Research paper

Surfactant concentration dependent metachromasy of an anionic cyanine dye in adsorbed and deposited Langmuir films

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ABSTRACT

This communication reports the metachromasy or the changes in shapes and positions of the absorption spectra of an anionic cyanine dye merocyanine 540 abbreviated as MC540, in aqueous solutions and in LB films when electrostatically interacted with a well-known cationic surfactant cetyltrimethylammonium bromide abbreviated as CTAB. Critical Micellar Concentration of CTAB affected largely on the dissociation of MC540 molecular aggregates in the CTAB-MC540 complex LB films. Spectroscopic studies of these LB films showed only monomeric absorption band and intense fluorescence band. This complex LB film can act as efficient fluorescence probe for several biological systems.

1. Introduction

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. Merocyanine 540 (MC540) is an anionic cyanine dye which shows Metachromasy under various conditions such as solvent polarity, physical state, temperature, etc. [1–4]. Metachromasy of a cyanine fluorescent dye in liquid phase and in solid phase are of great interest due to its potential applications in many technological fields [4–6].

In MC540 the heterocyclic aromatic groups are linked by a poly-methine chain (inset of Fig. 1). This dye has remarkable sensitivity to the surrounding medium, it is extensively used in many research areas such as photographic sensitizers, fluorescent probe, filters, textiles, chemotherapy [2,3,7]. MC-540 is widely used as a fluorescent probe in membrane studies, in clinical applications to differentiate normal and malignant cells and in photodynamic therapy [8,9]. It is also used as an external optical probe to study the structure and function of biological membranes as it binds specifically with cell membrane due to the presence of two methylene tails which are embedded easily inside membrane [9–11]. Both the photophysical and photochemical properties of this dye strongly depend on the changes in environmental factors and degree of aggregation. It is a useful probe in biophysical and photophysical research areas and determination of its photophysical properties in various environments is vital for technological applications. Due to the amphipathic nature, MC540 is soluble in solvents of a wide polarity range, including water and in chloroform.

UV–vis absorption spectrum of MC540 in aqueous solution shows two distinct absorption bands with peaks at 500 nm and 535 nm which have been assigned due to non-fluorescent H-dimeric and monomeric bands respectively [12]. With increasing dye concentration in the aqueous solution intensity of the dimeric band increases and monomeric band reduces in intensity. In chloroform solution, UV–vis absorption spectrum of MC540 shows metachromasy with respect to ‘water peak’ [13]. In dilute chloroform solution, MC540 shows monomeric band at 570 nm and dimeric band at 528 nm. With increasing dye concentration in chloroform solutions, the 528 nm band is further blue shifted [13]. This change in the spectrum is due to formation of higher order aggregates of the dye molecules at such dye concentrations. In aqueous solution MC540 binds to micelles and the non-fluorescent H-dimeric band dissociates to form fluorescent monomer and this is responsible for the fluorescence enhancement of MC540 [12,14]. Metachromasy was also observed in the complex Langmuir–Blodgett films of MC540 and a cationic surfactant Octadecylamine (ODA) [13]. Anionic MC540 interacted electrostatically with the cationic ODA and formed a stable Langmuir monolayer at the air–water interface. ODA-MC540 mixed Langmuir Blodgett (LB) films showed intense H-dimeric band at 528 nm and monomer band at 571 nm [13]. Multi-layered LB films showed intense H-dimeric band and the monomer band reduced to a weak shoulder. A new band was also developed in the high energy region.
which was the higher order aggregated band. However H-dimeric band being non fluorescent, fluorescence intensity was reduced to a larger extent. It may be mentioned in this respect that Langmuir-Blodgett technique is a unique thin film fabrication technique due to its ability to control the morphology and ultrastructure of the film by changing various film forming parameters [15–17]. It should be mentioned here that interaction of water soluble anionic dye with cationic lipid monolayer has been reported elsewhere.

In the solid state restricted geometry of ultrathin film, an effort should be given to obtain intense monomeric band in order to get intense fluorescence intensity in the ultrathin film. It may have profound applications in the field of biological stainer, photosensitizer, fluorescence probe, etc. [18–21].

