twisting δ angle is observed for the emission polarizer oriented in two orthogonal directions (horizontal, *H* right part of Fig. 3, and vertical, *V* left part). This is observed when the excitation polarizer is kept constant in both the horizontal (*H*, bottom part of Fig. 3) or the vertical (*V*, top part) direction.

These opposite tendencies evidence the anisotropic behavior of R6G/Lap films along the direction of the normal to the clay surfaces by fluorescence technique, corroborating previous conclusions derived from absorption spectra with polarized light [12]. From these evolutions, the orientation ψ angle of the transition moment (along the long molecular axis of the dye) of R6G monomers with respect to the normal to the Lap film can be evaluated [13]. Experimental data confirm that the recorded fluorescence

intensity with parallel excitation and emission polarizer is higher than the corresponding perpendicular configuration, i.e., $I_{HH} > I_{HV}$ and $I_{VH} < I_{VV}$. This effect will be corrected later by means of the isotropic *G* factor.

According to the experimental setup shown in Fig. 2, after excitation with horizontally ($H = X$ -axis) and vertically ($V = Y$ -axis) polarized light, the fluorescence intensities recorded with the emission polarizer oriented in the horizontal ($H = X'$ -axis) and vertical ($V = Y'$ -axis) directions are given by

$$I_{VH} \propto I_{\text{abs}}(Y) \cdot k_{\text{flu}}(X') \quad (1)$$

$$I_{VV} \propto I_{\text{abs}}(Y) \cdot k_{\text{flu}}(Y') \quad (2)$$

$$I_{HH} \propto I_{\text{abs}}(X) \cdot k_{\text{flu}}(X') \quad (3)$$

$$I_{HH} \propto I_{\text{abs}}(X) \cdot k_{\text{flu}}(X') \quad (4)$$

where $I_{\text{abs}}(U)$ is the absorption intensity for vertically ($U = X$ -axis) and horizontally ($U = Y$ -axis) polarized excitation light at the excitation wavelength, and $k_{\text{flu}}(W)$ is the radiative rate constant for the vertical ($W = X'$ -axis) and the horizontal ($W = Y'$ -axis) fluorescence polarization. The fluorescence dichroic ratio, defined as the ratio between the fluorescence intensity of two orthogonal polarizations for a constant excitation polarization, i.e., $D_{HV} \equiv I_{VH}/I_{VV} \equiv I_{HH}/I_{HV}$, can be simplified to

$$D_{HV} = k_{\text{flu}}(X')/k_{\text{flu}}(Y') \quad (5)$$

On the other hand, k_{flu} is proportional to the square of the transition dipole moment (M), and for a given polarization orientation, it is proportional to the square of the projection of M in that direction. Therefore, $k_{\text{flu}}(X')$ and $k_{\text{flu}}(Y')$ are proportional to the square of the projection of M in the X' - and Y' -axes, $M_{X'}^2$ and $M_{Y'}^2$, respectively. In order to obtain an expression relating the dichroic ratio and the emission transition moment of the fluorophore, it is necessary to align the x , y , and z axes of the sample with the X' , Y' , and Z' of the detection coordinates.

$$k_{\text{flu}}(X') \propto M_{X'}^2 = S_H [M_x \cos(xX') + M_y \cos(yX') + M_z \cos(zX')]^2 \quad (6)$$

$$k_{\text{flu}}(Y') \propto M_{Y'}^2 = S_V [M_x \cos(xY') + M_y \cos(yY') + M_z \cos(zY')]^2 \quad (7)$$

where S_H and S_V are the sensitivities of the emission channel for the vertically and horizontally polarizer components, respectively. The angles between (x, y, z) and (X', Y', Z') -axes are related with the α , β , and γ Euler angles [15–17], which for the present setup conditions take the values: $\alpha = \gamma = 0^\circ$ and $\beta = 180 - (22.5 + \delta)^\circ$ where 22.5° is the angle between the excitation and the emission beams in the front-face configuration of the Fluorolog 3–22 SPEX fluorimeter used in the present study. Then, the director cosines take the values [15–17]:

$$\begin{aligned} \cos(xX') &= \cos \alpha \cos \beta \cos \gamma - \sin \alpha \sin \gamma = \\ &= -\cos(\delta + 22.5) \end{aligned} \quad (8)$$

$$\cos(yX') = -\sin \alpha \cos \gamma - \cos \alpha \cos \beta \sin \gamma = 0 \quad (9)$$

$$\cos(zX') = \cos \alpha \sin \beta = \sin(\delta + 22.5) \quad (10)$$

$$\cos(xY') = \cos \alpha \sin \gamma + \sin \alpha \cos \beta \cos \gamma = 0 \quad (11)$$

