Matrix dependent changes in metachromasy of crystal violet in Langmuir-Blodgett films

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Abstract

This communication reports the effect of building matrices and nano clay platelets on the aggregation pattern of a water soluble cationic fluorescent dye Crystal Violet (CV) in Langmuir-Blodgett (LB) Films. When stearic acid (SA) was used as a building matrix, pressure induced changes in metachromasy was observed in the SA-CV complex LB films with a characteristic J-band formed at the longer wavelength side. On the other hand, clay incorporated hybrid LB films showed high degree of control over H-dimeric band. Isotherm characteristics, Brewster Angle Microscopic (BAM) images, UV–vis absorption and fluorescence characteristic studies were employed to investigate this result.

1. Introduction

Several dyes show Metachromasy in solid state, in ultrathin films, in the staining of biological tissues and even in solution. Metachromasy is a change in the shape of the absorption spectrum and the color of the dye with concentration or adsorption but with no chemical change in the dye itself [1]. This phenomenon can be seen in concentrated and even in dilute aqueous solutions of crystal violet (CV). In dilute aqueous solution of CV, UV–vis absorption spectrum shows intense band with peak at 590 nm and a shoulder at 550 nm [2]. The 590 nm peak is due to the presence of monomeric sites. With increasing solution concentration, 550 nm shoulder becomes intense and the 590 nm absorption band reduces to a shoulder. At higher concentration the 550 nm peak is further blue shifted [3]. The change in the spectrum is due to the aggregation of the dye molecules into dimmers and higher order aggregates. For dimeric sites, the absorption peak is at 550 nm. For higher order aggregates it is further blue shifted [4]. CV is soluble in water because it is an ionic compound. The dye ions themselves, however, are made up of large hydrophobic groups, and the charge is delocalized over most of the molecule. Thus, even at relatively low dye concentrations, the ions have a tendency to aggregate to minimize the interaction with water molecules, and the resulting π-electron interactions cause the aggregates to have a different absorption maxima [5]. A similar effect is responsible for Metachromasy of dyes when adsorbed on a preformed oppositely charged Langmuir monolayer at the air-water interface by electrostatic interactions [6]. A complex/hybrid Langmuir monolayer is thus formed at the air-water interface which can be transferred onto solid substrates to form mono- and multilayer Langmuir Blodgett (LB) films [6–10]. Depending on the various film forming parameters and molecular organizations in the LB films various kinds of dye Metachromasy were observed [11]. Red shifted absorption band with respect to the monomer absorption band is generally referred to as J-band formed due to J-type molecular aggregates. H-aggregates result in the blue shifted absorption band with respect to the monomer and it generally leads to the formation of H-dimmer resulting in the quenching of fluorescence intensity [12–14]. It reduces the fluorescence efficiency of a biological stainer. On the other hand H-aggregation of CV shows higher optical coefficients for third order nonlinear susceptibility and is used to study non-linear optical phenomena [15]. It may be mentioned in this context that several works reported the incorporation of water soluble ionic dyes into LB films and studied their characteristics. Aggregation behavior of a water soluble cationic dye Nile Blue A (NB) onto the inorganic nano clay sheets Laponite was investigated by LB technique [16]. H-aggregation of water soluble anionic dye merocyanine 540 (MC540) mixed with amphiphilic Octadecylamine (ODA) in LB films fabricated by co-spreading method was also reported [17]. Interaction of water soluble anionic dye Congo Red (CR) and cationic surfactant CTAB in presence of Ag+ ions and AgNPs was investigated and found that CR formed stable complex in presence of AgNPs [18].
Crystal Violet (CV, (tris[p-(dimethylamino)phenyl]methyl) has been the subject of interest in various fields of science due to its molecular structure, electronic states and the relaxation dynamics of the electronic states and good optical properties in the solid state [19–21]. It is also used as a biological stainer [21], dermatological agent [22] and considered as a drug for use in photodynamic therapy [23–25]. Being cationic, it can interact with negatively charged cell membrane surfaces and can enter into cells and concentrate in cytoplasm [26]. CV is carcinogenic and categorized as a recalcitrant molecule [27,28]. It is also widely used to test Gram-positive and Gram-negative bacteria [29,30]. CV is also used as a fluorescent tracer. It has a large molecular hyperpolarizability [31]. This important optical property has made this dye an interesting element for NLO – experiments [19,31] and near-field spectroscopy [32].

Smectite clay such as Laponite (inorganic sheet-like natural mineral) has its potential applications over the few years, due to its high aspect ratio, unique optical, thermal, mechanical properties, high Cationic Exchange Capacity (CEC), special swelling properties, large surface areas, surface reactivities, adsorptive properties, colloidal size, permanent structural charge, high viscosity and transparency in solution, etc. [33,34]. They are generally used as spacer/inert modifiers and film-forming agents with amazing properties. Recently organo-clay hybrid films have various technological applications due to their unique semiconducting, conducting, non-linear, dielectric properties [35,36].

In the present communication detailed investigations have been carried out to investigate the controlling of aggregates in the ultra-thin films of CV fabricated by Langmuir-Blodgett technique. Effect of nano clay platelets and ionic nature of the surfactants in controlling the aggregation of CV in the LB films have also been investigated.

2. Experimental section

2.1. Chemicals

Crystal Violet (CV), Stearic Acid (SA) and Octadecylamine (ODA) were purchased from Aldrich Chemical Co. and used as received. Solvent chloroform (SIL, India) was of spectroscopic grade and its purity was checked by fluorescence spectroscopy before use. The clay mineral Laponite used in this study was obtained from the source clays repository of the clay minerals society.

2.2. Instruments

Surface pressure vs. area per molecule (π–A) isotherm measurements of complex/hybrid Langmuir monolayers and preparation of monolayer LB Films were done by a commercially available Langmuir-Blodgett film deposition instrument (Apex 2006C). The Brewster Angle Microscope (BAM) images of complex/hybrid films were taken by a commercially available in-situ Brewster Angle Microscope (Accurion, nanofilm_EP4) attached to a KSV Langmuir-Blodgett instrument. Ultra-pure Milli-Q (18.2 MΩ-cm) water was used for the aqueous subphase of the Langmuir trough, for the preparation of aqueous clay dispersion also used in the Langmuir trough and for the preparation of aqueous solution of CV. Aqueous clay dispersion was prepared at various clay concentrations ranging from 10 to 80 PPM. Aqueous clay dispersion was stirred for 24 h and then sonicated for 30 min prior to use. The temperature was maintained at 24 °C throughout the experiment. UV–vis absorption and fluorescence spectra were recorded by UV–vis absorption spectrophotometer (Lambda 25, Perkin Elmer) and Fluorescence spectrophotometer (LS-55, Perkin-Elmer) respectively. The quartz and silicon substrates used for LB film preparations were thoroughly cleaned prior to use. The substrates were first of all cleaned with soap solution for removal of grease/dirt. Then the slides were treated with chromic acid for 30 min and washed with de-ionized water. Further they were rinsed by acetone followed by chloroform and then stored in an oven for drying. Atomic Force Microscopic (AFM) image of ODA-Clay-CV hybrid LB monolayer was taken in air with a commercial AFM system (Innova AFM system, Bruker AXS Pte Ltd.). AFM image was obtained in intermittent-contact (‘tapping’) mode. Typical scan area was 2 × 2 μm². Monolayer on Si wafer substrate was used for the AFM measurement. Experiment was performed at room temperature (24 °C). Relative humidity was kept under 60% (non-condensing). The pH of the subphase was kept fixed at 6.5 in equilibrium with atmospheric carbon dioxide throughout the experiments.

2.3. Methods

For surface pressure vs. area per molecule (π–A) isotherm studies and for the recording of BAM images, first of all stock solutions of SA (0.5 mg/ml) and ODA (1 mg/ml) were prepared using Spectroscopic grade chloroform. Stock solution of CV was prepared by dissolving it into ultra-pure Milli-Q water (10⁻⁴ M). For the studies of π–A isotherms and LB films preparations, the total volume of the subphase was kept fixed at 400 ml in all the cases.