In the present communication detailed works have been carried out to investigate the surfactant micellar concentration on the metachromasy of the anionic dye MC540 in the aqueous solutions and in the LB films. The LB films were prepared from the complex Langmuir monolayer at the air-water interface having different CTAB concentrations in the aqueous subphase of the Langmuir trough. A well-known cationic surfactant cetyltrimethylammonium bromide (CTAB) has been used in our investigation. CTAB forms micelles at 0.92 mM concentration in aqueous solution [22]. Being anionic MC540 interacted electrostatically with the cationic CTAB molecules in the aqueous solution of the Langmuir trough and combined to form a complex molecule which is LB compatible. In Langmuir monolayer at the air-water interface, organization of the complex molecules led to the formation of distinct micro-domains as have been observed by in-situ Brewster Angle Microscopic (BAM) images. Mono- and multi-layered LB films have been prepared from this complex Langmuir monolayer at various CTAB concentrations in the aqueous subphase of the Langmuir trough. UV–vis absorption spectroscopic studies revealed the formation of monomeric sites for films prepared above critical micellar concentration (cmc) of CTAB in the aqueous subphase of the Langmuir trough. Multi-layered LB films also retained this structure. It was observed that at such multi-layered complex LB films fluorescence intensity of MC540 increased remarkably. Thus in the solid state restricted geometry of ultrathin film, MC540 can act as an efficient fluorescence probe for biological molecules.

2. Experimental

2.1. Chemicals

Merocyanine 540 (MC540), Octadecylamine (ODA), Cetyltrimethylammonium Bromide (CTAB) were purchased from Sigma Aldrich Chemical Company. Solvent chloroform (SLR, India) was of spectroscopic grade and its purity was checked by fluorescence spectroscopy before use.

2.2. Instruments

Mono- and multi-layered LB films of CTAB-MC540 complex Langmuir monolayer were prepared by using a commercially available Langmuir-Blodgett film deposition instrument (Apex-2006C). The trough area of the Langmuir trough used in our experiment was 250 × 148 mm. Surface pressure vs. time (π-t) characteristics of spectroscopic grade and its purity was checked by fluorescence spectroscopy before use.

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 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.

2.3. Methods

For recording the surface pressure vs. time (π-t) characteristics studies and taking the BAM images at different concentrations of CTAB namely 0.7 mM (less than cmc), 0.92 mM (at cmc) and 1.0 mM (above cmc) in the subphase of the Langmuir trough, first of all desired amount of CTAB and MC540 was mixed so that in the mixed solution, the CTAB concentration was maintained at 24 °C throughout the experiment and the pH of the subphase was maintained at 6.5 in equilibrium with atmospheric carbon dioxide throughout the experiments.

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 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.
Langmuir balance with the passage of time to get surface pressure vs. time (π-t) characteristics curves. It may be mentioned in this context that the experiment was performed by using the aqueous solutions of the dye MC540 and CTAB separately in the Langmuir trough. No such increase of surface pressure was observed with the passage of time (figures not shown). It indicated that no stable Langmuir monolayer was formed at the air-water interface when the dye and CTAB were separately studied. A stable Langmuir monolayer was formed only when the aqueous solutions of anionic dye MC540 and the cationic CTAB were mixed in the Langmuir trough then surface pressure started increasing with the passage of time indicating the formation of complex Langmuir monolayer at the air-water interface. Several of such works were reported where water soluble oppositely charged materials were used to form stable Langmuir monolayer by electrostatic interaction [23,24].

In all three cases BAM images were taken at different surface pressures namely 5 mN/m, 15 mN/m and 20 mN/m during the formation of Langmuir monolayer at the air-water interface.

To study the photophysical characteristics (UV-vis absorption and fluorescence spectroscopy), CTAB-MC540 complex Langmuir monolayer was deposited onto quartz substrates at 20 mN/m surface pressure to obtain mono- and multi-layered (11 layer) Langmuir-Blodgett (LB) films. Lifting speed was kept at 5 mm/min. The transfer ratio was estimated by calculating the ratio of decrease in the subphase area to the actual area on the substrate coated by the layer and was found to be 0.98 ± 0.02.

3. Results and discussions

3.1. Surface pressure vs. time (π-t) characteristics studies of CTAB-MC540 complex Langmuir monolayer at different CTAB concentration in the subphase of the Langmuir trough

Fig. 1 shows the surface pressure vs. time (π-t) characteristics curves of CTAB-MC540 complex Langmuir monolayer at the air-water interface at different subphase concentrations namely 0.7 mM (graph-i), 0.92 mM (graph-ii) and 1.0 mM (graph-iii) of CTAB.