$$\cos(yY') = \cos \alpha \cos \gamma - \sin \alpha \cos \beta \sin \gamma = 1 \quad (12)$$

$$\cos(zY') = \sin \alpha \sin \beta = 0 \quad (13)$$

On the other hand, the x , y , and z components of M (M_x , M_y , and M_z) are related with the polar coordinates M , ψ , and ϕ (Fig. 4)

$$M_x = M \sin \psi \cos \phi \quad (14)$$

$$M_y = M \sin \psi \sin \phi \quad (15)$$

$$M_z = M \cos \psi \quad (16)$$

ψ defines the angle between the transition moment M and the normal to the film (z) and ϕ is the angle formed by the projection of M into the xy -plane (M_{xy}) and the x -axis. Applying Eqs. (8)–(16) to Eqs. (6) and (7):

$$k_{\text{flu}}(X') = S_V M^2 [\sin \psi \cos \phi \cos(22.5 + \delta) + \cos \psi \sin(22.5 + \delta)]^2 \quad (17)$$

$$k_{\text{flu}}(Y') = S_H M^2 [\sin \psi \sin \phi]^2 \quad (18)$$

Because the xy plane of the clay layers is an isotropic system, the adsorbed fluorophores have a preferential orientation with respect to the z -axis of the film. Then, the ψ angle actually represents the preferential orientation of the fluorescent molecules adsorbed into the film, whereas the ϕ angle can take any value between 0 and 2π . So, the

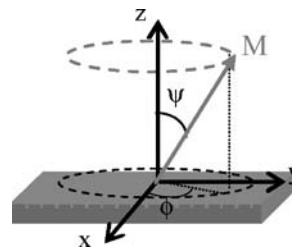


Fig. 4. Polar coordinated of the transition moment (M) with respect to the xyz coordinates of the clay layer.

average values in all these functions dependent on the ϕ parameter have to be considered. For instance,

$$\overline{\sin \phi} = \overline{\cos \phi} = \int_0^{2\pi} \cos \phi d\phi = 0 \quad (19)$$

$$\overline{\sin^2 \phi} = \overline{\cos^2 \phi} = \int_0^{2\pi} \cos^2 \phi d\phi = 1/2 \quad (20)$$

Applying Eqs. (19) and (20) to Eqs. (17) and (18):

$$k_{\text{flu}}(X') = S_V M^2 [1/2 \sin^2 \psi \cos^2(22.5 + \delta) + \cos^2 \psi \sin^2(22.5 + \delta)] \quad (21)$$

$$k_{\text{flu}}(Y') = S_H M^2 [1/2 \sin^2 \psi] \quad (22)$$

Therefore, the fluorescent dichroic ratio becomes

$$D_{HV} = \frac{I_{VH}}{I_{VV}} = \frac{S_H}{S_V} \frac{1/2 M^2 \sin^2 \psi \cos^2(22.5 + \delta) + M^2 \cos^2 \psi \sin^2(22.5 + \delta)}{1/2 M^2 \sin^2 \psi} \quad (23)$$

The first term is related to the different sensibilities of the instrumentation to the H - and V -polarizations. To correct these effects, the fluorescence intensity of an isotropic system (i.e., a R6G liquid solution) has to be recorded for H and V polarization, I_{HH}^{iso} and I_{HV}^{iso} or I_{VH}^{iso} and I_{VV}^{iso} . The so-called isotropic G factor can be calculated from these experimental data, $G \equiv I_{HV}^{\text{iso}}/I_{HH}^{\text{iso}} \equiv I_{VV}^{\text{iso}}/I_{VH}^{\text{iso}} = S_V/S_H$. However, two isotropic G factors, $G_H \equiv I_{HV}^{\text{iso}}/I_{HH}^{\text{iso}}$ and $G_V \equiv I_{VV}^{\text{iso}}/I_{VH}^{\text{iso}}$, must be distinguished because the recorded emission intensity depends on the parallel or perpendicular configuration of the excitation and emission polarizers. For instance, the parallel configuration is in the numerator for vertically polarized excitation (I_{VV}^{iso}) and in the denominator for horizontally polarized excitation (I_{HH}^{iso}). The isotropic factor must be recorded for all the orientations of the sample compartment (δ angle from -20 to 50°) in order to also correct the variation of the instrumental excitation and fluorescence signals with the sample arrangement.

Finally, the corrected dichroic ratio of the sample is related to the preferential orientation of the fluorophore ψ angle by means of:

$$D_{HV}^{\text{cor}} = \frac{I_{VH}}{I_{VV}} G_V = \frac{I_{HH}}{I_{HV}} G_H = 2 \cot^2 \psi + (1 - 2 \cot^2 \psi) \cos^2(22.5 + \delta) \quad (24)$$

From the linear relationship between D_{HV}^{cor} and $\cos^2(22.5 + \delta)$, the tilted ψ angle can be evaluated according to Eq. (24).

