Orientation of Adsorbed Dyes in the Interlayer Space of Clays. 2 Fluorescence Polarization of Rhodamine 6G in Laponite Films

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A new fluorescence polarization method is applied to evaluate the preferential orientation of rhodamine 6G (R6G) adsorbed into laponite (Lap) clay mineral in supported films. For this purpose, the fluorescence spectra for the horizontal and vertical directions of the emission polarizer were recorded for a common horizontally polarized excitation light as a function of the twist angle δ of the film with respect to the excitation (and emission) beam. The orientation of the fluorescent species adsorbed in the clay films can be evaluated from the linear relationship between the fluorescence dichroic ratio and the twist angle δ recently established.[1,2] For low dye-loading R6G, molecules are adsorbed into the Lap surface as monomeric units with a tilt ψ angle of 28° with respect to the clay layer, in agreement with results obtained by visible absorption with polarized light. Fluorescent coplanar J type dimers of R6G are also observed in Lap films for moderate dye loadings with a similar orientation angle around 30°. The presence of coplanar R6G H type dimers and high-order aggregates in moderate- and high-loading R6G/Lap films causes a depolarization for the fluorescence anisotropy of the J dimer, ascribed to an excitation energy-transfer process and reducing the applicability of this fluorescence method. However, present experimental results confirm a more-perpendicular disposition of these H aggregates with respect to the Lap layer. The new fluorescence polarization method can be extrapolated to determine the orientation of any adsorbed fluorophore in rigid and ordered 2D host materials.

Introduction

Nowadays, many scientific efforts are focused on the construction of ordered supramolecular assemblies for the development of photonic devices with interesting applications such as antenna systems, optical storage devices, dichroic filters, frequency doubling, waveguides, etc.[3−17] Most of these systems are based on the procedure of embedding photofunctional molecules, chromophores, and fluorophores into host materials.

Many supporting materials are considered for this type of use, including organic, inorganic, and more recently organo−inorganic hybrid matrixes.[11,12,16−23] Inorganic frameworks can provide one-, two- and three-dimensional architectures for the guest molecules. In this sense, clay minerals, with a lamellar structure, offer a bidimensional space for intercalating photoactive molecules. Clay minerals are characterized by their high capacity for adsorbing a multitude of organic and inorganic compounds, their high area/volume ratio (because of their layered nature), and their cation-exchange capacity (CEC).[24,25]

Most of the photonic and optoelectronic applications of photofunctional host/guest materials require the development of macroscopic ordered arrangements. Many efforts are focused on producing oriented guest arrangements, such as by applying electric/magnetic fields, covalent anchoring of guest molecules in self-organized matrixes, or using liquid crystals as host systems.[26−33] In this sense, a parallel macroscopic distribution of clay layers can be obtained by

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elaborating supported thin films on glass substrates by the spin-coating technique.[34] The incorporation of cationic dyes into these ordered clay assemblies directly achieves a homogeneous and ordered distribution of the photoactive molecules on a macroscopic scale. This spontaneous self-orientation of the guest molecules is mainly governed by dye−dye and dye−clay interactions.[3,7,18,35]

In a previous paper,[36] the orientation of rhodamine 6G (R6G) intercalated into ordered laponite clay (Lap) films was studied by visible absorption spectroscopy with linear polarized light at different dye loadings. The preferential orientation of the dye molecules depends on the dye concentration. For very diluted R6G/Lap films (i.e., 0.1% CEC), the long molecular axes of R6G monomers are disposed at a tilt angle of 62° with respect to the normal to the film.[36] But in moderate dye loadings (1−22% CEC), the dye aggregation leads to two different coplanar type dimers: a long displaced coplanar dimer, with a tilt angle of around 61° with respect to the layer normal, was identified as a J type dimer with an absorption band placed at lower energies than that of the monomer band; and a short displaced coplanar H type dimer, with a more tilted angle with respect to the clay surface (around 48−46°), was characterized by an absorption band at higher energies. For high dye loadings (>40% CEC), more-perpendicular coplanar higher-order aggregates with tilt angles around 30° with respect to the clay layer were characterized by hypsochromic absorption bands.

More recently,[1,2] a new fluorescence polarization method was developed for evaluating the preferential orientation of the R6G monomers in Lap layers. This method is based on the different responses of the fluorescence spectra of the sample to the horizontal (I_{VH}) and vertical direction (I_{VV}) of the emission polarizer (keeping the excitation polarizer constant in the vertical direction) for different orientations of the sample with respect to the excitation beam (the normal to the film was twisted at a δ angle). A linear relationship between the fluorescence dichroic ratio (D_{HV} defined as the ratio of H and V polarized emission spectra, $D_{HV} \equiv I_{VH}/I_{VV}$) and the twist angle δ was established by means of

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

From the corresponding slope and/or intercept, the relative orientation of the transition moment of the dye and the normal to the clay layer (defined by a ψ angle) can be evaluated. The validity of eq 1 was check for a diluted 0.1% CEC R6G/Lap film,[1,2] and a preferential orientation of R6G monomers of 62° with respect to the Lap normal was obtained. This angle agrees with that of 62° determined by visible absorption with linearly-polarized light.[36]

Equation 1 was derived in the absence of any depolarization phenomena affecting the recorded anisotropy behavior in the fluorescence.[37−40] In this sense, the following aspects are required in the molecular system for eq 1 to be applicable: parallel orientation between the absorption and fluorescence transition dipole moments of the fluorophore; absence of rotational motions of adsorbed fluorophores during their fluorescence lifetime; the absence of any excitation energy migration and/or transfer processes or any reabsorption/reemission phenomena, which could induce a change in the orientation of the transition moment from the excited state directly populated in the excitation process to the fluorescence excited state of the recorded emission intensity.