From the figure, it was observed that the π-t curve obtained at 0.7 mM CTAB subphase concentration, the surface pressure rose about 28 mN/m within a time span of about 100 min and then almost flat region came. Whereas in case of 0.92 mM and 1.0 mM subphase concentrations of CTAB, surface pressure rose about 25 mN/m and 23 mN/m. In the latter two cases the lower surface pressures might be due to the formation of CTAB micellar structure. This has been discussed in detail in the schematic representation portion in the next section.

3.2. Schematic representation of CTAB-MC540 complex Langmuir monolayer at lower and higher subphase concentrations of CTAB

The schematic representation of MC540 and CTAB is shown in Fig. 2(a) and (b). At below micellar concentration (0.7 mM), CTAB molecules initially lying at the air-water interface pointing their hydrophobic parts upward. When anionic MC540 molecules were adsorbed to the CTAB molecules, hydrophobic complex molecules were formed at the air-water interface as shown schematically in Fig. 2(c). After completion of the reaction process a stable complex Langmuir monolayer was formed at the air-water interface and shown schematically in Fig. 2(d). Thus surface coverage at the air-water interface became large. As a result higher surface pressure was obtained for same barrier fixed position. At 0.92 mM (at cmc) and 1.0 mM (above cmc) CTAB concentrations, CTAB molecules formed spherical micelle structure with their hydrophilic parts pointing upwards and shown schematically in Fig. 2(e) in 2-dimensional representation. When anionic MC540 molecules were adsorbed electrostatically on the micellar surface, a hydrophobic complex spherical micelle structure was formed. These complex hydrophobic spheres came to the surface and formed a stable complex Langmuir monolayer at the air-water interface. However these spherical domains, while came to the surface of the aqueous subphase of the Langmuir trough, a portion of it still lying below the surface as shown schematically in Fig. 2(f). Thus surface coverage became less. This proposition was also explained by in-situ Brewster Angle Microscopic (BAM) images discussed in the next section.

3.3. In-situ Brewster Angle Microscopic (BAM) images of CTAB-MC540 complex monolayer at the air-water interface

In-situ Brewster Angle Microscopic [BAM] images give visual evidence of the complex/hybrid Langmuir monolayer at the air-water interface [25,26]. Phase behaviour, domain shapes and organizations in the Langmuir monolayer can be visually demonstrated by BAM images [27,28]. During the process of formation of Langmuir monolayer at the air-water interface, BAM images showed the development of films structures at the microscopic level. In Fig. 3 BAM images of Langmuir monolayer at the air-water interface at different subphase concentrations of CTAB were shown. Images were taken at different surface pressures marked in the π-t curves during the formation of the Langmuir monolayer. BAM images in Fig. 3a were taken for Langmuir monolayer at 0.7 mM subphase concentration (below cmc) of CTAB. At 5 mN/m surface pressure, BAM image in Fig. 3a-i showed almost uniform surface and no domain structure was observed indicating the gaseous phase of the monolayer. Images taken at 15 mN/m and 20 mN/m surface pressures gave compact films structure having 1–2 μm spherical domains organised compactly. Short range ordering in the organization of the domains was also observed in the Langmuir monolayer. Images in Figs. 3b and 3c showed the BAM images of two Langmuir monolayer at 0.92 mM (at cmc) and 1.0 mM (above cmc) CTAB concentrations in the aqueous subphase of the Langmuir trough. In both the cases, at 5 mN/m surface pressure distinct circular domains were observed in Figs. 3b-i and 3c-i. With the passage of time more and more complex molecules came out at the air-water interface and density and dimension of the circular domains increased (Figs. 3b-ii and 3c-ii). At 20 mN/m surface pressure in both the cases domains were organised in a regular pattern as observed from the BAM images (Figs. 3b-iii and 3c-iii). These domain organizations were completely different from the compact film structure of Fig. 3a which was prepared below critical micelle concentration (cmc) of CTAB in the aqueous subphase. This domain organization gave visual evidence of the formation of CTAB-MC540 complex micellar structure at the air-water interface.

BAM image of pure CTAB Langmuir monolayer formed above CTAB cmc in the aqueous subphase of the Langmuir trough showed a compact organization (Fig. 3d). Distinct dissimilarities between the BAM images of pure CTAB Langmuir monolayer and CTAB-MC540 complex monolayer also supported the thesis.

In the next section of UV-vis absorption spectroscopic studies, it was shown that the micellar structure of the complex molecules led to the formation of distinct monomeric sites of MC540 in the LB films.

3.4. UV-vis absorption and fluorescence spectra of aqueous solution of MC540 and mixed aqueous solution of CTAB and MC540

Fig. 4(a) shows that UV-vis absorption spectra of the aqueous solution of MC540 at different sample concentrations and thin cast
microcrystal film of MC540. In aqueous solution, UV–vis absorption spectrum of MC540 gives two distinct absorption bands with peaks at 535 nm and 500 nm and have been assigned as monomeric and H-dimeric bands respectively [12]. It may be mentioned here that, the critical interaction distance between the dye molecules to form aggregated species is 0.35 nm. When the dye molecules arrange themselves in such a way that the distance between them is greater than 0.35 nm, then only monomeric...
species exists [29]. It was also observed that with increasing sample concentrations in aqueous solution, the monomeric band decreased while the H-dimeric band increased in intensity. The existence of the dimeric band even at very low concentration in the aqueous solution may be due to the geometrical structure of the molecule. It favours the closer association of MC540 molecules even at very low dye concentration in the aqueous solution. UV–vis absorption spectrum of thin cast microcrystal film of MC540 showed intense dimeric band at 500 nm which was due to the closer association of dye molecules into the restrict geometry of ultrathin films. It may be mentioned in this context that in solution state, the band position of MC540 is strongly dependent on the nature of the solvent. In chloroform solution [13] monomeric band was observed at 570 nm and dimeric band at 528 nm. With increasing sample concentration in chloroform solution, the monomeric band decreased and dimeric band increased in intensity. Shifting of band maxima was also observed. At higher dye concentration in chloroform solution a new band was observed in the high energy region and assigned as higher order H-aggregates.

The inset of Fig. 4(a) shows the fluorescence spectrum of MC540 in aqueous solution at $10^{-7}$ M concentration which is same as reported earlier [30]. An intense featureless fluorescence band with peak at 570 nm was observed in aqueous solution. The presence of intense non-fluorescent dimeric band of MC540 drastically reduces the fluorescence intensity of the dye. In chloroform solution the fluorescence band was observed at 589 nm [13]. It is also influenced by the sample concentration in the solution phase. At higher concentration the intensity of this band is strongly quenched due to the presence of non-fluorescent H-aggregated sites. When MC540 molecules were adsorbed onto the oppositely charged surfactants or polymer, its non-fluorescent H-dimeric band dissociates leading to a strong fluorescence band [12,14].

Fig. 4(b) shows the normalised UV–vis absorption spectra of aqueous solution of MC540 ($10^{-7}$ M) and mixed aqueous solution of CTAB-MC540 at different concentrations of CTAB namely 0.1 mM, 0.5 mM, 0.7 mM, 0.92 mM and 1.0 mM. As discussed earlier, the absorption spectrum of aqueous solution of MC540 has two absorption bands at 535 nm and 500 nm which are monomeric and H-dimeric bands respectively.

An interesting metachromasy was observed when different concentrations of CTAB aqueous solution was mixed with aqueous solution of MC540 ($10^{-7}$ M). At 0.1 mM CTAB concentration, the UV–vis absorption spectrum (graph-ii) shows an intense band at 460 nm which has been assigned as due to higher order H-aggregates, along with two longer wavelength weak humps at 524 nm and 564 nm which are H-dimeric and monomeric bands respectively. With increasing CTAB concentration into the mixed solution (graph-iii to graph-v), the H-aggregated band gradually decreased whereas the H-dimeric and monomeric band increased sequentially. At 1.0 mM CTAB concentration, the UV–vis absorption spectrum shows (graph-vi) an intense monomeric band at 564 nm along with weak H-dimeric hump at 524 nm whereas the H-aggregated band is completely diminished.

The presence of intense monomeric band above critical micellar concentration (cmc) of CTAB indicates the influential role played...
by the CTAB micelle in increasing the monomeric site of MC540 in the solution phase. In pure aqueous solution of MC540 both dimeric and monomeric band exist. It may be mentioned in this context that on spherical micellar structure of CTAB, when MC540 molecules were adsorbed due to electrostatics interaction, the intermolecular distance was increased sufficiently to prevent dimer formation. The complex micellar structure was shown schematically in Fig. 2(f).

3.5. UV–vis absorption and fluorescence spectra of CTAB-MC540 complex LB monolayer

The complex monolayer at the air-water interface was transferred onto the solid substrates (quartz plate) at 20 mN/m surface pressure to form mono- and multilayer Langmuir-Blodgett (LB) films. Fig. 5(a) shows the UV–vis absorption spectra of complex monolayer LB films prepared at different CTAB concentrations namely 0.1 mM, 0.5 mM, 0.7 mM, 0.92 mM and 1.0 mM in the aqueous subphase of the Langmuir trough. Unlike the solution phase, at lower CTAB concentration only two bands at 570 nm and 530 nm were observed and due to the presence of monomeric and H-dimeric sites in the complex LB films. The most interesting observation is that the LB film prepared from the complex Langmuir monolayer above micellar concentration of CTAB in the Langmuir trough, intense monomeric band with peak at 570 nm band was observed and 530 nm H-dimeric band almost reduced to negligible hump. Inset of Fig. 5(a) shows the Gaussian deconvolution of this two bands, the deconvolution curve shows intense monomeric band at 570 nm indicating the existence of a large number of monomeric sites of MC540 in complex LB film.

Inset of Fig. 5(a) also shows plot of ‘Ratio of Dimer to Monomer intensities’ vs. CTAB mole concentration. The intensities of dimer and monomer bands at different CTAB mole concentration were obtained using Gaussian deconvolution process by resolving each spectrum at 530 nm and 570 nm which were dimeric and monomeric peak positions respectively. From the plot it was observed...
Fig. 4. (a) UV–vis absorption spectra of MC540 in aqueous solutions at different concentrations namely $10^{-5}$ M, $10^{-6}$ M and $10^{-7}$ M along with microcrystal (MC) spectrum. Inset shows the fluorescence spectra of aqueous solution of MC 540 at $10^{-7}$ M concentration; (b) UV–vis absorption spectra of (i) Aqueous solution of pure MC540 and at different concentrations of CTAB mixed aqueous solutions, (ii) 0.1 mM, (iii) 0.5 mM, (iv) 0.7 mM, (v) 0.92 mM and (vi) 1.0 mM. In all the cases concentration of MC540 remained fixed at $10^{-5}$ M.

Fig. 5. (a) UV–vis absorption spectra of CTAB-MC540 complex monolayer LB films lifted at different subphase concentrations of CTAB (i) 0.1 mM, (ii) 0.5 mM, (iii) 0.7 mM, (iv) 0.92 mM and (v) 1.0 mM; Left inset shows the Gaussian deconvolution spectrum of monolayer LB film of CTAB-MC540 complex film at 1.0 mM CTAB subphase concentration; Right inset shows 'Dimer to monomer intensities' vs. CTAB mole concentrations. (b) Fluorescence spectra of CTAB-MC540 complex monolayer LB films lifted at different subphase concentrations of CTAB ranging from 0.1 to 1.0 mM.
that with increasing CTAB mole concentration, the contribution of
H-dimeric band decreased and above CTAB cmc concentration the
H-dimeric band was completely diminished.

Fig. 5(b) shows the fluorescence spectra corresponding to the
absorption spectra of LB films shown in Fig. 5(a) and the excitation
wavelength was at 520 nm. Fluorescence band in MC540 is highly
sensitive to the solution concentration [13]. With increasing con-
centration monomeric band in fluorescence spectrum is red shifted
due to aggregation [13].

When the monolayer LB film was prepared at 0.1 mM CTAB
concentration in the aqueous subphase of the Langmuir trough,
the absorption spectrum (Fig. 5a) showed both monomeric band
at 570 nm and H-dimeric band at 530 nm. The corresponding fluo-
rescence spectrum showed small intense bands at 598 nm and
630 nm band respectively. The origin of the 598 nm band is due
to the monomeric emission which is red shifted with respect to
the solution fluorescence band. In chloroform solution at high con-
centration the monomer band was observed at 589 nm [13]. In the
LB film along with the 598 nm monomeric band, a longer wave-
length weak band at 630 nm was observed which cannot be readily
explained.

In the monolayer LB film prepared at higher CTAB concentration
in the aqueous solution of the Langmuir trough, the absorption spectrum showed diminished H-dimeric band at 530 nm along
with increased monomeric absorption band at 570 nm. The corre-
sponding fluorescence spectrum also showed an increased mono-
meric band at 598 nm along with decreased longer wavelength
band at 630 nm. This 630 nm band was totally absent in the
fluorescence spectrum of the monolayer LB film prepared above
CTAB cmc in the aqueous solution of the Langmuir trough. There-
fore it can be inferred that the origin of the longer wavelength
weak fluorescence band at 630 nm is due to the presence of aggre-
gated sites in the LB film. With decreasing aggregated sites this
band was also reduced in intensity and finally disappeared when
only monomeric sites were prevailed in the LB film.

The pre-dominance of MC540 monomeric sites in complex
micellar structure has been discussed before and shown
schematically in Fig. 2(f). When MC540 were adsorbed on the
spherical micellar structure of CTAB, monomeric sites increased.
In LB film this complex micellar structure was also retained the
originality leading to the formation of intense monomeric band.

3.6. UV–vis absorption and fluorescence spectrum of multi-layered LB
films lifted at lower and higher subphase concentrations of CTAB

Fig. 6(a) and (b) show the UV–vis absorption spectra of different
layered CTAB-MC540 complex LB films at higher and lower concentrations of CTAB in the aqueous subphase of the Langmuir trough. The
surface pressure of lifting was kept fixed at 20 mN/m.

The UV–vis absorption spectrum of monolayer LB film prepared
at 1.0 mM CTAB concentration showed monomeric band at 570 nm
along with a negligibly weak H-dimeric hump at 530 nm. With
increasing layer number in the LB film, the monomeric band
became intense with almost diminished H-dimeric band. It became
clearly evident that the spherical micellar structure retained the
monomeric sites and no interaction between the adjacent layers
took place. The inset of Fig. 6(a) shows the fluorescence spectra
of the corresponding LB films. The fluorescence spectrum has
intense peak at 590 nm owing due to the monomeric emission.
With increasing layer number, the intensity of the fluorescence
band increased indicating the prevailing of monomeric sites even
at higher layered LB films. Absorption spectra of different layered
LB films also supported this.

However the UV–vis absorption spectra of CTAB-MC540 com-
plex LB films lifted at 0.1 mM (lower than cmc) CTAB concen-
trations showed remarkable changes in the absorption profile with
increasing layer number (Fig. 6(b)). An intense high energy band
was developed at 490 nm owing to the increased H-aggregated
sites in the multi-layered LB films. Thus it became evident that
in the multi-layered LB films of the CTAB-MC540 complex
Langmuir monolayer prepared at lower CTAB concentration in
the subphase of the Langmuir trough, inter-layer interaction took
place triggering the molecular aggregates with increasing layer
number. Complex Langmuir monolayer prepared below CTAB
cmc concentration in the aqueous subphase of the Langmuir trough is shown schematically in Fig. 2(d). CTAB-MC540 complex molecules showed amphiphilic nature with hydrophobic tail pointing upward. LB films prepared from this Langmuir monolayer contained organization of these amphiphilic molecules. With increasing layer number, molecules from the consecutive layers could interpenetrate. This resulted in the closer association of MC540 molecules leading to the higher order aggregates with increasing layer number.

It may be mentioned in this context that due to the predominance of non-fluorescent species in such multi-layered LB films, fluorescence intensity also quenched remarkably and was not presented here. Therefore, it is clearly evident that multi-layered LB films prepared above CTAB cmc concentration in the aqueous subphase of the Langmuir trough retained only the monomeric sites and took part in the enhancement of the fluorescence intensity. These films can act as an efficient biological fluorescent probe.

4. Conclusion

In conclusion our results showed that the critical micellar concentration (cmc) of CTAB played an important role in controlling the non-fluorescent H-dimeric sites of MC540 in the complex LB films. Films fabricated below the cmc of CTAB in the aqueous subphase could not effectively control the H-dimeric sites resulting in the decrease in fluorescence intensity. However in the LB films fabricated above cmc of CTAB in the subphase of the Langmuir trough, non-fluorescent H-dimeric sites were almost dissociated and monomeric sites became pre-dominant. It resulted in intense fluorescence band. Multi-layered LB films of the complex Langmuir monolayer prepared above CTAB cmc in the subphase of the Langmuir trough retained this characteristics as has been observed from the UV–vis absorption spectrum. Fluorescence intensity also increased remarkably with layer number. In-situ Brewster Angle Microscopic (BAM) images of Langmuir monolayer at the air-water interface prepared at below and above CTAB cmc showed distinct dissimilarities indicating different nature of organization. Thus CTAB micellar concentration play important role in demonstrating the metachromasy of MC540 dye in complex LB films. It may be used as an efficient fluorescence probe for several biological systems.

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