Chapter 5

Behavior of Methylene Blue With Arachidic Acid in the Restricted Geometry of Langmuir–Blodgett Film
Abstract

Anti-malarial methylene blue (MB) doped in arachidic acid (AA) have been incorporated in the Langmuir monolayer at the air-water interface and also in the Langmuir-Blodgett films deposited on quartz substrate. The pressure-area (π-A) isotherm studies at different concentrations of MB pointed out that pure MB could not form stable monolayer at the air-water interface and collapse readily at very low surface pressures. However, mixture of MB with AA formed stable monolayers and they could be transferred on solid quartz substrate to form mono/multi layered films of MB. The area per molecule of floating mixed monolayers was systematically decreased with the increasing concentrations of MB in the mixture. The pressure-time (π-t) isotherm study indicated the interaction kinetics between MB and AA. Higher the concentration of MB, higher is the increase in pressure. The spectroscopic characteristics of the mixed LB films have been compared with that of pure MB solution, microcrystal and Layer-by-Layer self assembled films of MB-PAA. Surface morphology of the mixed LB and LbL film of MB was measured with Atomic Force Microscopy (AFM).
5.1: Introduction:

The insoluble floating layer formed at the air-water interface is called Langmuir monolayer (LM). LM can act as a model of naturally occurring biological cell membrane and offer a unique system to study molecular interaction at the air-water interface with reduced dimensionality. The study of LM is based on the alignment of amphiphilic molecules at the air-water interface in a monomolecular layer, where the hydrophilic groups anchor onto the aqueous subphase and the hydrophobic groups point towards the air [1]. A lot of works on LM were performed using long chain fatty acid [2-3]. Although materials suitable for LM formation are the amphiphilic, several studies indicate that some water soluble dye molecules can also form stable monolayers when a suitable matrix is used as supporting medium [4-5].

Methylene blue (MB) is a cationic organic dye exhibiting significant antimicrobial properties. It inhibits the growth of various organisms such as bacteria [6], parasites [7] and viruses [8]. In addition to the antimalarial potency of MB [9], it is widely used in the medicine for its antiseptic properties [7]. In recent decades, several approaches have been proposed for applying MB and other photosensitizers in medicine and related industries, namely for the disinfection of blood, photochemotherapy, and photodynamic antimicrobial chemotherapy [10]. As MB possesses good electrochemical properties, it can be adsorbed onto single walled carbon nanotubes (SWCNT) to form electrochemically functional nanostructure and nanocomposite useful for the development of novel electronic nanodevices such as biosensors and photovoltaic cells [11].

MB molecular assemblies in various chemical systems have been extensively studied e.g. micellar systems [12-13], solutions of polyelectrolytes [14-15] and colloid of clay minerals [16]. As the molecular aggregation and properties of dye molecular assemblies depends strongly on the properties of the interface [17], study of the behavior of MB in the monolayer at air-water interface and also in the thin LB film at air-solid interface is of fundamental importance. Prieto and Giner-Casares independently studied the formation of LB films of MB mixed with dimyristoyl phosphatidic acid (DMPA) and reported their results in two different literatures [18-19]. However, there is no report on
the behavior of MB with arachidic acid in the LM and LB film. The molecular structure of phospholipid (dimyristoyl phosphatidic acid, DMPA) is similar to that of fatty acid (AA), but with some differences: DMPA has two aliphatic chains instead of one as in AA and also different head group. We have studied the mixed LB film of MB with a supporting matrix of different structure and checked the difference observed in the physico-chemical behavior of the mixed LB films of MB/AA and MB/ DMPA. This is the first report on the study of mixed LB film of MB and AA using AFM. This study was undertaken in order to reveal the adsorption behavior and organization of MB on the mixed monolayer film at the air-water interface. Here, MB-AA interaction was studied at the air-water interface by measuring the surface pressure ($\pi$) versus area (A) and surface pressure ($\pi$) versus time (t) isotherms. Spectral properties of mixed (MB-AA) LB films have been studied with respect to aqueous solution and microcrystal film of MB using absorption spectroscopic data. Moreover, the absorption spectrum of Layer by Layer (LbL) self assembled films of MB and poly acrylic acid (PAA) have been compared with mixed LB film of MB and AA. Surface morphology of the mixed (MB-AA) LB film and LbL film deposited onto silicon wafer were studied by AFM.

