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Confocal Fluorescence Microscopy and AFM of Thiacyanine J Aggregates in Langmuir-Schaefer Monolayers

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The influence of surface pressure and adsorption time on the size and shape of J aggregates of 3,3′-disulfopropyl-5,5′-dichlorothiacyanine sodium salt (THIAMS) adsorbed onto dioctadecylammonium bromide (DODAB) monolayer were investigated. After deposition of the monolayers on a hydrophobic glass substrate by the Langmuir−Schaefer (LS) technique, confocal fluorescence microscopy and atomic force microscopy (AFM) were used to study their fluorescence and topography. The difference in size and shape of the J aggregates formed at different surface pressure and adsorption times may be caused by a different rigidity and charge density of the DODAB film. The total thickness of the films amounts to 4.5 ± 0.2 nm which confirms that THIAMS forms 2D aggregates with edge-on orientation when adsorbed to a DODAB monolayer. In the bulk of the J aggregate the fluorescence was polarized in all films investigated. THIAMS molecules adsorbed to the dark areas between the fluorescence domains remained mainly as monomers and dimers.

Introduction

In the 1930s Scheibe and Jelley[1,2] independently discovered a new narrow, intense and red-shifted absorption band in the visible spectrum of some cyanine dyes at high concentration. This narrow absorption band (J band) was attributed to delocalized Frenkel-type exciton states[3,4] present in assemblies of dye molecules interacting by electrostatic forces. Due to their large oscillator strength and fast electronic response, J aggregates are of interest to many fields, such as photographic sensitizers, models for antenna's in photosynthetic reaction, nonlinear optics related to superradiance, and solar energy conversion.[5−15]

It was found the J aggregation could be observed in concentrated aqueous solution,[1,2] in polymer matrixes,[16,17] in liposomal membranes,[18,19] and upon adsorption to metal surfaces.[20,21] Since 1970, the LB technique has been used extensively to prepare J aggregates in Langmuir−Blodgett (LB) films.[22,23] Those aggregates were assumed to form a virtual, two-dimensional (2D) crystal at the air/water interface assisted by the amphiphile monolayers.[24−27] The monolayers obtained in this way can be transferred to the surface of a suitable hydrophilic or hydrophobic substrate.

In previous work, the J aggregates formed by a thiatrimethine cyanine dye (THIATS) adsorbed onto Langmuir monolayers or self-organized polymer films of oppositely charged amphiphiles were studied in detail.[28−33] The influence of the amphiphile structure, the adsorption time, and the deposition method on the molecular and mesocopic organization of the J aggregates were discussed. The monolayer deposited onto a substrate by the Langmuir−Schaefer (LS) technique preserved the structure of the domains as formed at the air/water interface. The influence of the surface pressure on the structure of the

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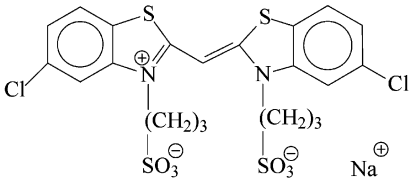
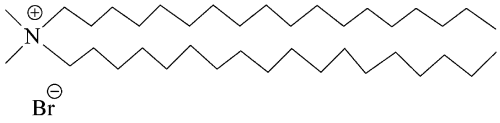
Scheme 1. Chemical Structures of THIAMS and DODAB.



5,5'-dichloro-3,3'-disulfopropylthiacyanine sodium salt

THIAMS



dioctadecyldimethylammonium bromide

DODAB

domains in the deposited films was however not investigated.

In the present study, we made Langmuir films of the thiamonomethine cyanine dye, 3,3′-disulfopropyl-5,5′-dichlorothiacyanine sodium salt (THIAMS) adsorbed onto a compressed monolayer of the amphiphile dioctadecylammonium bromide (DODAB) at the air/water interface at two different surface pressure of 15 and 30 mN/m. While the film is still expanded at 15 mN/m, it is clearly condensed at 30 mN/m.[29] After deposition of the films on a hydrophobic substrate by the LS technique, confocal fluorescence microscopy and atomic force microscopy (AFM) were used to determine their spectra and morphologies.

Experimental Section

Chemicals. The dye 3,3′-disulfopropyl-5,5′-dichloro-thiacyanine sodium salt (THIAMS) was purchased from FEW Chemicals GmbH (Germany) and was used as received (Scheme 1). The amphiphile dioctadecylammonium bromide (DODAB) (Scheme 1) was purchased from Sigma-Aldrich (purity 95%) and recrystallized from $CHCl_3/Et_2O$ before use to remove a fluorescent impurity. The ultrapure water, used for all experiments and all cleaning steps, was obtained by using a filter system (Millipore, catalog no. CFOF 012 05) to remove ions, organic materials and small particles and had a resistivity of 18.2 $M\Omega \cdot cm^{-1}$, a pH of 5.5, and a surface tension of 71 mN/m. Dichlorodimethylsilane was purchased from ACROS. Other solvents used in the experiments were of spectrophotometric grade.

Sample Preparation. The deposited films were prepared on a commercially available LB trough (KSV Instruments Ltd., size: 150 × 528 mm) at 20.5 ± 0.1 °C. The surface pressure was measured with a Wilhelmy type balance. To make the Langmuir films at the air/water interface, DODAB was spread on the Milli-Q water from 10^{-3} M chloroform solution. After 10 min evaporation time of the chloroform, the DODAB film was compressed to the target surface pressure (15 or 30 mN/m) at a speed of 5 mm/min. Then 3 mL of a concentrated THIAMS solution (300 mg/L) in water was injected into the subphase (1500 mL of water) to reach the target concentration (10^{-6} M).

To clean the glass substrates, the cover glasses (22 × 22 × 0.1 mm) were placed in a specially constructed beaker and sonicated in acetone for 15 min followed by sonication (2 × 5 min) in aqueous 10 wt % NaOH solution. The beaker was thoroughly rinsed with water after each step. After sonication in water for 10 min the glasses were dried and put in a UV-photoreactor for 30 min. To prepare the hydrophobic substrate, the cleaned cover glass was immersed in 1% dichlorodimethylsilane solution in chloroform for 15 min and dried by nitrogen after being rinsed twice with acetone. To deposit the LS film, a hydrophobic cover glass was mounted on a T-shaped metal plate held by the clamp of the mechanical arm, to position the substrate horizontally. When the position of the arm was moved close to the air/water interface, a part of the film could be lifted and deposited onto the hydrophobic substrate.

Methods. Steady-state absorption spectra were recorded on a double-beam UV/vis spectrophotometer of the Lambda 40 series (Perkin-Elmer Instruments). Fluorescence spectra were recorded with a fluorimeter (Spex Fluorolog model 1691) under front face conditions (26°) to eliminate as much scattered light as possible.