None of these phenomena are present for the 0.1% CEC R6G/Lap film.[1,2] The main absorption band and the fluorescence emission of R6G involve common S_0 and S_1 singlet states. Indeed, both transitions are oriented along the long molecular axis, as quantum mechanic calculations suggest, and it is illustrated in the molecular structure of the dye (see Chart 1). On the other hand, the reorientation of the adsorbed R6G molecules during their fluorescence can be ruled out, as the time-correlated fluorescence decay curves of R6G/Lap films do not exhibit any dependence on the orientation of the emission polarizer after excitation with vertically polarized pulses.[41] The absence of time-dependent fluorescence anisotropy reveals a static adsorption of the dye molecules in the time scale of the fluorescence lifetime (around a nanosecond). Finally, bimolecular processes causing fluorescence depolarization are neglectable in the dye diluted sample of the 0.1% CEC R6G/Lap film, in which only monomeric species of the dye are adsorbed.[41,42] In the present paper, the fluorescence polarization method is applied to evaluate the preferential orientation of monomers and different aggregates of R6G adsorbed in the interlayer space of Lap films. For this purpose, the fluorescence anisotropy of R6G/Lap films with different dye loadings, from the most-diluted 0.1% CEC (R6G monomer) to the dye-saturated 60% CEC (high-order aggregates) films, are checked by recording the emission spectra with horizontal and vertical polarization with respect to a common horizontally polarized excitation light. Because of the high dye content and the presence of

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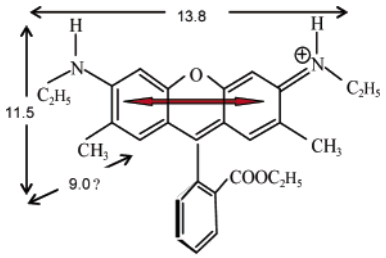
Chart 1. Molecular Structure of R6G



Molecular Structure

[a] The transition moment along the long molecular axis of the xanthene ring is also included.

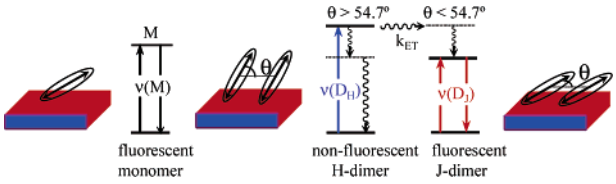


Figure 1. Photophysical characteristics of short ($\theta > 54.7°$) and long ($\theta < 54.7°$) displaced coplanar dimers obtained from the exciton theory.[43,44]

several absorbing and fluorescent species of some of the R6G/Lap samples, bimolecular processes such as energy migration/transfer can occur during the fluorescence lifetime of the adsorbed species, leading to fluorescence depolarization phenomena affecting the validity of eq 1.

The absorption and fluorescence properties of R6G monomer and dimers adsorbed in the Lap films, derived from the exciton theory[43,44] and confirmed by experimental results with unpolarized light,[41,42] are illustrated in Figure 1. R6G monomers adsorbed in the Lap surface have common lowest-energy absorption and fluorescence bands with maximal intensities at 528 and 548 nm, respectively.[41,42] The short displaced coplanar R6G dimer with a tilt angle $\theta > 54.7°$, characterized by an absorption H band centered at 503 nm,[42] is nonfluorescent, because the radiative transition from the lowest excited state to the ground state is forbidden.[43,44] Generally, this dimer is an efficient quencher for the fluorescence emission of monomers. Finally, long displaced coplanar R6G dimers with a tilt angle $\theta < 54.7°$ have active absorption and fluorescence J bands centered at 545 and ~580 nm, respectively.[36,41,42] Although the transition to the second excited state (shorter wavelengths) of this aggregate is spectroscopically forbidden, fluorescence emission of this J type aggregate can still be recorded after excitation of R6G/Lap films at short wavelengths (e.g., 495 nm) because the fluorescence excited state of the J type dimer can be populated via a quenching process mechanism from the excited state of monomers and/or H type aggregates directly populated in the excitation process at this wavelength (Figure 1).

Experimental Section

Rhodamine 6G dye (R6G, laser grade) and the sodium form of synthetic laponite clay (Lap) were supplied by Kodak and Laporte Industries, respectively, and were used as received.

Transparent and oriented thin films were obtained by a spin-coating procedure (2500 rpm, 60s) by applying some drops of a 2 wt % Lap aqueous suspension onto glass substrates. The incorporation of the dye (via an ion-exchange mechanism) was performed by immersing the clay films into R6G solutions in water/ethanol mixtures, with a water molar fraction of $x = 0.80$. The dye loading in the films was controlled by the immersion time (from a few minutes to 2 days) and the concentration of the dye solution (10^{-5} to 10^{-3} M). Samples with dye loading in the 0.1−60% range of the total cation-exchange capacity (CEC) of the Lap clay (77.3 meq/100 gr)[23] were considered in the present work. After the adsorption of the dye, the films were rinsed with ethanol and water and finally dried in an oven at 35 °C overnight. Further details of the preparation of R6G/Lap films and their characterization are described elsewhere.[34]

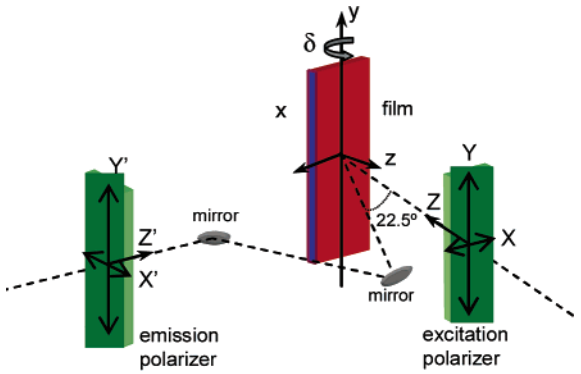


Figure 2. Three-dimensional perspective for the experimental setup to record fluorescence spectra with linear polarized light in the front-face configuration of a Fluorolog 3-22 fluorimeter. The twist angle δ of the sample with respect to the excitation beam is also illustrated.

Fluorescence spectra were recorded in a SPEX spectrofluorimeter (model Fluorolog 3-22), equipped with a double-monochromator in both excitation and emission channels. The emission spectra of the R6G/Lap films were collected in the front-face configuration, where the emission intensity was monitored at 22.5° with respect to the excitation beam (see Figure 2), after excitation at 495 nm and scanning the emission wavelength in the 510−670 nm range every 2 nm with a response time of 2 s nm^{-1}. The applied excitation and emission slits were 8 and 2 nm, respectively, except for the highest dye-loading samples (40 and 60% CEC), where the slits were increased to 10 and 3.5 nm, respectively, to improve the signal-to-noise ratio for these very low fluorescent samples.