5.2. Experimental:

The molecular structures of MB, AA and PAA (Poly acrylic acid) are shown in fig. 5.1. These chemicals were purchased from Aldrich Chemical Company (U.S.A.) and used as received. Teflon-bar-barrier type LB trough (model 2007DC, Apex Instruments Co., India) was used for the preparation, characterization and deposition of mono and multi-layer films. The subphase used throughout this study was double distilled water. The monolayer studies were conducted with distilled water. All the measurements were performed at room temperature (24°C).

150µl of arachidic acid (AA) having concentration (0.5mg/ml) in chloroform was spread to prepare a Langmuir monolayer on the air-water interface. After allowing sufficient time (about 15 minute) to evaporate the solvent, a dilute solution of pure MB was spread on the pre-formed AA monolayer by a micro syringe. After that the barrier is compressed slowly to record surface pressure-area isotherm / surface pressure-time isotherm. When the water soluble MB$^+$ ions come into contact with the preformed...
monolayer of AA within the barrier the reaction started and it is observed that the surface pressure increases with the passage of time. For the fabrication of LB films, the complex monolayer was transferred to the fresh quartz substrate when the desired surface pressure is attained.

Layer-by-layer (LbL) self-assembled film of MB was prepared by dipping quartz substrate alternately in aqueous solutions of PAA and MB. The van-der-Waal interaction between the quartz substrate and the PAA molecules and charge transfer type interactions between PAA and MB play important role in this method of thin-film deposition. At first, quartz substrate was dipped in PAA solution for 15 min, followed by rinsing in a water bath for 2 min to wash off additional anions attached to the surface. It was then dipped in MB solution for 15 min, followed by the same rinsing procedure. After each deposition and rinsing process, sufficient time (about 10–15 min) was allowed to dry the film. The deposition of PAA (anion) and MB (cation) layers resulted in one-bilayer LbL film. The whole sequence was repeated for fabrication of multilayer LbL films. UV-Vis absorption spectra of MB solution in quartz cell, microcrystal and (MB-AA) complex LB films on quartz slide were recorded by a Perkin Elmer Lamda 25 absorption spectrophotometer. Surface morphology of mixed monolayer film and one bilayer LbL film were studied by an AFM (Innova, Bruker Inc., USA). The AFM images were captured in tapping mode under ambient atmosphere.

Figure 5.1: Molecular structures of methylene blue (MB), arachidic acid (AA) and Poly acrylic acid (PAA).

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5.3: Surface pressure –area per molecule (π-A) isotherms:

The surface pressure versus area per molecule (π-A) isotherms of pure AA as well as of MB-AA mixed monolayers at different concentrations of MB dye at the (A-W) interface are shown in the fig. 5.2. Each isotherm was obtained at least by averaging four runs. The abscissas of these figures represent the area of Langmuir film divided by the total number of the molecules, N = N_{MB} + N_{AA}, where N_{MB} and N_{AA} are respectively, the numbers of MB and AA molecules. The pure AA isotherm is consistent with the behavior expected from the previous measurements of this system. For the AA molecules, the solid line in the fig. 5.2 indicates that surface pressure increases rapidly and there exists a transition point at which molecular area is being compressed to its limit. This point marks the liquid-solid transition of the molecular layer, at which molecules are densely packed and the surface is no longer compressible [20]. The transition point from liquid to solid state for AA molecules occur at a pressure of about 27 mN/m. Isotherm of pure AA gives the limiting area of 0.22 nm² reported in the literature [20-21]. This value is reasonable; since it is well known that long-chain fatty acids occupy the molecular area of 0.20 nm² when stand vertically on water.

When a pure dilute solution of MB in water was slowly spread at air–water interface on LB trough and the barriers were compressed very slowly, it was observed that the surface pressure did not rise practically. Adding large amount of MB solution resulted in the solubilization of dye molecules into the water subphase. This result reveals that pure MB does not form stable monolayer at the air–water interface.