Because the fluorescence spectra of Fig. 3 are corrected for the instrumental response to the polarized light, the fluorescence dichroic ratio of the 0.1%CEC R6G/Lap films can be directly determined by the I_{VH}/I_{VV} or the I_{HH}/I_{HV} relations. However, in the last case the I_{HH}/I_{HV} ratio had to be corrected by the $I_{VV}^{\text{iso}}(0)/I_{VH}^{\text{iso}}(0) \times I_{HV}^{\text{iso}}(0)/I_{HH}^{\text{iso}}(0)$ term in order correct the higher recorded intensity of the parallel configuration of both excitation and emission polarizer in the numerator. Figure 5 shows the evolution of D_{HV}^{cor} with the emission wavelength as a function of the twisting angle δ of the sample. The D_{HV}^{cor} values have been obtained from both horizontal (top part of Fig. 5) and vertical (bottom part, Fig. 5) excitation. Except at shorter wavelengths, both dichroic ratios are wavelength independent for a given twisting angle δ . These results corroborate previous conclusions related to the exclusive presence of monomers of R6G species in the adsorption surfaces of the Lap film at this very low dye loading sample. However, at shorter wavelengths the D_{HV}^{cor} ratio obtained after excitation with horizontally polarized light increases as the emission wavelength is decreased (Fig. 5(top)), just the opposite effect observed for the fluorescence dichroic ratio obtained after vertically polarized excitation (Fig. 5(bottom)).

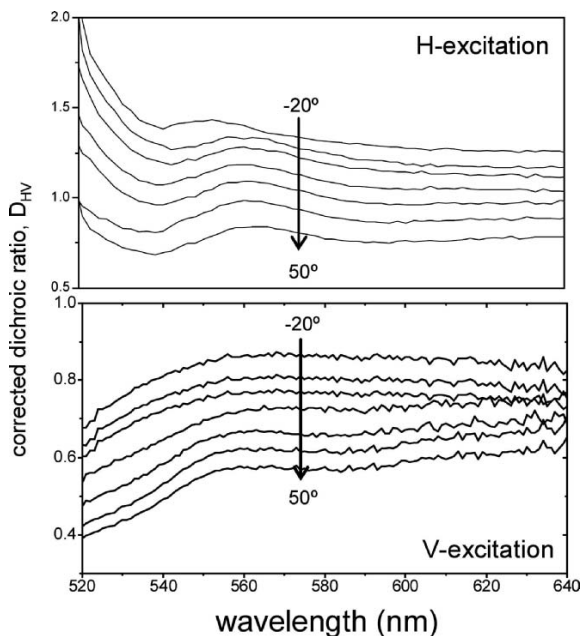


Fig. 5. Fluorescence dichroic ratio (corrected for the instrumental isotropic response) of the 0.1% CEC R6G/Lap film for horizontally (top) and vertically (bottom) polarized excitation as a function of the twisted angle δ of the sample.

These observations can be interpreted in terms of the polarization of the scattering light detected at those emission wavelengths close to the excitation wavelength (495 nm). Indeed, the scattering of the excitation light is very important in solid thin films of clays and can affect the detected fluorescence signal of dyes at those wavelengths close to the excitation. Since the scattering saves the polarization of the excitation light, it will affect the fluorescence polarization of the sample at shorter wavelengths. Thus, because the highest light scattering is detected for parallel (VV or HH) configuration of the excitation and emission polarizers, it increases the dichroic ratio for horizontally polarized excitation, $D_{HV}^{cor} = I_{HH}/I_{HV}$ where the parallel HH configuration is in the numerator, whereas the opposite behavior is expected in the vertically polarized light, where the parallel component VV is in the denominator of the dichroic ratio, $D_{HV}^{cor} = I_{VH}/I_{VV}$. The effect of the light scattering cannot be totally corrected by the isotropic system used in the present study (a liquid solution of R6G in a 1 mm quartz cell) because in this case the diffused light is much lower than that of Lap film. However, recording the isotropic signal of the light scattering (I^{sct}) by the pure Lap film (without dye) in identical experimental conditions, an extra correction of the effect of the polarization by light scattering is possible. This has been performed for horizontally polarized light, and the correction of the D_{HV}^{cor} ratio of Fig. 5 (top) by the $I_{HV}^{sct}/I_{HH}^{sct}$ term, D_{HV}^{rec} is illustrated in Fig. 6(bottom). As this figure suggests, this extra correction leads to a nearly constant D_{HV}^{rec} ratio through the complete wavelength range of the R6G emission, confirming the important influence of the light scattering in the determination of the fluorescence dichroic ratio, at least in dye/clay films.

Figure 6 (top) shows the linear relationship between the fluorescence dichroic ratio and the $\cos^2(22.5 + \delta)$ parameter for the 0.1%CEC R6G/Lap film. This linear relationship is observed for the D_{HV}^{cor} ratio obtained after the excitation with both horizontally (right) and vertically (left) polarized excitation and it is independent of the emission wavelength. The data shown in Fig. 6 (top) were obtained not exactly at the emission peak but rather at a longer wavelength in order to reduce any reminiscent influence of the excitation light scattering. The tilted angle ψ of the transition moment of R6G monomers adsorbed in Lap obtained from both the intercept and the slope of these relationships are listed in Table I. The average ψ values of 62° derived from this fluorescence polarization method perfectly agrees with that previously obtained by absorption spectroscopy with linearly polarized light [12], confirming the validity of the fluorescence polarization method to evaluate preferential orientation of fluorescent dye molecules adsorbed in 2D layered materials [13].

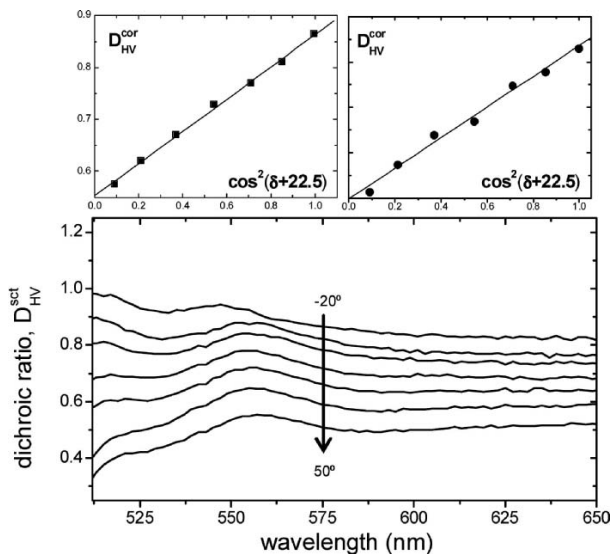


Fig. 6. Fluorescence dichroic ratio (corrected for the instrumental isotropic response and the scattering of the film) of the 0.1%CEC R6G/Lap film for horizontal excitation (bottom). Top: linear correlation of D_{HV}^{cor} vs $\cos^2(22.5+\delta)$ of 0.1%CEC R6G/Lap films obtained at the emission wavelength of 580 nm after excitation at 495 nm with vertical (left) and horizontal (right) polarization.

As is discussed above, the evaluation of the orientation of dye molecules in organized layered materials by fluorescence polarization is independent of the direction of the excitation polarization. However, with the experimental setup, the Spex Fluorolog 3–22 fluorimeter and the Glan-Thomson polarizers used in the present paper, the recorded emission intensity is higher for the horizontal than for the vertical polarization excitation (see ordinate scale in Fig. 3). Consequently, more sensitive signals are obtained under this configuration. This is reflected, for instance, in the D_{HV} data where a clearer D_{HV} vs emission wavelength distribution is observed for horizontally polar-

Table I Orientation angle ψ of R6G monomer with respect to the layer normal of Lap films obtained by different spectroscopies with polarized light

Absorption polarization [12]	Fluorescence polarization	
	Vertical excitation [13]	Horizontal excitation
62°	$62^{\circ a}$	$61^{\circ b}$

^aFrom the intercept of Eq. (24)

^bFrom the slope of Eq. (24)

ized excitation (Fig. 5(top)) while the vertical excitation results in a greater level of noise (Fig. 5(bottom)).

CONCLUSIONS

Fluorescence polarization is an adequate method to evaluate the angle of the preferential orientation of R6G monomers adsorbed in the interlayer space of organized thin films of Lap. The derived linear relationship between the fluorescence dichroic ratio (obtained from the emission polarizer horizontally and vertically oriented) with the twisting angle δ of the sample around its vertical axis allows the evaluation of the tilted angle of R6G monomers adsorbed in the surfaces of Lap films. The obtained preferential adsorption angle, around 62° , is independent of the direction of the excitation polarizer and agrees with the values previously determined from absorption with polarized light, justifying the validity of the present fluorescence method. The experimental conditions to determine the fluorescence dichroic ratio have to be adequately selected in order to avoid other phenomena affecting the fluorescence polarization of the dye. The new method can be used to evaluate the preferential orientation of fluorescent molecules in 2D layered materials [13,18,19].

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