To record the π–A isotherm of pure SA monolayer at the air-water interface, the subphase of the Langmuir trough was filled up with Milli-Q water and then 80 μl of chloroform solution of SA was spread on the aqueous subphase of the Langmuir trough by using a micro-syringe. After waiting for sufficient time to evaporate the solvent, the barrier of the Langmuir trough was compressed slowly to study the isotherm characteristics. For the π–A isotherm studies of SA-CV complex monolayer, first of all SA-CV complex Langmuir monolayer was prepared at the air-water interface of the Langmuir trough. For this purpose 2000 μl of aqueous solution of CV (10⁻⁴ M) was dissolved in the aqueous subphase of the Langmuir trough and then 60 μl of chloroform solution of SA was spread on the aqueous subphase of the Langmuir trough by using a micro-syringe. After waiting for sufficient time (1 h) to complete the reaction, the monolayer was compressed slowly to obtain π–A isotherm. The complex monolayer at the air water interface was also transferred onto solid substrates at different surface pressures (10, 15, 18, 20 and 25 mN/m) to form monolayer LB films. Lifting speed was kept at 5 mm/min.

To incorporate anionic nano clay platelets in the LB films, first of all aqueous clay dispersions were prepared at various PPM clay concentration (namely 10–80 PPM). The aqueous clay dispersion was stirred overnight followed by 30 min sonication prior to use in the Langmuir trough. Preformed Langmuir monolayer of cationic ODA molecules was prepared for the adsorption of anionic nano clay platelets onto the ODA monolayer by electrostatic interactions. Thus ODA-Clay hybrid Langmuir monolayer was formed at the air-water interface. For the preparation of ODA-Clay-CV hybrid monolayer, 2000 μl of aqueous solution of CV (10⁻⁴ M) was mixed with the aqueous clay dispersion (various clay PPM concentrations namely 80–100 PPM) and sonicated prior to use in the Langmuir trough. Being cationic CV molecules interacted electrostatically with the anionic SA molecules and formed SA-CV complex Langmuir monolayer at the air-water interface. For the preparation of ODA-Clay-CV hybrid monolayer, 2000 μl of aqueous solution of CV (10⁻⁴ M) was mixed with the aqueous clay dispersion (various clay PPM concentrations namely 80–100 PPM) and sonicated prior to use in the Langmuir trough. Being cationic CV molecules interacted electrostatically with anionic nano clay platelets and got adsorbed onto the nano clay platelets. Thus Clay-CV hybrid molecules were formed in the aqueous dispersion of the Langmuir trough. When cationic ODA molecules were spread at the air-water interface of the Langmuir trough, then CV tagged nano clay platelets were further adsorbed onto the preformed cationic ODA monolayer and thus ODA-Clay-CV hybrid monolayer was formed at the air-water interface. After waiting for sufficient time (1 h) to complete the reaction, the hybrid
monolayer was compressed slowly to obtain \( \pi \)-A isotherm. The hybrid monolayer was also transferred onto solid substrates at a desired surface pressure to form mono- and multilayered LB films.

3. Results and discussion

3.1. Molecular structure and area of the CV

Inset of Fig. 1(a) shows the molecular structure of CV. One cationic charge is associated with the \( N^+ \) ions. In the solution as well as in the solid phase CV form planer structure and the molecular area of CV was found to be 1.2 nm\(^2\) [37]. SA-CV complex molecule was formed when anionic head group of SA molecule was attached electrostatically to the \( N^+ \) cation of the CV molecule. This is also shown in the inset of Fig. 1(a). At the air-water interface of the Langmuir trough when SA-CV complex monolayer was formed, if CV molecules lying flat at the air-water interface of the Langmuir trough, area per molecule would have been of the order of 1.2 nm\(^2\). Any deviation of this area per molecule indicates the tilted orientation of the CV molecules in the complex monolayer at the air-water interface. The area per molecule of any monolayer can be calculated from the surface pressure vs. area per molecules (\( \pi \)-A) isotherm characteristics curve. It was discussed in Section 3.2, that the area per molecule at any surface pressure remained far below 1.2 nm\(^2\), which is the area per molecule of CV in lying flat conformation. It strongly indicated the tilted orientation and even vertical orientation of CV molecules at higher surface pressure.