However, when MB solution is spread on AA monolayer, the limiting area per molecule (A₀) of the isotherms decreases with the increase in concentration of MB in the solution. From fig. 5.2 a, b and c, the limiting area, A₀, of the mixed Langmuir films upon the MB containing subphase can be estimated by extrapolating the linear part of each observed isotherm to the zero surface pressure. Comparing the behavior of AA-MB with that of AA on the distilled water, the ionic repulsion between the –COOH groups of AA molecules in the monolayer is obviously reduced by adding MB.
Figure 5.2: The plots of Surface pressure versus area per molecule isotherms for mixed Langmuir monolayer of MB with AA at room temperature (24°C) with three different concentrations of MB (a:10^{-2}M, b:10^{-3}M and c:10^{-4}M) and three different volume ratios of MB and AA in the mixture (I, II and III denote MB: AA volume ratio of 1:9, 1:1 and 9:1).

The decrease in A₀ value compared to the pure AA monolayer indicates condensation in the AA-MB complex monolayers. The narrowing of the area per molecule with the increase in concentration of MB indicates the presence of an aggregation process with increasing concentration of MB. Besides aggregation solubilization of MB molecules cannot be excluded. To check whether MB molecules at the air-water interface were lost through solubilization or not in the bulk of the subphase, a small amount of water were sucked out from just below the air-water interface and its absorbance was recorded. The failure to detect any absorbance ruled out the possibility of solubilization of the MB molecules in the subphase. Some researchers have reported that molecular aggregation process is easier for the planar dye molecules than the non-planar molecules [22-23]. The structure of MB dye which belongs to the phenothiazine group is similar to acridine dye has a planar structure; hence MB dye molecules experience aggregation at all surface pressure. According to the pressure-area isotherm studies, one possible explanation seems to be MB molecules were present among AA molecules such as MB-AA complex, MB aggregates or monomers as a result of electrostatic interactions between negatively charged head group of AA and cationic MB dye molecules. It is relevant to mention here that the previous study of MB with DMPA [18] reveals that...
area per molecule increases when MB is mixed with DMPA, our study of MB with AA gives a decrease in area per molecule upon adsorption of MB onto AA monolayer, which is a new result.

5.4: Adsorption kinetics:

To record (π-t) curve, first of all 150µl of AA (0.5mg/ml) in chloroform was spread at the air-water interface of the LB trough, then about 15 minutes are allowed to evaporate the highly volatile solvent chloroform. Then, a dilute solution of MB in water was spread on the preformed AA monolayer with the barrier fixed without disturbing the AA monolayer. As the cationic MB⁺ ions come in contact with –COOH group of AA, they start to interact with each other. As a result, a complex is formed which is manifested as an increase in surface pressure. As the time elapses, pressure increases. Higher the concentration of MB in the subphase, higher is the rise in pressure. The increase in surface pressure (π) versus time (t) was recorded to get the (π-t) curve. Fig. 5.3 showed the π-t curves for two different concentration of MB, viz., 10⁻³M and 10⁻⁴M along with the curve for pure AA. In all the cases, the amount of AA (150 µl) and concentration of AA (0.5mg/ml) are fixed.

![Adsorption kinetics of MB with two different MB concentrations (10⁻³M and 10⁻⁴M) in the subphase and also for pure AA.](image)

Figure 5.3: Adsorption kinetics of MB with two different MB concentrations (10⁻³M and 10⁻⁴M) in the subphase and also for pure AA.
The pressure of pure AA monolayers on air-water subphase does not increase with the passage of time and it is also observed that it remain parallel to the time axis. It indicates that AA form a stable monolayer at the air-water interface. When interaction starts between MB and AA and a complex is formed, it is observed that surface pressure ($\pi$) increases. Surface pressure is reached to a maximum in the first 30min, then decreases to reach a pseudo-equilibrium value. From the $\pi$-t graph, it is observed initially the reaction takes place with higher speed, but when time is passed the reaction takes place slowly and become flat at the end. In this context, it is relevant to mention that there are several molecules viz poly (NIPAM) [24], lysozyme [25] which showed such initial steep raising and flat plateau like region at the end of the reaction kinetics.