Fluorescence polarization was studied by recording the fluorescence spectra with the emission polarizer horizontally (H, along the X' axis) and vertically (V, along the Y' axis) oriented, see Figure 2. The excitation polarizer was kept constant in the horizontal direction (X axis), and the fluorescence signals were recorded for different orientations of the films (i.e., the angle between the normal of the film, z-axis, and the excitation beam, Z axis) by twisting the sample compartment around its y axis at a δ angle from −20 to 50°. The instrumental response (optics, monochromator, detector, etc.) of the detection channel to the direction of the emission polarizer was corrected by considering the fluorescence spectra of an isotropic system for both H and V orientations of the emission polarizer, recorded in the same experimental conditions. In the present case, two liquid solutions, rhodamine 6G (10^{-5} M) in ethanol and pyrromethene 650 (2×10^{-5} M) in c-hexane with emission maxima at 550 and 600 nm (covering the whole emission range of this work), respectively, recorded in the front-face mode by means of a 1 mm pathway quartz cell were used as the isotropic system. The instrumental correction G factor is then obtained by

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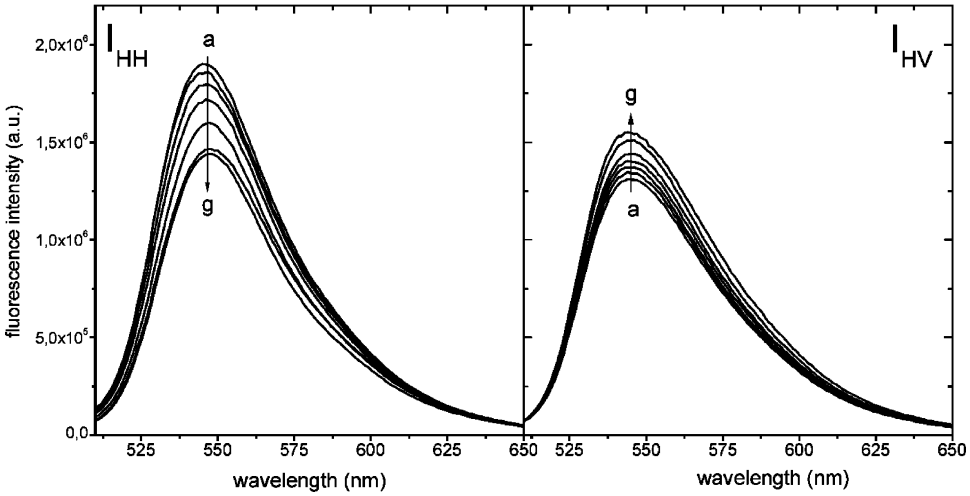

Figure 3. Evolution of the H (left) and V (right) polarized fluorescence spectra of the 0.1% CEC R6G/Lap film with the following twist angles δ of the sample: (a) −20, (b) 0, (c) 10, (d) 20, (e) 30, (f) 40, and (g) 50°. Spectra were recorded after excitation with horizontally polarized light.

the $(I_{HV}/I_{HH})^{iso}$ ratio, where I_{HV} and I_{HH} are the fluorescence intensity for V and H polarized emission light, respectively, for a common H polarized excitation light.

Results and Discussion

The fluorescence polarization is now applied to verify the anisotropic behavior of R6G molecules adsorbed in Lap layers and to evaluate the preferential orientation of the dye molecules in the interlayer space of Lap films. The fluorescence anisotropy is studied for four representative dye loadings in which different R6G species adsorbed in Lap films were previously characterized:[34,36,41,42] the 0.1% CEC sample, in which the adsorption of R6G molecules on the Lap film is performed mainly as monomeric units; the 5.7 and 22% CEC samples, in which different proportions of H and J type dimers of R6G coexist with monomers; and, finally, the saturated 60% CEC films, where higher-order aggregates of R6G adsorbed in Lap layers have been reported.

For this purpose, the emission spectra of R6G/Lap films have been recorded with the emission polarizer horizontally (H, along the X' axis) and vertically (V, along the Y' axis) oriented. This fluorescence anisotropy is carried out for a common horizontally (H, along the X axis) polarized excitation light, although it can be also performed using a vertically (Y) polarized excitation light.[1] These fluorescence spectra are called I_{HH} and I_{HV}, where the first and second subindex refers to the direction of the excitation and emission polarizers, respectively.

Experimental results in the present work evidence more intense fluorescence signals by using a horizontally polarized excitation beam in our fluorimeter. This improves the signal-to-noise ratio of the recorded spectra. Generally, in a right-angle configuration between the excitation and emission beams, the vertically polarized excitation is required. This is not the case for the present experimental setup, in which the front-face configuration of the fluorimeter implies a 22.5° angle (instead of the more extensively used right angle) between the excitation and detection directions. In this study, the horizontally polarized excitation light is recommended, because the fluorescence anisotropy was collected for different orientations of the samples with respect to the excitation beam by turning the solid sample holder around its vertical y axis (Figure 2). With this sample twisting direction and excitation with horizontally polarized light (X axis), the average orientation of the dipole moment of the dyes along the normal to the films (z axis) is changed with respect to both the excitation and emission polarizers. With this experimental arrangement, a higher anisotropic response is obtained.

Figure 3 shows the fluorescence spectra of the 0.1% CEC R6G/Lap film recorded with the emission polarizer in the H (I_{HH}) and V (I_{HV}) directions for different twist δ angles. These fluorescence spectra were corrected for the instrumental response to the emission H and V polarizer by considering the evolution of the fluorescence band of an isotropic system (a 10^{-5} M R6G solution in ethanol) with the twist angle δ recorded in identical conditions (front-face configuration in a 1 mm pathway cell). The fluorescence intensity for the emission H polarizer decreases by increasing the twist angle δ from −20 up to 50° (Figure 3, left), whereas an opposite behavior is observed for the V polarized emission light (Figure 3, right). This opposite evolution corroborates the fluorescence anisotropy behavior of R6G/Lap films, which is assigned to the preferential orientation of the R6G molecules (monomers in this very low dye content R6G/Lap film) adsorbed in Lap films.

The fluorescence anisotropy is analyzed by means of the dichroic parameter, D_{HV}, defined as the ratio between the H and V polarized emission intensity, i.e., $D_{HV} \equiv I_{HH}/I_{HV}$. Because of the intrinsic instrumental response to the plane of the polarized light, the D_{HV} parameter has to be corrected for the response of the detection channel to the H and V polarization, by means of $(D_{HV})^{cor} = I_{HH}/I_{HV} \times G$, where G is the instrumental G factor determined by the recorded fluorescence anisotropy of an isotropic system, $G \equiv (I_{HV}/I_{HH})^{iso}$. In this work, two liquid solutions of dyes covering the 520−670 nm emission range are used as the isotropy system (see the Experimental Section for further details).

The evolution of the fluorescence dichroic ratio with the emission wavelength of the 0.1% CEC R6G/Lap film for

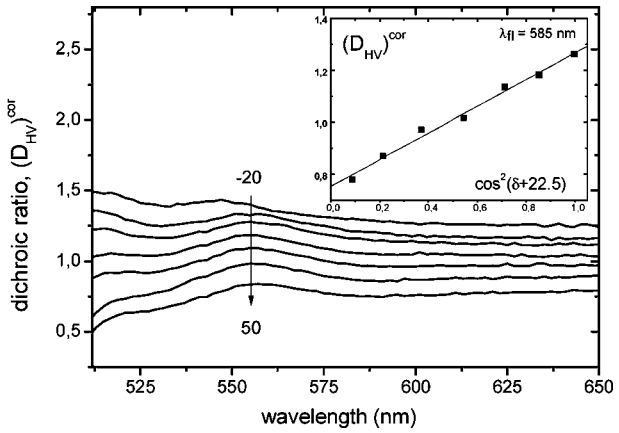

Figure 4. Evolution of the fluorescence dichroic ratio of the 0.1% CEC R6G/Lap film with the emission wavelength for different twisting δ angles of the sample (see Figure 3 caption). The linear relationship between the dichroic ratio and $\cos^2(22.5 + \delta)$ at 585 nm is also included.

different twist δ angles is shown in Figure 4. For a given δ angle, the $(D_{HV})^{cor}$ value is practically independent of the emission wavelength, confirming the presence of only one type of R6G species (monomer) for this very diluted dye sample. The augmentation of the $(D_{HV})^{cor}$ value at shorter wavelengths for small δ angles can be attributed to an incomplete correction of the scattering of the excitation light observed in film samples. Indeed, at those emission wavelengths close to the excitation wavelength, 495 nm in the present case, and in film orientations in which the specular reflection of the excitation beam is close to the emission detection channel, i.e. $-20° > \delta > 0°$, high-level scattering light was detected for all solid film samples used in the present work. Nor can this be corrected for the isotropic factor, because the light scattering of a liquid solution in a quartz cell (our isotropic sample) is much lower than that of solid films.

For a given emission wavelength, the dichroic ratio of the 0.1% CEC sample linearly correlates with the $\cos^2(22.5 + \delta)$ values, as is shown in the inset of Figure 4 at 585 nm. According to the previously developed eq 2,[1,2] the orientation ψ angle between the transition moment of the fluorescent species (along the long molecular axis of the rhodamine chromophore) and the normal to the clay layer can be evaluated from the slope and/or intercept of this linear relationship

$$(D_{HV})^{cor} \equiv \frac{I_{HH}}{I_{HV}} \times G = 2\cot^2\psi + (1 - 2\cot^2\psi)\cos^2(22.5 + \delta) \quad (2)$$

Equation 2 was derived on the basis of the absence of any reorientation of the fluorescent molecules during their lifetime after sample excitation and, consequently, it is only valid for systems with immobile fluorescent molecules. Present results suggest that the R6G monomers are statically adsorbed in the interlayer space of Lap films, at least at the fluorescence lifetime scale of nanoseconds, corroborating previous conclusions derived from time-resolved fluorescence spectroscopy. Indeed, the fluorescence decay curves of R6G/Lap films do not exhibit any time-dependent anisotropic response.[41]

From the slope and intercept of the $(D_{HV})^{cor}$ vs $\cos^2(22.5 + \delta)$ linear relationship shown in the inset of Figure 4, a tilt ψ angle of 62° is derived for the very diluted 0.1% CEC R6G/Lap film. This preferential orientation of R6G monomers in Lap films is identical to that previously obtained by linearly polarized absorption spectroscopy[36] and proves the efficiency of the polarized fluorescence technique to determine the orientation of adsorbed molecules in ordered 2D host materials. Moreover, the tilt angle obtained from fluorescence polarization with horizontally polarized excitation light is also identical to that previously obtained with vertically polarized excitation,[1] suggesting the absence of any depolarization phenomenon in the excitation process, e.g., the absorption and fluorescence transition moments of the adsorbed molecules are collinear, at least with respect to the film normal. Because the excitation at 495 nm corresponds to the same absorption and emission S_0-S_1 electronic transition, present results rule out any reorientation of R6G monomers in the Lap films during their fluorescence lifetime.

Polarization by the fluorescence technique provides a more sensitive and versatile method than that by absorption spectroscopy, because of the possibility of controlling both the excitation and emission wavelengths and polarizers. However, the fluorescence technique requires the adsorption of fluorescent molecules and the absence (or the minimization) of other phenomena occurring in the excited states that can provide depolarization in the fluorescence signal[37−40] (i.e., anisotropy in the excitation process and/or depolarization during the fluorescence lifetime, as is discussed below). Table 1 summarizes the preferential orientation of R6G molecules adsorbed in Lap films for different dye loadings obtained by both absorption and fluorescence spectroscopies with polarized light.

As the R6G loading in Lap films is increased, the dye molecules tend to self-aggregate in the clay layers. In the 1−25% CEC loading range, the coexistence of H and J type dimers of R6G in equilibrium with monomers was previously characterized by absorption and fluorescence spectroscopies with unpolarized light.[34,42] The J type dimer was identified by absorption (λ_{ab} = 545 nm) and fluorescence (λ_{fl} = 585 nm) bands placed at longer wavelengths than the corresponding spectral bands of the monomers (528 and 548 nm, respectively). On the other hand, the H type dimer is not fluorescent. In fact, it acts as an efficient quencher of the fluorescence emission of the monomer and is characterized by a hypsochromic absorption band (503 nm) with respect to the monomer absorption band. The proportion of the J over H dimer decreases as the loading of the dye is increased in the 1−25% CEC range.[34,42]

Figure 5 illustrates the response of the fluorescence spectra to the emission polarizer with respect to the horizontally polarized excitation light as a function of the twist angle δ for the 5.7 and 22% CEC R6G/Lap films. In the former sample (Figure 5 (left)), in which the monomer is the predominant fluorescent species, the increase in the twist angle δ leads to an important decrease in the monomer emission band (centered at around 550 nm) and a slight increase in the fluorescence intensity at longer wavelengths,

Table 1. Orientation Angle of R6G Species Adsorbed in Lap Films for Different Dye Loadings Obtained by Absorption[36] and Fluorescence Spectroscopy with Linearly Polarized Light

	absorption		fluorescence	
% CEC	species (λ range)	tilt angle ψ (deg)	species (λ range)	tilt angle ψ (deg)
0.1	monomer (λ_{ab} all range)	62	monomer (λ_{fl} all range)	62[a]
5.7	H dimer (λ_{ab} < 500 nm)	48	monomer (λ_{fl} < 570 nm)	61
	J dimer (λ_{ab} > 510 nm)	61	J dimer (λ_{fl} > 600 nm)	49 (λ_{exc}= 495 nm)[b]
				56 (λ_{exc}= 545 nm)[b]
				62 (λ_{exc}= 570 nm)
22	H dimer (λ_{ab} < 500 nm)	46	monomer (λ_{fl} < 570 nm)	60
	J dimer (λ_{ab} > 510 nm)	61	J dimer (λ_{fl} > 600 nm)	50 (λ_{exc}= 495 nm)[b]
				53 (λ_{exc}= 545 nm)[b]
				60 (λ_{exc}= 570 nm)
60	H dimer (λ_{ab} < 500 nm)	28	J dimer (λ_{fl} > 600 nm)	49 (λ_{exc}= 495 nm)[b]
	J dimer (λ_{ab} > 510 nm)	60		52 (λ_{exc}= 545 nm)[b]
				50 (λ_{exc}= 570 nm)[b]

[a] Also obtained for vertically polarized excitation light.[1] [b] Affected by fluorescence depolarization.

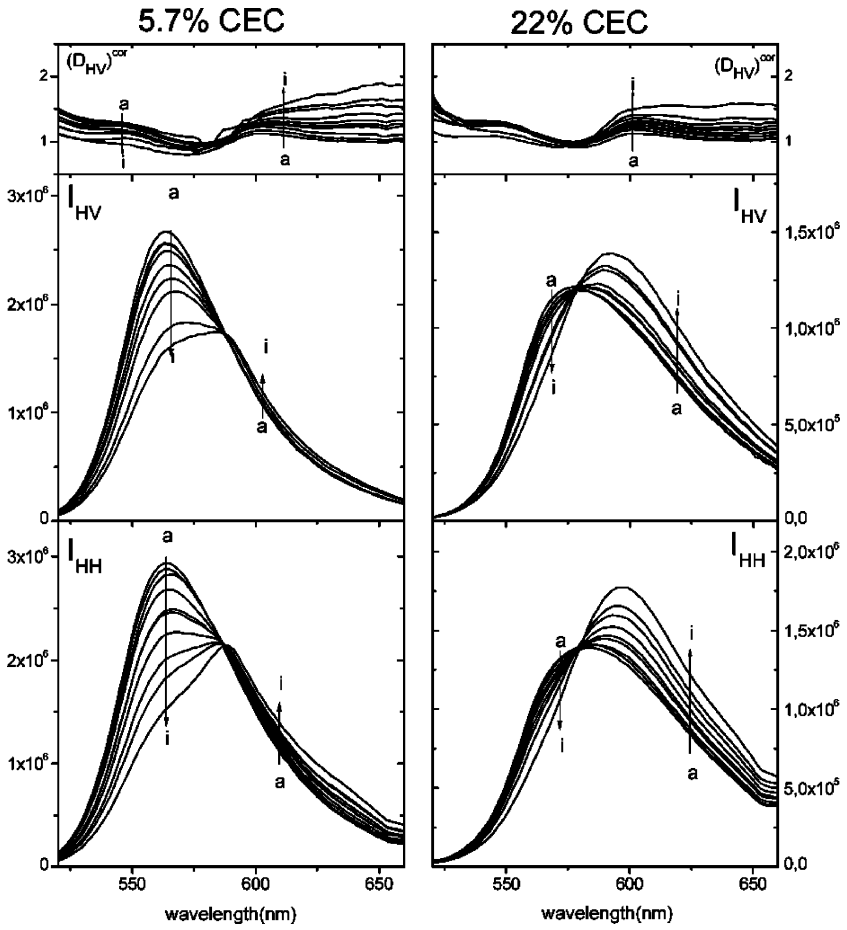

Figure 5. Evolution of the H (bottom) and V (middle) polarized fluorescence spectra and the dichroic ratio (top) of the 5.7 (left) and 22% (right) CEC R6G/Lap films with the following twist angles δ: (a) −20, (b) 0, (c) 10, (d) 20, (e) 30, (f) 35, (g) 40, (h) 45, and (i) 50°.

attributed to the emission of the J type R6G aggregates. These evolutions are observed in both H (Figure 5 (left, bottom)) and V (Figure 5 (left, middle)) polarized emission light and are characterized by an isoemissive point at around 585 nm.

The fluorescence band of the J dimer and its response to the emission polarizer are better characterized in the 22% CEC sample (Figure 5 (right)), because the dye aggregation is enhanced by increasing the dye loading in the clay layers. The isoemissive point obtained for this sample (around 580 nm) is similar to that observed for the 5.7% CEC sample, confirming that the fluorescence of these 5.7 and 22% CEC samples is due to both the emission from the locally excited state of R6G monomers and the emission from the J type dimers. Because of the poor emission of the 10^{-5} M ethanolic solution of R6G in the higher-wavelength range (> 600 m), the isotropic correction G factor at this wavelength range was better recorded by means of the mixed solution, recorded in the same front-face configuration.

Because of the presence of at least two fluorescent species, the dichroic ratio in the 5.7 and 22% CEC samples presents a wavelength dependence with two different evolutions of the $(D_{HV})^{cor}$ value with the twist angle δ (Figure 5 (top)). In the monomer emission region (<570 nm), the dichroic ratio decreases with the δ angle (similarly to that obtained in the

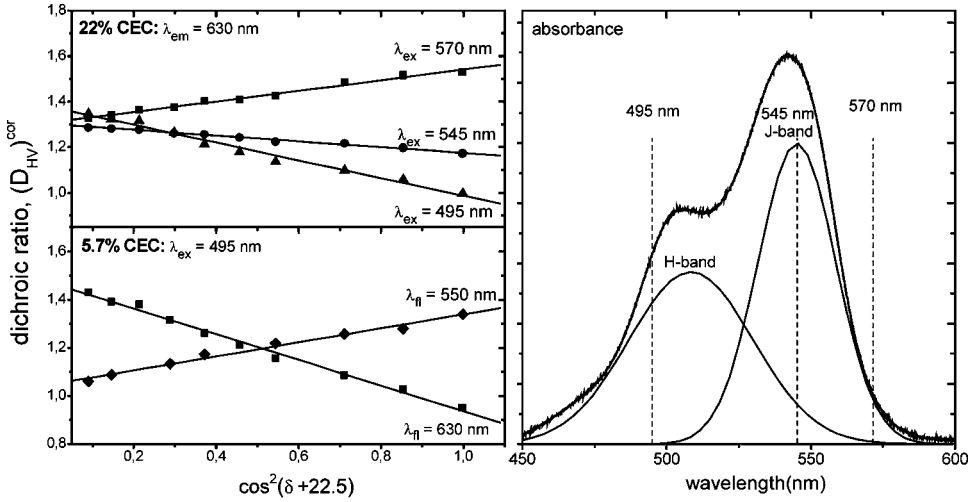

Figure 6. Left: linear relation of the fluorescence dichroic ratio vs $\cos^2(22.5 + \delta)$. Bottom: 5.7% CEC R6G/Lap films at two emission wavelengths 550 (fluorescence from R6G monomer) and 630 nm (J coplanar R6G dimer). Top: 22% CEC film at three excitation wavelengths, 495 (monomer and H dimer), 545 (H and mainly J dimers), and 570 nm (exclusively J dimer). Right: average absorption spectrum (deconvoluted in two H band and J band Gaussians) for the dimer observed in the 1−25% CEC range.

0.1% CEC film, Figure 4), whereas the opposite evolution is observed in the emission band of the J type dimer (>600 nm). The tilt angle derived from the slope of the $(D_{HV})^{cor}$ vs $\cos^2(\delta + 22.5)$ linear relationship (Figure 6(bottom)) in the monomer emission region ($\psi = 61$ and $\sim 60°$ for the 5.7 and 22% CEC samples, respectively, Table 1) is nearly the same as that obtained by polarized absorption spectroscopy,[36] corroborating once again the validity of the fluorescence technique in evaluating the preferential orientation of fluorescent dyes adsorbed in ordered clay layers. The slight decrease in the ψ value with respect to the most-diluted 0.1% CEC sample ($\psi = 62°$) was also observed by the absorption spectroscopy[36] and suggests a slight tendency of the R6G monomers to be oriented toward the normal to the Lap layers by increasing the dye content. On the other hand, the tilt angle obtained for the emission of the J dimer ($\psi = 49$ and 50° for the 5.7 and 22% CEC films, respectively, Table 1) does not correspond to the angle derived by absorption spectroscopy for the J band ($\psi = 61°$ for both samples),[36] but it is close to the tilt angle of the H absorption band ($\psi = 48$ and 46° for the 5.7 and 22% CEC samples, respectively, Table 1).

These different results could be attributed to the depolarization of the fluorescence light in the excitation process due to the presence of several absorbing R6G species in equilibrium. In fact, the fluorescence anisotropy is affected by the excitation of other species with different orientations present in the system. The fluorescence spectra of R6G/Lap films shown in Figure 5 were recorded by excitation at 495 nm, where the fluorescence excited state of the J dimer is not directly populated in the excitation process but the locally excited state of the monomer and the nonfluorescence excited state of the H type dimer are (Figure 6, right). Therefore, the observed fluorescence emission of the J dimer is the consequence of a transfer of the excitation energy from the monomer and/or the H dimer excited states to the fluorescence excited state (see Figure 1). H and J dimers are differently oriented with respect to the Lap normal (see ψ value in Table 1), and the excitation to the H band would imply a depolarization for the fluorescence emission of the J dimer.

This depolarization process would depend on the quenching rate constant of the H dimer and monomer excited states by the fluorescent J dimer. Indeed, this depolarization process can be applied to estimating the probability of the energy transfer in liquid systems.[45,46] Following the Förster energy-transfer expression,[39] the rate constant for the migration of the excited energy would depend on the relative orientation of the donor and acceptor moieties.[47] The probability of energy transfer from X polarized excited states to 22.5° twisted X' polarized and the 90° twisted Y' polarized (see the experimental setup in Figure 2) fluorescence excited states should be different. Consequently, although the excitation polarization is kept constant, it will affect the fluorescence dichroic ratio.

Fluorescence depolarization by energy transfer is performed during the population process of the fluorescence excited state. The population of the fluorescence state will depend on the absorption intensity of the sample at the excitation wavelength (I_{abs}) and on the probability of the quenching of the excited states directly populated in the excitation process by the fluorescence state. This probability is given by the rate constant of the energy transfer between the donor (D) and acceptor (A) moieties multiplied by the acceptor concentration ($k_{ET}(D-A)[A]$) over the corresponding unimolecular expression of all deactivation processes of the directly populated donor excited state ($\Sigma_i\, k_D(i)$). The fluorescence intensity polarized along the vertical direction (Y' axis, Figure 2) of an emissive excited state populated by an energy transfer from an excited state directly excited by horizontally (X axis) polarized light will then be proportional to

(45) Scherer, P. O. J.; Seilmeier, A.; Kaiser, W. *J. Chem. Phys.* **1985**, *83*, 3948−3957.
(46) Kawski, A.; Kuten, E.; Kamisnki, J. *J. Phys. B* **1973**, *6*, 1907−1916.
(47) Strictly, the Förster expression of the energy-transfer rate constant depends on the overlapping of the fluorescence band of the donor and the absorption band of the acceptor. The fluorescence from the donor H dimer excited state is not experimentally observed in the present case because of the very fast nonradiative deactivation from this state. This, however, does not discard the energy transfer via an interaction between the dipole moment of the donor and acceptor moieties, which should depend on their relative orientations.

$$I_{HV} \propto I_{abs}(X) \frac{k_{ET}(D_X-A_{Y'})[A]}{\sum_i k_D(i)} k_{fl}(Y) \quad (3)$$

where X and Y' refer to the orientation of the excitation and emission polarization, respectively, and k_{fl} is the radiative rate constant of the fluorescence state in the corresponding emission polarization (k_{fl} is proportional to the square of the transition dipole moment projected in the polarization direction[39]). Actually, $1/\sum_i k_D(i)$ is the lifetime of the quenched excited state of the donor, τ_D. In the present case, the polarization of the excitation light is along the horizontal direction (i.e., along the X axis) and the absorption intensity is referred to as $I_{abs}(X)$. Similarly, if the excitation polarization is kept in the X direction and the emission polarizer is moved in the horizontal (X') direction, the fluorescence intensity should be given by

$$I_{HH} \propto I_{abs}(X) k_{ET}(D_X-A_{X'}) \tau_D [A] k_{fl}(X') \quad (4)$$

The dichroic ratio corrected for the instrumental response to the polarization light is then transformed in

$$(D_{HV})^{cor} \equiv \frac{I_{HH}}{I_{HV}} G = \frac{k_{ET}(D_X-A_{X'})\tau_D(D_X-A_{X'})}{k_{ET}(D_X-A_{Y'})\tau_D(D_X-A_{Y'})} \frac{k_{fl}(X')}{k_{fl}(Y')} \quad (5)$$

If it is taken into account that the Förster expression for the energy-transfer rate constant depends on the relative orientation of the dipole moments between the donor and acceptor moieties,[39] then $k_{ET}(D_X-A_{Y'}) \neq k_{ET}(D_X-A_{X'})$ and $\tau_D D_X-A_{X'}) \neq \tau_D(D_X-A_{Y'})$, and the dichroic ratio would depend on the excitation polarization. The $k_{fl}(X')/k_{fl}(Y')$ ratio corresponds to the fluorescence dichroic ratio in the absence of any depolarization phenomena and for a constant excitation process, and consequently is given by eq 1.

For R6G/Lap films with moderate dye contents (in the 1−25% CEC range), monomers (M) and H (H) and J type (J) coplanar dimers coexist[41,42] and at the general excitation wavelength all these species can absorb light and populate the corresponding excited state. If the emission wavelength is selected at a wavelength in which only the fluorescence from the J dimer is detected, then the fluorescence dichroic ratio is given by eq 6, where a_V represents the probability of absorbing the excitation light by the V species (with V = M, H, and J), $k_{ET}(W_X-J_U)$ is the energy-transfer rate constant from the excited state of the W species (with W = H and M) directly populated in the X polarized excitation process to the fluorescence excited state of the J type dimer oriented in the U direction (with U = X' and Y'), $\tau_W(W_X-J_U)$ is the lifetime of the corresponding quenched excited state of the donor, and [J] is the concentration of the acceptor J dimer.

To confirm depolarization of the fluorescence J band by excitation to other species, we checked the anisotropy response of the fluorescence intensity at 630 nm for different excitation wavelengths. Figure 6 (top) shows an illustrative example of this effect for the 22% CEC sample, where the emission from the J dimer is predominant. As is discussed above, excitation at 495 nm (predominant absorption of the H dimer) implies a depolarization in the fluorescence emission, leading to a reduction in the recorded ψ angle (50°) with respect to that obtained by absorption spectroscopy[36] ($\psi = 61°$, Table 1). Increasing the excitation wavelength to 545 nm (maximal absorption of the J band, see the average absorption spectrum of the dimer in Figure 6 (right)) reduces the excitation depolarization, and a more realistic $\psi = 53°$ value is obtained. This value, however, does not match that proposed by the absorption data, probably because at this excitation wavelength there is still some excitation of the H dimer, inducing a small proportion of depolarization. Upon a further increase in the excitation wavelength to a value in which the excitation of the H band is negligible (e.g., 570 nm), the depolarization by the direct excitation of the H dimer disappears and the ψ angle obtained (60°) is close to that reported from the absorption spectra data[36] (61°). For the 5.7% CEC sample, this angle ($\psi = 62°$) is slightly larger, although this small difference could be accounted for in the experimental error.

This effect is included in eq 6. In fact, at 570 nm, the absorption capacity of the R6G monomer and H dimer are practically negligible, and thus $a_J = 1$ and $a_H = a_M = 0$ in eq 6, which transforms into eq 2. Under these experimental conditions, the 5.7 and 22% CEC R6G/Lap films do not present any fluorescence depolarization phenomena.

Therefore, it can be concluded that by an adequate choice of the experimental setup (excitation and emission wavelengths, excitation and emission polarizers, etc.), the fluorescence technique can provide information of the anisotropic behavior not only of fluorescent molecules but also of nonfluorescent species involved in the excitation process. Moreover, by developing adequate mathematical expressions, we could evaluate the preferential orientation of fluorophore molecules in macroscopically ordered host materials. The fluorescence technique is a more sensitive and versatile method than absorption spectroscopy for evaluating the preferential orientation of dyes adsorbed in ordered systems (in this case, supported clay films). Of course, the former technique requires the incorporation of unquenched fluorescent dyes or molecular probes in the system under study.

For the 60% CEC R6G/Lap film, the emission capacity is very low (the fluorescence efficiency, analyzed as the fluorescence intensity over the absorbance at the excitation wavelength, of this sample is 3 orders of magnitude less than that observed for the very dilute 0.1% CEC film with unpolarized light). The fluorescence intensity is enhanced by increasing the excitation slits, but a high-level scattering of the excitation light is also detected. So, special care has to be taken into account when manipulating and recording fluorescence anisotropy of this sample. The response of the fluorescence spectrum to the polarized light as a function of the twist angle δ, Figure 7, does not clearly lead to a new emission band, suggesting the presence of one

$$(D_{HV})^{cor} = \frac{a_J + a_H k_{ET}(H_X-J_{X'})\tau_H(H_X-J_{X'})[J] + a_M k_{ET}(M_X-J_{X'})\tau_M(M_X-J_{X'})[J]}{a_J + a_H k_{ET}(H_X-J_{Y'})\tau_H(H_X-J_{Y'})[J] + a_M k_{ET}(M_X-J_{Y'})\tau_M(M_X-J_{Y'})[J]} [2\cot^2\psi + (1 - 2\cot^2\psi)\cos^2(22.5 + \delta)] \quad (6)$$

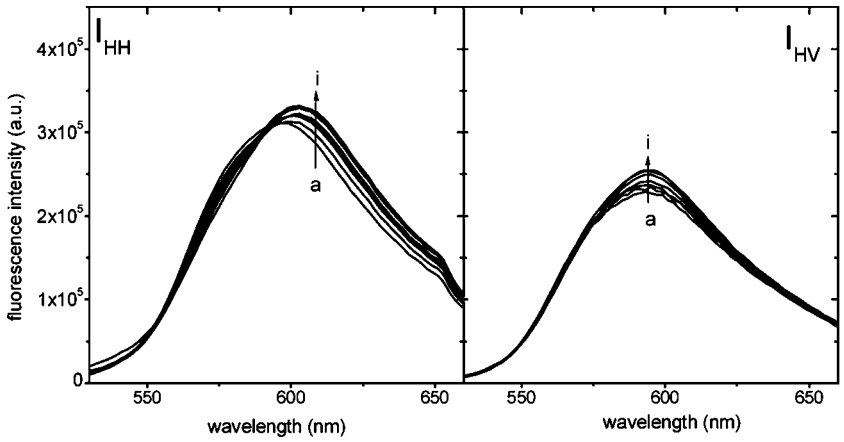

Figure 7. Evolution of the H (left) and V (right) polarized fluorescence spectra of the 60% CEC R6G/Lap film with the following twist angles δ of the sample: (a) −20 (b) 0, (c) 10, (d) 20, (e) 30, (f) 35, (g) 40, (h) 45, and (i) 50°.

fluorescence excited state. Considering previous results,[41] this state should correspond to the lowest excited state of a high-order R6G aggregates. However, it cannot discarded a reminiscent emission from the J type dimer in equilibrium with the high-order aggregate. For emission wavelengths >600 nm, the fluorescence dichroic ratio of this sample is wavelength independent (data not shown), and its evolution with the δ angle provides a tilt ψ angle of around 50° (Table 1). This angle is higher than that obtained for the J dimer using polarized absorption spectroscopy[36] but is similar to those obtained for the fluorescent J dimer in the 5.7 and 22% CEC samples after excitation at 495 nm (see data in Table 1). For the 60% CEC sample and contrary to the observation for the 5.7 and 22% CEC samples, the obtained ψ value does not depend on the excitation wavelength (Table 1). This observation can be interpreted in two different ways:

The reminiscent emission is due to a very low proportion of fluorescent J dimer but, independent of the excitation wavelength, the absorption of the excitation light is practically due to high-order aggregates. In this case, the excitation depolarization of the fluorescence signal should always be present and cannot be corrected (or reduced) by an adequate selection of the excitation wavelength.

The reminiscent emission is due to the lowest excited state of the high-order aggregates with a very low fluorescence capacity. Take, for instance, a coplanar displaced higher-order aggregate with a twisted angle between the dipole moment of the monomeric units. In this case, the fluorescence excited state should be populated not by an intermolecular energy transfer but rather by a very fast internal conversion from the highest excited states to the lowest excited state of the aggregates and/or to an intermolecular energy migration between high-order aggregates. Equation 6 then reduces to

$$(D_{HV})^{cor} = \frac{k_{ic}(H{-}J_{X'})}{k_{ic}(H{-}J_{Y'})}[2\cot^2\psi + (1 - 2\cot^2\psi)\cos^2(22.5 + \delta)] \quad (7)$$

where $k_{ic}(H{-}J_U)$ is the rate constant for the internal conversion from the H excited state of the higher-order aggregate directly populated by the excitation process to the fluorescence J excited state of the aggregate oriented in the U emission polarization ($U = X'$ and Y').

On the other hand, the adsorption of R6G molecules in Lap films is saturated for the 60% CEC R6G/Lap film.[36] So, in this sample, the interlayer space of the clay is saturated by dye molecules, and reabsorption/reemission and energy migration processes between adsorbed molecules can cause a global fluorescence depolarization phenomenon.

In any case, the emission of this 60% CEC sample is very weak, and the presence of several absorption species makes it more difficult to give a precise interpretation for the observed depolarization in fluorescence. However, with an adequate selection of the experimental conditions, the fluorescence technique with polarized light provides a powerful tool for evaluating the orientation of fluorescent molecules in ordered bidimensional host materials. In fact, the angle of the preferential orientation of the guest molecules can be evaluated by both excitation and emission processes, by precise selection of the excitation and emission wavelengths and the polarization of the excitation and emission lights.

Conclusions

Fluorescence polarization is a powerful tool for evaluating the preferential orientation of fluorescent dyes adsorbed in ordered clay layers in a macroscopic scale, for instance, in supported thin films obtained by the spin-coating procedure. This is a more sensitive and selective method than the alternative absorption spectroscopy with linearly polarized light. In those experimental conditions, in which the depolarization phenomena, both in the excitation process or during the lifetime of the fluorescence state, are negligible, a simple mathematical expression linking the fluorescence dichroic ratio and the twist angle of the sample can be used to evaluate the orientation of adsorbed fluorophores in rigid 2D layered materials.

Thus, R6G monomers are adsorbed in the Lap layers for low-loading films, with a preferential orientation of around 62° with respect to the normal of the film (around 28° with respect to the layer plane). Coplanar J type dimers, characterized by a fluorescence band placed at longer wavelengths,

are formed in moderate R6G/Lap loading films with a preferential orientation of the monomeric units around 30° with respect to the layer plane. This result agrees with the exciton theory, as fluorescent long displaced ($\theta < 54.7°$) coplanar dimers are formed. The direct excitation to the absorption band of nonfluorescent coplanar H type dimers causes a depolarization process in the emission intensity, probably by an energy-transfer mechanism, restricting the application of the fluorescence polarization technique for evaluating the preferential orientation of adsorbed fluorophores in layered host materials. However, extra information of the depolarization process (rate constant of energy transfer, rotational motion or angle between the absorption and emission transitions moments) could be derived from an exhaustive treatment of the depolarization processes, which is not the aim of this work. In any case, the R6G H type dimers are disposed more perpendicularly with respect to the Lap layers, leading to nonfluorescent short, displaced coplanar dimers in agreement with the exciton theory.

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