3.2. \( \pi \)-A isotherm studies of SA and SA-CV complex monolayer at the air-water interface

Fig. 1(a) shows the \( \pi \)-A isotherm characteristic studies of the Langmuir monolayer of pure SA (graph-I) and SA-CV complex (graph-II). The \( \pi \)-A isotherm of pure SA monolayer showed a rise of surface pressure with decreasing area per molecule and has the characteristic kink at 25 mN/m known as lateral transition pressure for long-chain fatty acids. The lift-off area of SA was found to be 0.276 nm\(^2\) determined by the method described by Ras et al. [38]. The molecular areas of 0.23 nm\(^2\) and 0.21 nm\(^2\) at surface pressures 15 mN/m and 25 mN/m of SA isotherm are in good agreement with the reported results [39]. The \( \pi \)-A isotherm of complex SA-CV monolayer showed a larger lift off area (0.37 nm\(^2\)) that of pure SA monolayer, which was the clear evidence of the formation of SA-CV complex monolayer at the air-water interface. At about 18 mN/m surface pressure, there was a phase transition which was due to the orientation of the complex molecules at the air-water interface. This orientation might lead to different types of molecular organizations in the ultrathin films. At 15 mN/m surface pressure the area per molecule of the complex monolayer became 0.25 nm\(^2\) which was greater than the area per molecule of pure SA monolayer (0.23 nm\(^2\)) at the surface pressure, but far below the area per molecule of CV when it was lying flat at the air-water interface (1.2 nm\(^2\)). At lower surface pressure although the lift of area (per molecule) of the complex monolayer was to some extent larger (0.37 nm\(^2\)) but still it was much smaller than the 1.2 nm\(^2\) area per molecule of CV in lying flat conformation. It indicated that in the complex monolayer, CV molecules did not lying flat at the air-water interface rather it formed tilted orientation. At 25 mN/m surface pressure, the area per molecule of the complex monolayer was 0.22 nm\(^2\) which was almost equal to the area per molecule of pure SA monolayer. It indicated almost vertical orientation of the CV molecules in the complex monolayer. When this monolayer was transferred onto solid substrates at different surface pressures to form LB monolayer, CV molecules were stacked keeping their vertical orientation and tilted orientation respectively. The organization of CV molecules in the LB monolayer lifted at lower and higher surface pressures are schematically shown in Section 3.4.

Fig. 1. [a] \( \pi \)-A isotherms of (i) SA monolayer, (ii) SA-CV complex monolayer; [b] C-\( \pi \) graphs of pure SA monolayer and SA-CV complex monolayer.
3.3. Compressibility studies of complex monolayer at the air-water interface

The compressibility can be extracted from the \( \pi-A \) isotherms of the Langmuir monolayer at the air-water interface. It is often used to characterize the nature of a monolayer's stable phases and has been correlated with particular phase behavior. Compressibility (C) is calculated using the following standard thermodynamic relation in two dimensions \( C = -(1/A)(dA/dr) \), where A is the area per molecule at the indicated surface pressure \( \pi \) \([40,41]\). Different phases of Langmuir monolayer are reflected as peaks in the compressibility vs. surface pressure \( C-\pi \) curve. The presence of a peak in the curve represents the inherent phase transitions involved in the system. Fig. 1(b) showed the \( C-\pi \) curve of pure SA monolayer and SA-CV complex monolayer at the air-water interface and calculated from the data of compression isotherms. At the lower surface pressure region of 0–15 mN/m, both the curves showed compressible in nature but complex monolayer showed more compressible than the pure SA monolayer. In the complex monolayer a sharp peak in the 18–22 mN/m surface pressure region indicated a phase transition which was absent in pure SA monolayer. This was also confirmed from isotherm characteristic studies. In SA monolayer a small peak in the \( C-\pi \) curve at 25 mN/m surface pressure indicated the starting of the solid phase. In the complex monolayer the sharp phase transition led to the orientational change and compact molecular packing. The change in the photophysical behavior in the light of this phase transition has been discussed later in the section UV–vis absorption spectra.

3.4. Schematic representation of CV molecules in the complex LB monolayer

When the complex LB monolayer was lifted at higher surface pressure of 25 mN/m, the stacking of vertically oriented CV molecules is shown schematically in Fig. 2 (H-aggregate). In the monolayer film this types of molecular stacking leads to the formation of H-aggregates \([16]\). H-aggregates generally form H-dimeric sites. It gives high energy H-dimeric band in the UV–vis absorption spectrum. On the other hand, CV molecules lying in a tilted orientation in the complex LB monolayer when lifted at a lower surface pressure Fig. 2 (J-aggregate). Stacking of tilted CV molecules in the LB monolayer most favorably forms J-aggregate \([42]\). It leads to the longer wavelength J-band in the UV–vis absorption spectrum. UV–vis absorption spectra of these films have been discussed in Section 3.6.

3.5. In-situ Brewster Angle Microscopic (BAM) images of SA-CV complex monolayer at the air-water interface

The phase behavior of the complex monolayer at the air-water interface is the most important aspect, which can be investigated by BAM images \([43,44]\). With increasing surface pressure, domains of different sizes and shapes are observed in the Langmuir monolayer indicating phase transition and formation of micro-domains at the air-water interface. In this investigation BAM images have been used for the direct visualization of micro-domains and morphological information of SA-CV complex monolayer formed at the air-water interface. After spreading chloroform solution of SA on the Langmuir trough containing desired amount of CV aqueous solution and then compressing the monolayer, BAM images were taken at different surface pressures namely (a) 15 mN/m, (b) 18 mN/m, (c) 20 mN/m and (d) 25 mN/m as shown in Fig. 3. Image (a) shows the presence of large number of small dimensional circular domain at the air-water interface. In some cases few circular domains were attached to form a chain like structure. At 18 mN/m surface pressure, image (b) shows a large number of linear worm like structure formed by the linear combination of circular domains. At 20 mN/m surface pressure, image (c) shows highly concentrated large dimensional circular domains. Linear arrays of the circular domains are also observed. At higher surface pressure of 25 mN/m, image (d) shows a highly concentrated closed pack structures indicating the attainment of solid phase. From the BAM images it becomes evident that the monolayer film at the air-water interface has gone through a phase transition just above 20 mN/m surface pressure. This was also supported by the \( \pi-A \) isotherms and compressibility studies of the complex monolayer at the air-water interface. UV–vis absorption studies discussed later showed clearly the changes in the aggregation pattern before and after the phase transition.

3.6. UV–vis absorption spectra of SA-CV complex Langmuir-Blodgett (LB) film

UV–vis absorption spectrum of CV in aqueous solution consists of two absorption bands arising due to \( \pi-\pi^* \) transitions \([45]\). A longer wavelength absorption band with intense peak at 590 nm is the monomeric band while the band at 550 nm is assigned as H-dimeric band.

Fig. 4(a) shows the normalized UV–vis absorption spectra of the aqueous solution of CV \((10^{-5} \text{ M})\) and SA-CV complex LB monolayer films lifted at different surface pressures namely 10, 15, 18, 20 and

![Fig. 2. Schematic representation of SA-CV complex LB films lifted at [a] 20 mN/m surface pressure; [b] 25 mN/m surface pressure.](image-url)
25 mN/m. These surface pressures have been chosen as there might have been some orientational/organizational changes of the molecules in the LB films at these surface pressures, as would be inferred from the isotherm characteristic studies, compressibility studies, schematic representation and BAM images.

UV–vis absorption spectrum in aqueous solution of CV (10⁻⁵ M) shows highly intense monomeric band at 590 nm and weak hump of H-dimeric band at 550 nm. Even at much lower concentrations of CV (10⁻⁷ M) in aqueous solution a small hump of H-dimeric band was found at 550 nm (figure not shown). Generally at such low concentration dimeric band does not exist. But with increasing solution concentration this band increased and in solid state of the LB film this band became highly intense and blue shifted. As discussed by several authors [4] the origin of this band is due to the formation of dimer and higher order aggregates. The existence of this band even at very low concentration in the aqueous solution may be due to the planer structure of the molecule. It favours the closer association of several CV molecules even at very low concentration in the aqueous solution.

Films lifted at 10 and 15 mN/m surface pressures showed a broad band with two overlapping peaks at around 610 nm and 540 nm. At 18 and 20 mN/m surface pressures, the UV–vis absorption spectra showed intense longer wavelength band with peak at 610 nm and comparatively low intense band with high energy shoulder at 540 nm. However for the LB film fabricated at 25 mN/m surface pressure an intense high energy band at 540 nm along with a weak hump at 610 nm was observed. The sharp changes in the UV–vis absorption spectra of the LB monolayer film lifted at 20 mN/m surface pressure and 25 mN/m surface pressure was an indication of pressure induced organizational changes of the complex monolayer. As discussed schematically in Section 3.1, in the LB monolayer lifted at higher surface pressure, stacking of CV molecules formed H-dimeric sites. Whereas in the LB monolayer lifted at lower surface pressure, J-aggregation became predominant. This thesis has also been confirmed from π–A isotherm, C–π characteristics studies and BAM images. Thus intense H-dimeric band was found in the UV–vis absorption spectrum of complex LB monolayer lifted at 25 mN/m surface pressure.

Another interesting point to note is that in the LB films lifted at 20 mN/m surface pressure, there was a shifting of about 20 nm in the longer wavelength UV–vis absorption band with respect to the monomeric band at 590 nm. Gaussian deconvolution of the two peaks showed sharp intense longer wavelength band with peak at 610 nm (Fig. 4b). This is the characteristic feature of the J-band [41].

Inset of Fig. 4(a) shows the layer effect of SA-CV complex LB films lifted at 20 mN/m surface pressure. From the graph it was observed that, with increasing layer number, the intensity of high energy H-dimer band was also increased. It may be that with increasing layer number, orientational changes of the complex molecules took place resulting in the favorable condition for increased H-dimeric sites.

3.7. Effect of nano clay platelets Laponite in controlling the H-dimer formation of CV

3.7.1. Inclusion of nano clay platelets in aqueous solution

Anionic nano clay platelets Laponite has a plate like structure with a large surface charge density [46]. As a result, cationic CV molecules were adsorbed on the surface of anionic nano clay platelets by electrostatic interactions and formed hybrid molecules [16]. Effect of clay on the UV–vis absorption spectra of CV molecules has been investigated both in the aqueous solutions and in the LB films.

Fig. 5 shows the UV–vis absorption spectra of CV in aqueous clay dispersion having clay concentrations varying from 10 to 80 PPM along with the pure aqueous solution. As discussed earlier, aqueous solution absorption spectrum consists of intense monomeric peak at 590 nm along with H-dimeric shoulder at 550 nm. At lower clay concentration of 10 PPM, monomeric peak was reduced to a weak shoulder and an intense high energy band with peak at 525 nm was observed. As discussed earlier, this blue shifted band at 525 nm was originated due to higher order aggre-
gates \[4\]. With increasing clay concentration the 525 nm band was again red shifted and at higher clay concentration of 80 PPM this band coincided with the dimeric band at 550 nm. The intensity was also reduced while the intensity of the monomeric band was increased to a considerable amount. It is clearly evident from this spectral data that at higher clay concentration, due to the presence of large number of nano clay platelets, CV molecules were adsorbed onto a large number of clay platelets thus reducing the chances of higher order aggregates. While at lower clay concentration chances of aggregating of large number of CV molecules onto the few clay platelets increased and thus forming the higher order aggregates. Also at higher clay concentration, intensity of monomeric band with peak at 590 nm is also appreciable. It indicated the existence of monomeric species of CV molecules.

3.7.2. In-situ Brewster Angle Microscopic images of ODA-Clay-CV hybrid monolayer at the air-water interface

BAM images of the ODA-Clay-CV hybrid Langmuir monolayer were taken at a surface pressure of 10 mN/m and at different PPM of clay concentrations in the aqueous subphase. Fig. 6 shows the BAM images at (1) 10 PPM, (2) 30 PPM, (3) 50 PPM and (4) 80 PPM clay concentrations in the aqueous subphase.

Unlike the BAM images of SA-CV complex Langmuir monolayer, in this case, distinct circularly shaped domains were observed. At 10 PPM clay concentration the domains were smaller in size. However with increasing clay concentration the domains became larger in size and density of domains were also increased. From the distinct dissimilarities in the BAM images before and after inclusion of clay platelets, it became clearly evident that inclusion of clay platelets affected the molecular organizations in the Langmuir monolayer at the air-water interface. Laponite has plate like structure having dimension varying from 25 nm to 30 nm. However the circular domains as observed in the BAM images have several micrometer dimensions. Therefore it became evident that several ODA-Clay-CV hybrid molecules aggregated to form microcrystalline circular domains. The circular plate like Laponite clay platelet has clear influence in the formation of circular domains as shown in the BAM images. The nature of the aggregated species could not be ascertained from the BAM images however UV–vis absorption and Fluorescence spectroscopic studies discussed in the next section clearly showed the nature of the aggregated species.

3.7.3. UV–vis absorption spectra of ODA-Clay-CV hybrid Langmuir-Blodgett (LB) film

Fig. 7(a) shows the normalized UV–vis absorption spectra of the ODA-Clay-CV hybrid monolayer LB films prepared at different clay concentrations of 80–10 PPM in the aqueous subphase of the Langmuir trough. The surface pressure of lifting was kept fixed at 10 mN/m.

The UV–vis absorption spectrum of monolayer LB film prepared at 10 PPM clay concentration showed two distinct overlapping bands with peaks at 590 nm and 550 nm. The two bands have been assigned as monomer and H-dimer. There was no shifting of the peak positions unlike that in case of SA-CV complex LB films. With increasing clay concentrations, dimeric band reduced in intensity while intensity of monomeric band increased. At 80 PPM clay concentration, the monomeric peak at 590 nm became intense and the dimeric band was totally absent. Thus it was clearly observed that inclusion of clay platelets in the LB film, could control the dimeric
band and increased the monomeric species. It might be that at higher clay concentration, CV molecules got adsorbed onto a large number of clay platelets and relatively less number of CV molecules got adsorbed on a single clay platelet and thus reducing the chances of formation of aggregates of CV molecules on a single clay platelet. Inset of Fig. 7(a) shows the plot of the ratio of intensity of dimeric and monomeric bands vs. Clay concentration (PPM) of monolayer LB films. It shows the presence of a larger number of dimeric species in the monolayer LB film prepared at lower PPM clay concentration. With increasing PPM clay concentration monomeric band increased in intensity.

Fig. 7(b) shows the UV–vis absorption spectra of different layered (1–11) layer ODA-Clay-CV LB films prepared at 80 PPM clay concentration. With increasing layer number dimeric band at 550 nm was observed along with the intense monomeric peak at 590 nm. It might be that with increasing layer number, stacking and overlapping of CV tagged clay platelets occurred in the LB films resulting in the changes in the molecular organizations. Thus dimeric species increased.

3.7.4. Fluorescence spectra of ODA-Clay-CV hybrid LB films lifted at different PPM clay concentrations

In an aqueous solution, CV fluoresces weakly due to rapid rotation of its phenyl rings which results a fast relaxation process and provides a non radiative decay path for the excited singlet state [17]. The fluorescence intensity increases when the dye is present in rigid media and when it is bound to the polymer like substrates and surfactants [20]. CV in a rigid media gives emission at around 620 nm and with increasing concentration of a surfactant, it is slightly shifted to 630 nm. Again the formation of H-dimer of dye molecules in the ultra-thin films reduces its fluorescence intensity greatly. So the controlling of H-dimer formation of the dye molecules in the ultra-thin film becomes utmost important.

Fig. 8 shows the fluorescence spectra of ODA-Clay-CV hybrid LB films lifted at different PPM clay concentrations varying from 10 to 80 PPM. From the figure it was observed that at 80 PPM clay concentration, the fluorescence intensity became maximum indicating the predominance of monomeric sites in the ODA-Clay-CV hybrid LB film. With decreasing PPM clay concentration, the dimeric band increased which reduced the fluorescence intensity as observed.
form the graphs and showed a minimum intensity at 10 PPM clay concentration.

4. Atomic force microscope

Fig. 9 shows the AFM image of ODA-Clay-CV hybrid monolayer LB film deposited onto smooth silicon substrate. The surface pressure was maintained at 10 mN/m. The Laponite nano clay platelets are clearly visible in the AFM image. The surface coverage is more than 90%. The dimensions of ODA and CV are beyond the scope of the resolution. AFM image gives clear visual evidence of the inclusion of nano clay platelets in the hybrid monolayer and thus formation of ODA-Clay-CV hybrid LB film.

5. Conclusion

In conclusion, our results showed the effect of building matrices and nano clay platelets on the aggregation pattern of CV molecules in the LB films resulting in metachromasy. At particular surface pressures of SA-CV complex monolayer, CV molecules were organized to form J-aggregates. This was happened at the phase transition surface pressure of the complex monolayer as was observed from the \( \pi-A \) isotherm studies. The longer wavelength sharp J-band was found in the UV–vis absorption spectra of the complex film. When nano clay platelets were incorporated in the LB film, then at high clay concentration, only monomeric species were available. This was inferred from the existence of intense monomeric absorption peak at 80 PPM clay concentration in the clay incorporated LB film. At such higher clay concentration, dimeric species of CV molecules remained absent in the LB film. It increased the fluorescence intensity as observed from the fluorescence spectroscopic studies. Thus clay incorporated LB film of CV
can be used as an efficient fluorescent tracer and biological fluorescent stainer.

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References


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