### 5.5: UV-Vis absorption spectroscopic study of (MB-AA) mixed LB film and (MB-PAA) LbL film:

The UV–Vis absorption spectra at room temperature of MB in distilled water (concentration $\sim 10^{-4}$M), LbL film of MB, 10-bilayer LB film (0.5 mole fraction of MB) and microcrystal are presented in the fig. 5.4. MB is a water soluble and electroactive polynuclear aromatic dye and its aqueous solution is blue in color. The UV-Vis spectrum of AA solution gives featureless absorption (not shown here), whereas MB in solution exhibits strong absorbance at about 650nm with a shoulder near 598nm, which is consistent with results reported elsewhere [26]. The main absorption band of isolated MB dye cations in the visible region is assigned to $\pi \rightarrow \pi^*$ electronic transitions. The shoulder at about 598nm is probably composed of two overlapping components associated with a vibronic state of the main 1-0 transition [27] and with H-dimmers. The strong adsorption of MB dye molecules onto the AA monolayer was observed through the large decrease in the absorbance of MB/ AA mixed LB film. A close inspection of the spectrum of pure MB solution and MB-AA mixed film reveals that there is a change in the spectrum of MB after its adsorption onto the AA monolayer. The prominent bands of MB solution spectrum are broadened in the mixed film and about 60 nm blue shifted in comparison to the solution. In the less polar arachidic acid, the shift and broadening observed in the mixed LB films is likely to originate from exciton splitting due to strong transition dipole–dipole interaction between the MB molecules.
It is well established that if in molecules sufficiently strong electronic transitions exist, the exciton splitting of excited states may be observed in molecules aggregates [28-29]. The molar extinction coefficient (ε) of dye molecules is quite large. Typical values of ε for dyes lie between 10,000 and 500,000M⁻¹ cm⁻¹, thus the exciton coupling is most likely to occur in MB dye adsorbed LB films.

![Absorption Spectra](image)

**Figure 5.4**: UV-Vis absorption spectra of aqueous solution of pure MB (1×10⁻⁴ M) in water (SOL), 10 layered (AA-MB) mixed LB film, 10 layered LbL film of MB-PAA and microcrystal (MC).

In our investigated mixed LB film system, we observed blue shifting along with the broadening of the spectral profile which is an indication of the formation of H-type aggregates according to the exciton model [28-29].

The absorption spectra of LbL film and microcrystal film presented in the fig. 5.4 also show the broadening and blue shift, indicating the formation of H-type aggregates. It is relevant to mention here that the photochemical and antimicrobial efficiencies of MB are significantly reduced upon the formation of sandwich type molecular dimmers (H-dimmers) or large assemblies. The H-type assemblies are not only less effective generators of an active chemical spices ¹O₂, but also quench coexistent photoactive...
spices [30]. The reactivity of $^1\text{O}_2$ is responsible for the destruction of microbial biofilms, which are resistant to a wide range of antimicrobial agents and cause serious problems in medicine.

Here we have also studied the dependence of photophysical characteristics of mixed LB films of MB and AA lifted at different surface pressures. The packing pattern of molecules can be controlled by changing the surface pressure of lifting of LB film, which may suit for any specific technical applications. Fig. 5.5 shows the UV-Vis absorption spectra of MB/AA mixed LB films at different surface pressure of lifting. No appreciable change in the position of absorption maxima except intensity is observed in the absorption spectra of the mixed LB films lifted at 15, 20 and 25 mN/m surface pressures. One plausible explanation of the change in intensity may be the change of packing density of MB molecules in the LB films.

![UV-Vis absorption spectra of MB/AA mixed LB films](image)

Figure 5.5: UV-Vis absorption spectra of MB/AA mixed LB films taken at three different surface pressures of lifting (15, 20 and 25mN/m).

With the rise in surface pressure, the density of MB molecules in the LB films increases, which in turn increases the absorbance.
Fig. 5.6a and b shows UV-Vis absorption spectra of Layer-by-Layer self assembled films of MB-PAA onto quartz substrate as a function of different dye deposition times ranging from 15 min to 90 min and layer numbers (5 to 20 alternate layer of PAA and MB) respectively. From the fig. 5.6a, it is observed that the absorbance intensity of the films increases with increasing dye deposition time and intensity is maximum when the dye deposition time is 90 min.