The wide range fluorescence microscopy of the LS films was monitored visually via an optical microscope OPTIPHOT-2 (Nikon OPTIPHOT-2) with an episcopic fluorescence attachment Nikon EFD-3. A CW Argon-ion laser (Spectra Physics, model 2025) was used for fluorescence excitation at 458 nm with circular polarized light. A dichroic mirror (DM 460, Nikon) was used to guide the excitation light to the sample. A notch filter (N 457.5) prevented the scattered light from passing into the registration port of the microscope. The size of the illuminated area could be adjusted with a diaphragm that functions as a pinhole and can be opened or closed manually.[34] In the experiments an MPlan 40× NA 0.5 dry objective lens (Nikon) was used. Photographs of the samples were made using a digital camera (Nikon 995).

The high-resolution fluorescence microscopy of the LS films was performed using a confocal microscope (Nikon Diaphot 200) with an oil immersion, high-numerical aperture lens (NA 1.4, 60×). A 458 nm line of an argon-ion laser with linear or circular polarization was used to excite the samples. The dichroic mirror of 460 nm (reflection ∼460−500 nm) which suppressed the emission spectrum of the J aggregate, with maximum at around 470 nm, was replaced by a glass plate (thickness = 0.1 mm). A notch filter (457.5 nm, Kaiser Optics) and a 460 nm long pass filter were used to suppress excitation light. For the combined measurements (spectra and micrographs) excited by linear polarized light, the emission of the samples was split with a beam splitter cube, guiding 50% of the light to the liquid nitrogen cooled, back-illuminated CCD camera (Princeton Instruments) and 50% to the avalanche photodiodes (APD, EG&G, Canada). The exposure time for the acquisition of each spectrum was 5 s. In the fluorescence polarization measurements the intensity of the mutually perpendicular polarized beams, separated by a polarizing beam splitter cube was detected by two independent APDs. When circular polarized excitation was used, the emission was split with a polarizing beam splitter cube and detected by two APDs. The first APD detected the s-polarized component and the second one detected the p-polarized component of the fluorescence light. The laser power was 100 nW in most experiments. By storing the 40 × 40 μm micrographs at 200 × 200 pixels, each pixel corresponds to a size of 200 × 200 nm. Due to the diffraction limit, the information stored in each pixel will come from a slightly larger area.

The morphology and thickness of the films was investigated with atomic force microscopy (AFM) using a Discoverer TMX2010 AFM system (ThermoMicroscopes, San Francisco, CA) operating in noncontact mode using Si probes (ThermoMicroscopes, San Francisco, CA) with a spring constant of 34−47 N/m and a resonance frequency of 174−191 kHz. A calibration silicon grating (TGZ01, pitch 3 μm, Δz = 26 ± 1 nm, MicroMasch, Tallinn, Estonia) was used to calibrate the piezo scanner. Measurements were done under ambient conditions. Image analysis was performed with Topometrix SPMLab 5.0 software.

Results and Discussion

Steady-State Absorption and Emission Spectra of the Films. The absorption and emission spectra of THIAMS/DODAB LS films deposited at 15 and 30 mN/m are shown in Figure 1. The spectral data are summarized in Table 1. THIAMS/DODAB LB films formed at the air/water interface were deposited onto glass substrates after adsorption time intervals of 15, 30, 45, 60, and 75 min. At a surface pressure of 15 mN/m, no absorption and emission of THIAMS could be observed at 15 and 30 min while no fluorescent domains were observed in fluorescence micrographs obtained after an adsorption time of

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Table 1. Spectral Data of THIAMS/DODAB LS Films Deposited at 15 and 30 mN/m

target pressure (mN/m)	adsorption time (min)	λ_{abs} (nm)	A_{abs}	$FW^2/_3M_{abs}$[a] (cm^{-1})	λ_{em} (nm)	$FW^2/_3M_{em}$[b]	N_{eff}[c]
15	45	470	0.004	460 ± 30	475	440 ± 30	12 ± 2
	60	470	0.01	430 ± 30	473	430 ± 30	15 ± 2
	75	469	0.03	420 ± 30	472	420 ± 30	15 ± 2
30	15		0				
	30	469	0.065	460 ± 30	471	450 ± 30	13 ± 2
	60	469	0.075	460 ± 30	471	450 ± 30	13 ± 2

[a] $FW^2/_3M(M)_{abs}$= 1630 ± 30 cm^{-1}. [b] $FW^2/_3M(M)_{em}$= 2100 ± 30 cm^{-1}. [c] Calculated from

$$N_{eff} = \left[\frac{FW^2/_3M(M)_{abs}}{FW^2/_3M(J)_{abs}}\right]^2$$

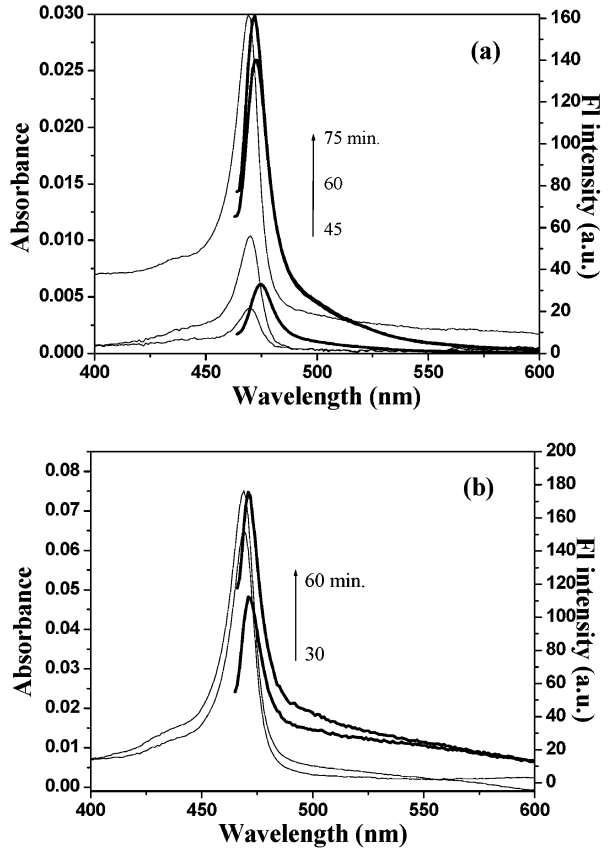

Figure 1. Absorption (thin lines) and emission (thick lines, excited at 458 nm) spectra of THIAMS/DODAB LS films deposited at 15 (a) and 30 mN/m (b) with different adsorption times.

15 min. A weak band around 470 nm appeared after an adsorption time of 45 min which increased 8-fold at an adsorption time of 75 min where saturation occurred. At a surface pressure of 30 mN/m, there was still no observable absorption after an adsorption time of 15 min while a very intense absorption band around 470 nm was obtained at 30, 45 and 60 min where saturation occurred. The narrowed ($FW^2/_3M_{abs} \sim 420-460 \pm 30\ cm^{-1}$) and red-shifted absorption band (470 nm) in respect to the monomer absorption (428 nm, $FW^2/_3M_{abs}(M) = 1630 \pm 30\ cm^{-1}$) confirmed the formation of J aggregates which is assisted by electrostatic interactions between the negatively charged dye molecules and the positively charged layer of amphiphiles.[35]

The formation of a complete J aggregate monolayer from a 10^{-6} M solution may take 20−25 min or more if the rate of adsorption and aggregate formation is controlled by diffusion of the dye to the amphiphile surface.[36] This time span is a minimum and can be much longer if the organization of the adsorbed dye molecules into J aggregates is rate-limiting step. This can explain why no J band can be observed at an adsorption time of 15 and 30 min at 15 mN/m and at an adsorption time of 15 min at 30 mN/m. For the films deposited at the same surface pressure, the effective coherence length (N_{eff}),[37] which means the number of effectively coupled dye molecules in the aggregate is almost independent of the adsorption time over the time span investigated (∼13−15 ± 2). That means that the effective delocalization in the aggregates remains unchanged during the growth of the aggregates. Since it is possible that besides homogeneous and inhomogeneous spectral broadening the formation of dimers also contributes to the width of absorption spectrum at two-thirds of the maxima ($FW^2/_3M(M)_{abs}$) of a dilute THIAMS solution, the actually exciton delocalization in THIAMS J aggregates could be smaller than 13 ± 2 dye molecules.

In the fluorescence spectra, the maxima of the J bands show a small Stokes shift (∼2−5 nm) compared to those of the absorption spectra and the bandwidth at two-thirds of the emission maximum ($FW^2/_3M(J)_{em} \sim 420-450 \pm 30\ cm^{-1}$) is almost same as in the absorption spectra. While the J band in the THIAMS/DODAB films deposited at 15 mN/m experiences a little blue shift (3 nm) at longer adsorption times, for the films deposited at 30 mN/m, no change in the position of the J band is observed at longer adsorption times.

When measuring the absorption and emission spectra of each film deposited at 15 mN/m, there were detectable spatial variations of the J band intensity at different sites in each film which persisted even after adsorption times of 60 or 75 min. For the films deposited at 30 mN/m, no such phenomenon was found. In Figure 1, the most intense spectrum of each film is shown.

Fluorescence Microscopy. Fluorescence micrographs, obtained with the epifluorescence microsocope for the films deposited at 15 mN/m and adsorbed during different time spans, reveal very large irregularly shaped and sometimes ragged fluorescent domains (Figure 2a−c) with a random orientation. Upon increasing the adsorption time from 45 (Figure 2a) to 60 min (Figure 2b), the size of the domains increased from ∼30 × 50 to ∼50 × 80 μm. Upon extending the adsorption time to 75 min (Figure 2c), the fluorescence

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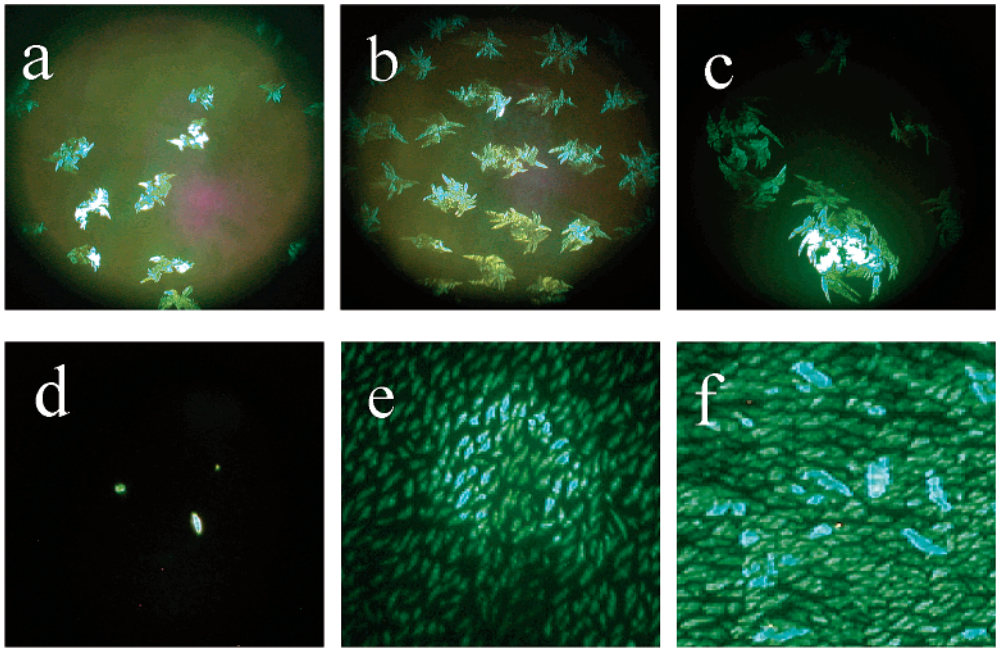

Figure 2. Fluorescence micrographs of THIAMS/DODAB LS films deposited at 15 mN/m ((a) 45, (b) 60, and (c) 75 min; image size ~ 300 μm × 300 μm) and 30 mN/m ((d) 15, (e) 30, and (f) 60 min; image size ~ 200 μm × 200 μm) recorded with an epifluorescence microscope. (λ_{ex}= 458 nm circular polarized light, notch filter = 457.5 nm, DM = 460 nm.)

domains coalesced to very large domains with a size of about 100 × 150 μm, almost three times those in Figure 2a. From these micrographs, it is easy to understand the variation between spectra recorded at different position in each film mentioned before. The nonfluorescence areas between the adjacent domains were so large that the J band will be less intense if a larger part of the illuminated area does not contain fluorescent J aggregates, as happens, e.g., at a surface pressure of 15 mN/m and an adsorption time of 45 min.

As shown in Figure 2d−f, the fluorescence micrographs of THIAMS/DODAB LS films deposited at 30 mN/m with different adsorption time indicate the formation of the spindle shaped domains with a regular shape and a much smaller size (2 × 4 μm). Hence their size and features differ strongly from those of domains formed at 15 mN/m. At an adsorption time of only 15 min (Figure 2d), sporadic fluorescence domains can be found although no J band absorption and emission was observed in the steady-state spectra. There were no changes of the shape and size of the domains at an adsorption time of 30 min (Figure 2e), but the number of domains in the field of view increased acutely. When the adsorption time was 60 min (Figure 2f), the arrangement of the domains in the film was more congested and apparently multilayers of fluorescence domains were formed locally. For the fluorescence micrographs of all LS films, the distribution of domains over the glass surface was homogeneous which indicates the suitability of horizontal lifting to deposit Langmuir film at the air/water interface onto a hydrophobic substrate. In Figure 2e,f, the orientation of the domains is apparently correlated over a length scale of at least 200 μm (field of view). As the excitation was circularly polarized and no polarizing elements (with exception of the dichroic mirror which has a slightly different reflectivity for s- and p-polarized light) were used, the abundance of the orientation of the observed domains will resemble that of the domains present in the film.

For a more detailed study of the J aggregates a confocal fluorescence microscope which combines a better spatial resolution with a simultaneous determination of the fluorescence spectra and the fluorescence polarization was used. Figure 3 shows the fluorescence micrographs (40 × 40 mm) of the THIAMS/DODAB LS films deposited at 15 mN/m recorded by the confocal fluorescence microscope using linear polarized excitation. As the size of the fluorescent domains observed with the epifluorescence microscope ranged from 30 × 50 to 100 × 150 μm, only images of parts of the domains are shown in each micrograph. The setup does not allow one to obtain micrographs of larger areas. The complementarity of the micrographs obtained with the two APDs' indicates that, as already suggested in Figure 2a−c, the large domains are a conglomeration of smaller domains each characterized by a homogeneous orientation of the transition dipoles. Hence the transition dipoles of the THIAMS molecules preserve their orientation over several micrometers in the small domains. The preferred direction of their orientation varies however in a random way between different small domains.

Typical micrographs (5 × 5 μm) of a selected small section of a fluorescent domain of THIAMS/DODAB LS film deposited at 15 mN/m after an adsorption time of 75 min are shown in Figure 4. These micrographs show again that the large domains consist of smaller domains of which the fluorescence is polarized homogeneously. This means that small single crystalline domains with a size up to 10 × 5 μm (Figure 3c) are formed where all dye molecules have an identical orientation. One should note however that the small single crystalline domains contain a large number of cracks (Figure 4) and show often ragged edges (Figure 3). Fluorescence spectra of small domains with different orientation (Figure 4) have identical features. The bandwidth of the fluorescence spectra of individual small domains ($FW^2/_3M(J)_{em}$) was around 350 ± 30 cm $^{-1}$ which is 30% smaller than that of the emission spectrum of a bulk sample. (Table 1). The intensity of the spectra obtained at the edge of the bright domains was too low to allow a determination of the spectral width.

The fluorescence micrographs (40 × 40 μm) of THIAMS/DODAB LS films deposited at 30 mN/m with different adsorption times and recorded by confocal fluorescence

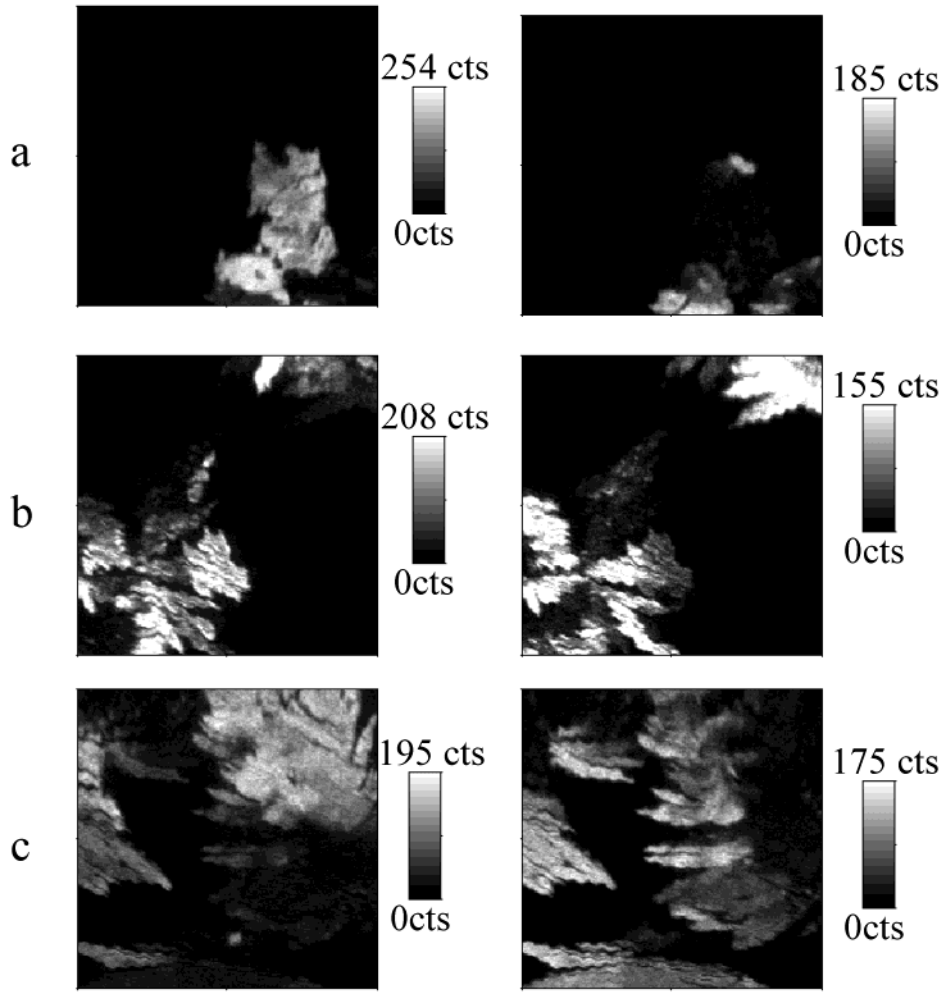

Figure 3. Fluorescence micrographs of THIAMS/DODAB LS films deposited at 15 mN/m with different adsorption time: (a) 45 min; (b) 60 min; (c) 75 min. (Excited by linear polarized laser at 458 nm, laser power = 100 nW, glass plate instead of dichroic mirror, 457.5 nm notch filter, 460 nm long pass filter. Size: 40 μm × 40 μm; left and right columns, detection of s- and p-polarized light.)

microscopy with linear polarized excitation are shown in Figure 5. The micrographs show, in agreement with the data obtained with the epifluorescence microscope, the presence of small spindlelike domains with a size of 2 × 4 μm and with a homogeneous fluorescence polarization. One observes, when comparing Figure 5b with Figure 5c, that, independent of the polarization of the emission, most domains in Figure 5b have a similar "horizontal" orientation. Although on one hand this could be due to bias introduced by photoselection by the linearly polarized excitation, Figure 5c shows on the other hand that most domains have again a similar but "oblique" orientation which differs from that of the majority of the domains in Figure 5b. This indicates that the orientation of neighboring domains is correlated and that the orientational correlation length of the domains exceeds the viewing field of the microscope.

For the monolayer deposited after an adsorption time of 15 min the intensity of the bright domains (Figure 5a) was still too low to get a sufficient signal-to-noise ratio to obtain spatially resolved fluorescence spectra. Figure 6 shows the enlarged micrographs of the film deposited at 30 mN/m after an adsorption time of 30 min. The $FW^2/{}_3M(J)_{em}$ of the spectra taken in the center and at the edge of the single crystalline domains were all around 350 ± 30 cm^{-1} which is identical with those of the films deposited at 15 mN/m. After the laser power was increased, also a spectrum with a maximum at 474 nm and an $FW^2/{}_3M_{em}$ of 500 ± 10 cm^{-1} (Figure 6d) could be obtained from the dark area of the film. Besides the J aggregate emission, all spectra clearly show a shoulder around 500 nm which is more important at the edge of (Figure 6c) or between (Figure 6d) the domains. Similar observations could be made for LS-films deposited at 15 mN/m (not shown). This suggests that in all aggregates formed at 15 and 30 mN/m THIAMS sandwich dimers are incorporated as already suggested by Duschl.[38] The broader spectrum and more important shoulder observed for the area between the domains could suggest that in this region a large part of the dye molecules is adsorbed as monomers or dimers. As a 458 nm laser line was used to excite THIAMS J aggregates in the films, the absorption cross section of monomers and dimers at this wavelength was much weaker than that of the J band. Hence the emission spectra will reflect a bias in favor of the J aggregates. Although the emission spectra suggest that the presence of adsorbed monomers and dimers of THIAMS in the dark areas between the fluorescent domains, the latter contribute little to the bulk spectra due to their small number density and/or fluorescence quantum yield.[39,40]

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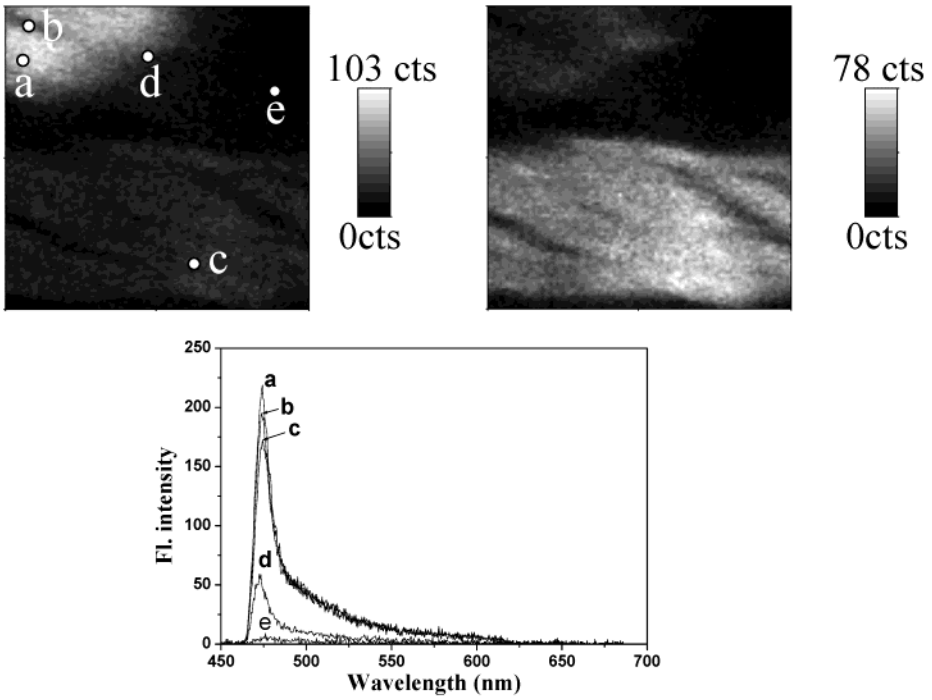

Figure 4. (Top) Small scale fluorescence micrographs of THIAMS/DODAB LS films deposited at 15 mN/m with adsorption time of 75 min detected by two APD (size: 5 μm × 5 μm). Left and right columns detection of s- and p-polarized light. (Bottom) Emission spectra recorded at different positions indicated in the upper micrographs. (Excited by linear polarized laser at 458 nm, laser power was 100 nW.)

To remove the bias of the fluorescence micrographs due to photoselection, also linear polarized fluorescence micrographs generated by circular polarized excitation were recorded. The fluorescence micrographs of the LS films deposited at 15 mN/m with an adsorption time of 75 min and at 30 mN/m with an adsorption time of 30 min, obtained under those conditions, are shown in Figure 7 which shows clearly that when the s-polarized fluorescence was detected, the emission of "vertical" oriented domains was much brighter than that of "horizontal" oriented domains, and vice versa. These micrographs indicate that the transition dipole of the THIAMS molecules is parallel to the long axes of the small domains in the aggregates. Using circular polarized excitation the intensity of s- and p-polarized fluorescence, obtained from "vertical" and "horizontal" domains respectively is the same within experimental error.

The different structures formed at a target pressure of 15 and 30 mN/m can be related to a different rigidity and charge density of the DODAB film. On one hand the small size and regular shape of the small domains formed at the surface pressure of 30 mN/m would be caused by frequent nucleation of the J aggregates at high surface pressure. The more frequent nucleation can be due to a larger surface charge density of the condensed DODAB leading to a better electrostatic screening of the dye molecules assembled in the aggregate and a larger 2D concentration of the absorbed dye. Both factors will directly increase the rate of nucleation and growth. They will furthermore allow the nucleation of smaller domains characterized by a larger line tension energy per monomer. On the other hand at a surface pressure of 30 mN/m the DODAB film is in a condensed state[41] and hence is fairly rigid. The small domains, the size of which is limited by the (positional, bond or tilt) correlation length[42] of DODAB cannot assemble to large aggregates. Following this line of thought it is however less clear why the mesoscopic structures of the J aggregates do not reflect the dendritic structure of the crystalline phase domains of the DODAB film.[43] The situation here resembles that encountered by Vranken[28] after injection a dye solution below a compressed DODAB layer on a water surface. At 15 mN/m the pressure−area diagram of a DOBAB layer is less steep[41] and the molecular area is larger, which implies a lower charge density.[29] This will decrease the rate of nucleation while, the DODAB monolayer is still fairly mobile. Hence the packing of the DODAB molecules will be organized by the J aggregates of THIAMS. Here the situation is intermediate between the compression of DODAB on a THIATS subphase[29] and injection of a THIATS solution under a completely compressed DODAB layer.[28] The results obtained for THIAMS under the former conditions will be published separately. Hence as well a different rigidity of the DODAB layer as a Volmer−Weber mechanism[44,45] for the domain formation[46] can explain the different number density and size of domains formed at 15 and 30 mN/m.

Comparison with the Mesoscopic Structures of Other J Aggregates in Monolayers. When comparing the results obtained for THIAMS with those obtained for THIATS under identical conditions (surface pressure of 30 mN/m, addition of the dye after compression of the DODAB monolayer), we observe in both cases an agglomeration of small (1−4 μm) domains. While for THIAMS, the domains are spindlelike, they are apparently circular for THIATS.[28] It is unlikely that this difference is due to the comparison of data obtained for a floating

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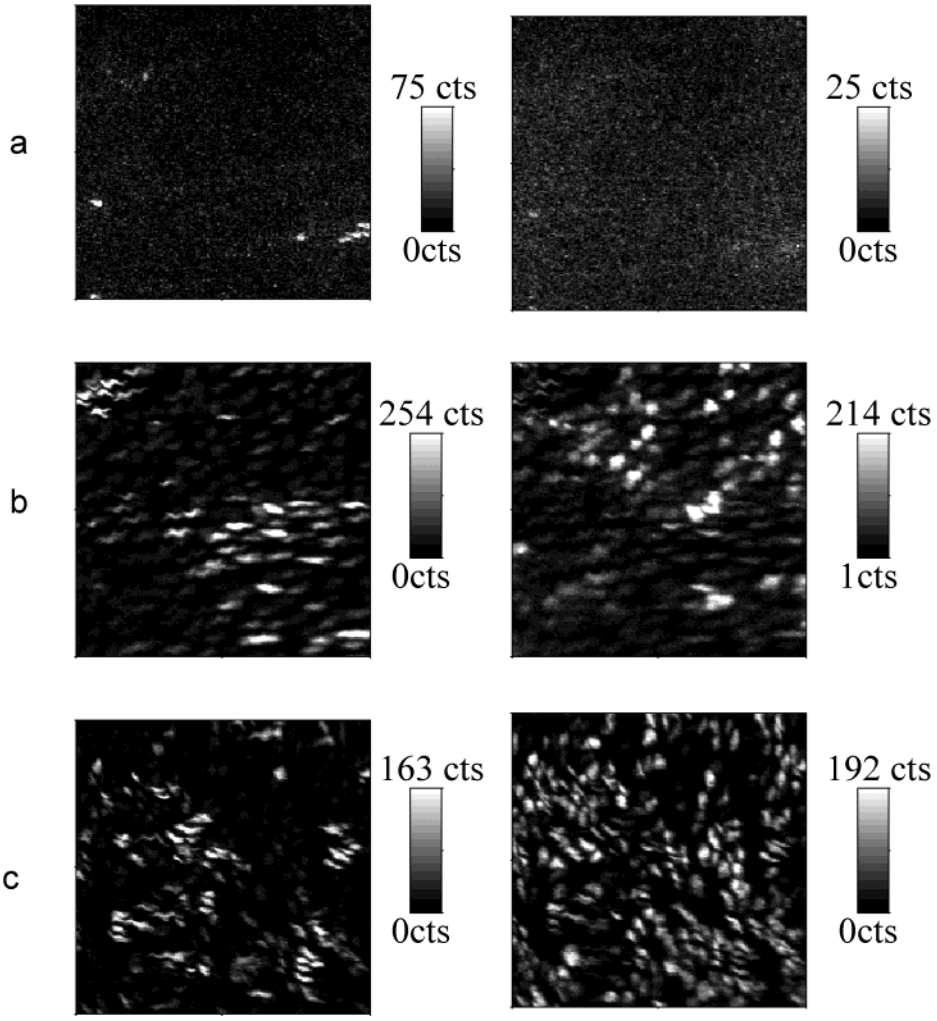

Figure 5. Fluorescence micrographs of THIAMS/DODAB LS films deposited at 30 mN/m with different adsorption time: (a) 15 min; (b) 30 min; (c) 60 min. (Excited by linear polarized laser at 458 nm, laser power = 100 nW, glass plate instead of dichroic mirror, 457.5 nm notch filter, 460 nm long pass filter. Size: 40 μm × 40 μm; left and right columns, detection of s- and p-polarized light.)

Langmuir film (THIATS) and a deposited film of THIAMS. Actually earlier experiments[29,30] indicated that deposition by the Langmuir−Schaeffer method does not alter shape, size or packing of the THIATS domains. Small (a few micrometers) spindlelike or circular domains were also observed upon compression of a monolayer of DODAB on a subphase with 10^{-6} M 1,1′-diethyl-3,3′-disulfopropyl-5,5′,6,6′-tetrachlorobenzimidazolotrimethinecyanine[41] using Brewster angle microscopy.

The shape of the monocrystalline domains observed at 30 mN/m resembles that observed for J aggregates of pseudoisocyanine (PIC) adsorbed on mica. However the size of the latter domains is significantly smaller (800 × 300 nm).[46,47] Furthermore while the latter aggregates were three-dimensional this is not suggested by the AFM micrographs obtained for the THIAMS−DODAB system (cf. infra). The shape of the aggregates formed at 15 mN/m resembled those formed by J aggregates in mixed monolayers of octadecanoic acid and of octadecyl-substituted merocyanines[48] at 15 mN/m.

Kirstein et al.[26,49,50,51] observed upon first compression of DODAB in the presence of a 9-methylthiatrimethinecyaninethe formation of nearly circular domains, which were in contrast with our data[29,30] not polarized. Stopping the compression for 70 min after the initial compression resulted in a reorganization of the circular domains into elongated domains. The fluorescence of those domains was polarized along the long axis.[49,51] The size of those domains of 50 × 10 μm[26,49,51] differs clearly from the irregular monocrystalline small domains formed at 15 mN/m as from the spindlelike domains formed at 30 mN/m. One should keep in mind that domains observed for the 9-methylthiatrimethinecyanine are made up of differently packed dye molecules as they show an absorption spectrum characteristic for a herringbone aggregate.[26,49] Furthermore the data of Kirstein and Möhwald were obtained for a Langmuir film with a different history of compression and relaxation.[26] While monomethines[46,47] or trimethines with a 9-ethyl substituent adopt mainly a brickstone packing, 9-methyl-substituted trimethinecyanines[49] adopt a columnar packing (H aggregates) or a herringbone packing.[52] However it is not yet clear how this has to be related to the mesoscopic structures of 2D J aggregates.

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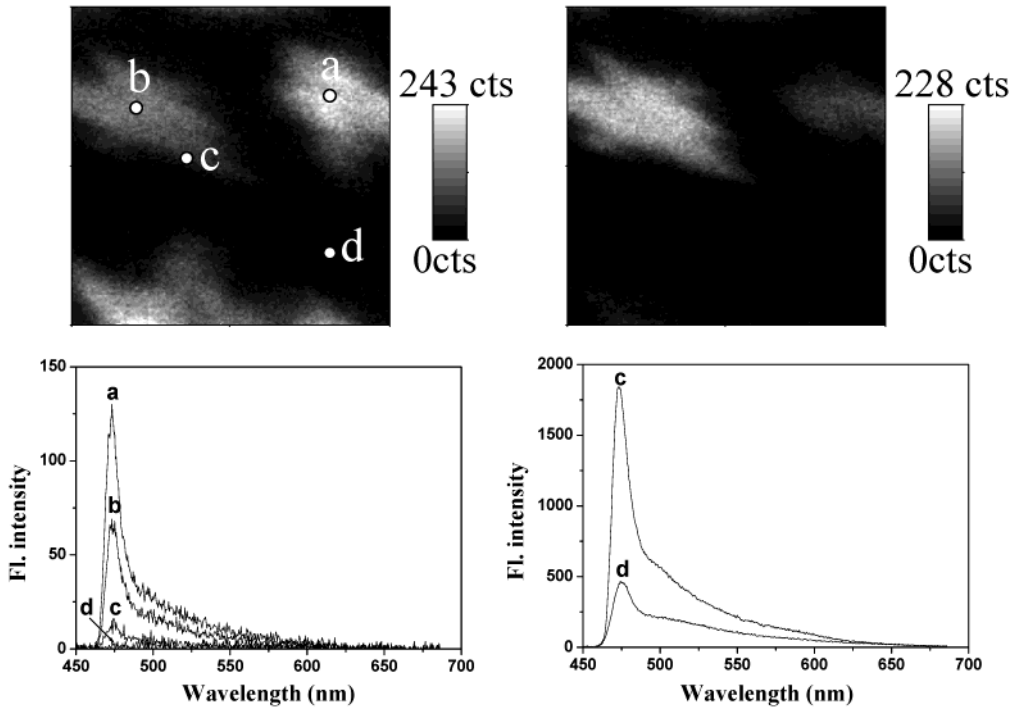

Figure 6. (Top) Small scale fluorescence micrographs of THIAMS/DODAB LS films deposited at 30 mN/m with adsorption time of 30 min (size: 5 μm × 5 μm); left and right columns, detection of s- and p-polarized light. (Bottom) Emission spectra recorded at different positions indicated in the upper micrographs. (Excited by linear polarized laser at 458 nm; laser power was 100 nW (lower left) and 2 μW (lower right).)

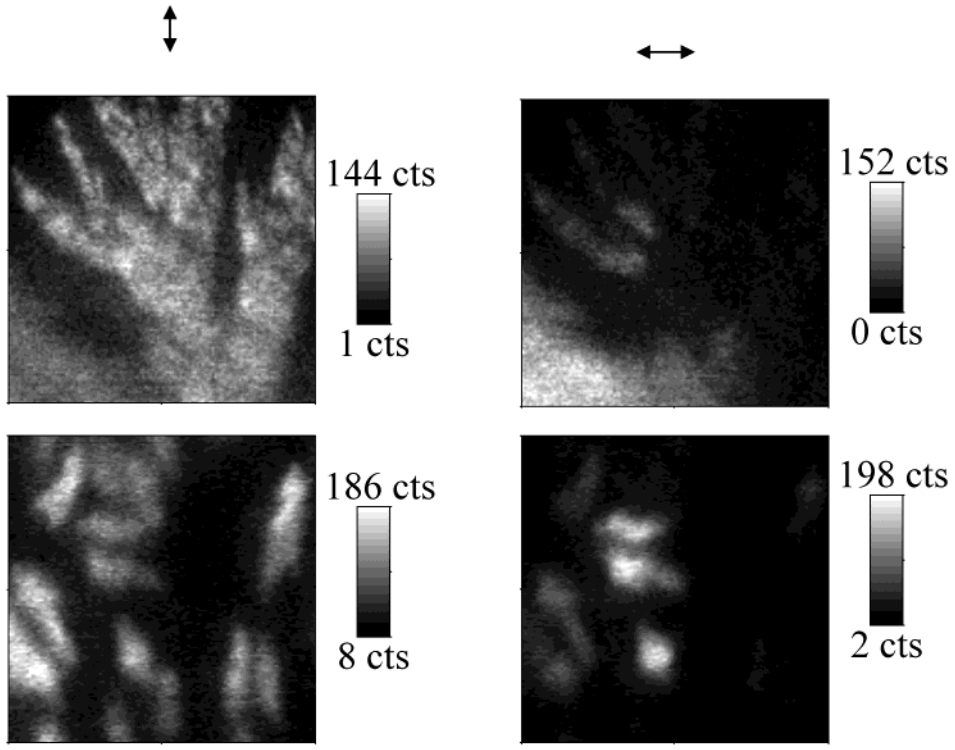

Figure 7. Fluorescence micrographs of THIAMS/DODAB LS films deposited at (upper) 15 mN/m with adsorption time of 75 min (size: 25 μm × 25 μm) and (lower) 30 mN/m with adsorption time of 30 min (size: 10 μm × 10 μm). (Excited by circular polarized laser at 458 nm, glass instead of dichroic mirror, 457.5 nm notch filter, 460 nm long pass filter. The arrows show the direction of the polarization of polarizers in the path of the detectors.)

Atomic Force Microscopy (AFM). The morphologies and thickness of the THIAMS/DODAB LS films were investigated by AFM using the noncontact mode. AFM observations of bare glass and silianized glass revealed a flat surface with a number of high spots (8−12 nm height, not shown) and with a cross section of 70−200 nm. The morphology of the THIAMS/DODAB LS films deposited at 15 and 30 mN/m are shown in Figures 8 and 9 respectively and differ strongly from those of bare glass or silanized glass. The figure in the lower-right corner shows the cross section of AFM image along the line shown in the upper-right figure. The morphologies of the domains observed in the AFM micrograph have almost the same shape of the domains in fluorescence micrographs obtained

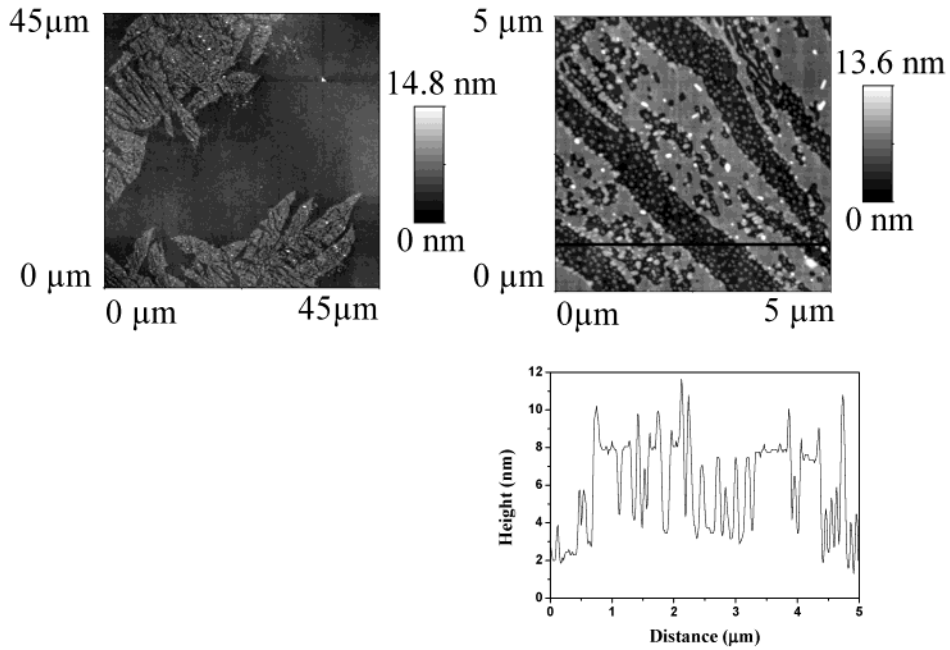

Figure 8. (Top) Topography images of THIAMS/DODAB LS film deposited at 15 mN/m with adsorption time of 60 min. (Bottom) Line scan recorded at the position of the horizontal line in the upper right figure.

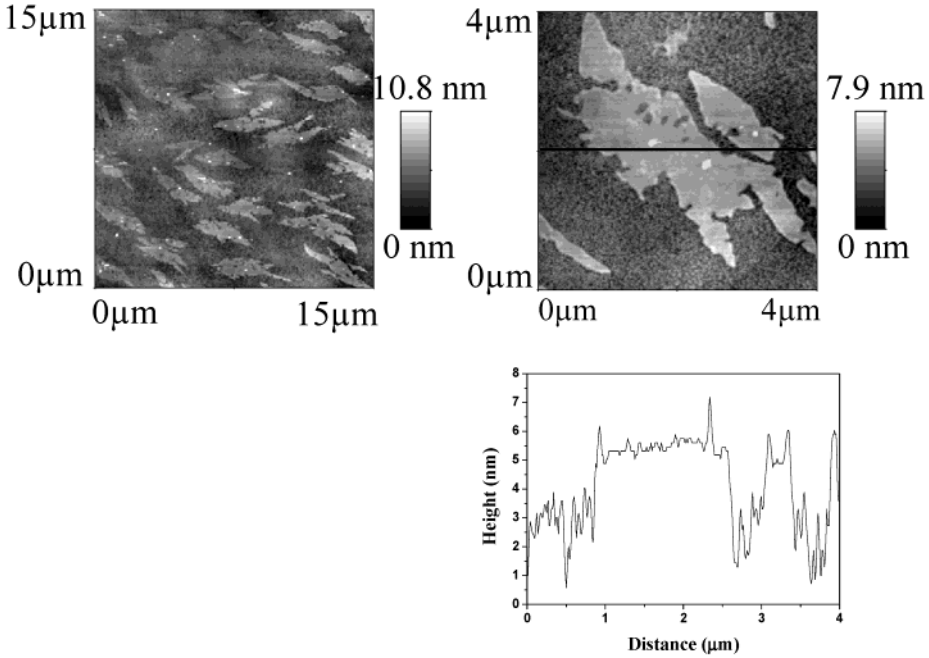

Figure 9. (Top) Topography images of THIAMS/DODAB LS film deposited at 30 mN/m with adsorption time of 30 min. (Bottom) Linescan recorded at the position of the horizontal line in the upper right figure.

at the same surface pressure shown before. This indicates that the structures observed in the AFM micrographs correspond to J aggregates.

The thickness of the big flat islands, yielding J aggregates fluorescences was at both 15 and 30 mN/m around 4.5 ± 0.2 nm, which corresponds to the thickness of one DODAB layer (3 nm)[53,54] and the thickness of a monolayer of an edge-on oriented thiacyanine dye (1.5 ± 0.2 nm).[55] Although the compound investigated by Asanuma et al.[55] had a different structure this will mainly reflect in an increase of the long axis of the dye. These results agree with earlier studies on LS film of THIATS adsorbed DODAB.[30] The morphology of the areas between the large flat domains shows many small circular islands with a height of about 4.0 ± 0.5 nm for the film deposited at 15 mN/m (cross section) which may also consist of DODAB monolayer and THIAMS. In the film deposited at 30 mN/m, a layer with a few grooves, where the thickness amounted to 3 ± 0.5 nm, was formed between the large islands. As the thickness is almost same as that of the DODAB monolayer and as we observed fluorescence of monomers and dimers of THIAMS from this part of the film, we assume that at those sites the adsorbed THIAMS

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molecules are not densely packed while their molecular plane is more or less parallel to the air/water interface.

Conclusions

Monolayers of J aggregates of THIAMS adsorbed onto the DODAB observed after transfer to a glass substrate by the Langmuir−Schaefer technique, showed domains with a shape depending upon the surface pressure. In the LS film deposited at 15 mN/m, the J aggregates were irregular shaped and grew larger upon extending the adsorption time. When the deposition occurred at a surface pressure of 30 mN/m, much smaller spindle-shaped J aggregates formed. Increasing the adsorption time did not increase the size of domains but their number density. The THIAMS J aggregates in LS films are two-dimensional domains with a thickness of about 4.5 ± 0.2 nm, which consisted of one DODAB monolayer and one THIAMS layer. In all LS films, the polarization of the fluorescence indicated that the domains consist of smaller monocrystalline domains where orientation of the transition dipoles of all THIAMS molecules is correlated over several micrometers. In the dark areas between the fluorescence domains in the films, the number density of the adsorbed THIAMS molecules is much lower and a large fraction of the adsorbed THIAMS molecules are present as monomers and dimers.

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