The cationic dye/polyanion deposition time for all cases was taken as 15 minutes while absorbance was recorded for different layer number LbL films (fig. 5.6b). Absorption spectra of different layer number LbL films show similar band profile irrespective of layer number except an increase in intensity distribution of the peak observed at about \( \lambda_{\text{max}} = 592 \) nm in visible region. From the fig.5.6b, it is also observed that the intensity of absorbance at \( \lambda_{\text{max}} \) increase linearly in proportion to the number of dye-polymer pair layers. This reveals that charge compensation during adsorption was sufficient to proceed with each subsequent deposition and MB-PAA complex species increases with the increase in film thickness, which confirms the successful incorporation of MB molecules in the PAA-MB LbL films.

![Figure 5.6](image)

**Figure 5.6**: (a) UV-Vis absorption spectra of 10 bilayered (PAA-MB) LbL films for different dye deposition time (15 min to 90 min), Concentration of MB solution = \( 10^{-4} \) M.

5.6: (b) UV-Vis absorption spectra of different bilayered (5 to 20 bi-layers) PAA-MB LbL films, concentration of MB solution = \( 10^{-4} \) M.
5.6: Surface morphology of (MB-AA) mixed monolayer LB film:

AFM has been used to study the surface morphology of the mixed LB film of MB. Fig. 5.7 shows the AFM image of Y-type mixed monolayer of MB and AA. The picture clearly depict that domains of different sizes are formed in the mixed LB film of MB/AA. Giner-Casares et al. [31] reported different domain morphologies in the LB film of MB mixed with Dimyristol-Phosphaditic Acid (DMPA) revealed by BAM image. Domain formation in the LB films of two water soluble dyes, namely, pyronin B and pyronin Y mixed with stearic acid (SA) separately was shown by Meral et al. using AFM [32]. In a different study, Acharya et al. observed the crystalline domains in the mixed LB films of 4-methyl 3-phenyl coumarin (MPC) mixed with SA by SEM and they are identified as aggregated MPC [33]. These results are in good agreement with the interpretation of aggregate formation in the mixed LB films of MB and AA.

Figure 5.7: a) AFM image of single layer (AA-MB) mixed LB film lifted at 20 mN/m pressure, b) AFM image of 1 bi-layer (PAA-MB) LbL film.
Moreover, the broadening along with the blue shift of the absorption maximum of mixed LB film of MB/AA compared to the absorption spectrum of pure MB solution also supports the thesis of aggregation. Though the mixed LB film appeared homogeneous in optical microscope, the AFM picture reveals a clear heterogeneous morphology. The homogeneous smooth background is due to an AA layer, and MB molecules seem to be in islands above that. This situation is attributed to the electrostatic interactions of dye molecules themselves and between the cationic sites of MB molecules and negatively charged carboxyl groups of the AA molecules.

AFM of one bi-layer MB-PAA LbL film is shown in fig.5.7b. This picture clearly shows the nano-dimensional aggregates of dye-polymer complex with sharp and distinct edges. From two AFM images 5.7a and 5.7b, it is evident that the size of aggregates in MB-PAA LbL film is smaller than MB-AA mixed LB film.
5.7: Conclusion:

In the present work, the formation of stable and easily transferable monolayers has been demonstrated for a water soluble dye methylene blue (MB) using a long chain fatty acid, arachidic acid (AA) as supporting matrix. The pure MB is incapable to form any stable film on the water surface. The mixed monolayers of MB and AA were characterized by surface pressure versus area and surface pressure versus time isotherms. The mean area per molecule was found to decrease with the increasing concentration of MB, probably because of condensation / aggregation. A uniform layer by layer transfer of MB/AA mixed monolayers was inferred through the linear increase of maximum absorption with increasing number of layers. The observed blue shift in the absorption maximum for LB films, in comparison to the solution spectrum, may indicate H-type aggregation of MB molecules. Similar type of aggregates was observed to be formed in Layer by layer (LbL) self assembled films of MB. AFM image certainly confirms the some kind of aggregate formation in the mixed LB film of MB and AA. Since methylene blue is widely used across many disciplines, our results have many potential applications in sensors, data storage, electronic devices, etc.
References: