Layer-by-Layer self assembled films of rose bengal (RB) have been fabricated onto quartz substrate by the alternative adsorption of poly cation poly (allyl amine hydrochloride) (PAH) and RB. UV-Vis absorption studies reveal the formation of RB dimmer in PAH-RB LbL films. SEM picture confirms the aggregation of RB molecules in LbL films. Almost 15 minute is required to complete the interaction between RB and PAH molecules in the one bilayer LbL film. The dye (RB) was found to come off the film during the subsequent poly cation (PAH) deposition. As an alternative approach RB was anchored to the poly cation PAH via physiadsorption and controlling the concentration of the combination of RB and PAH was used as poly cation and poly (acrylic acid) (PAA) as poly anion for film deposition. The absorption spectra after each deposition showed that there was no material loss during layer depositions via second method.

Key words: Adsorption, deposition process, multilayer, Layer-by-Layer (LbL) self assembled films, UV-Vis absorption spectroscopy.

1. Introduction:
In recent years studies on ultrathin organic films have received technological importance owing to their potential applications in electronic and optical devices. The most recent method used for obtaining molecular assemblies on the nanometer scale is based on Layer-by-Layer (LbL) self adsorption of oppositely charged polyions. Although the LbL technique was first applied to assemble the oppositely charged polyelectrolytes, however it is being rapidly extended to other systems such as polymeric nanocrystals, metal and semiconductor nanoparticles, dyes, and dendrimers. Also noteworthy is the use of the LbL method for immobilization of biological components such as proteins, enzymes, DNA, cell membranes and viruses. Applications of LbL films have been suggested for optics and optoelectronics, drug delivery and electrochemistry. In recent years in depth studies have been carried out on deposition procedure and applications of such films.

In this article we report the preparation of ultrathin films of rosebengal (RB) by Layer-by-Layer (LbL) self assembled technique and their spectroscopic characterizations. Rosebengal (RB) is a Xanthene molecular dye that is highly visible and easily absorbed having strong absorbance and fluorescence. Due to the fluorescence quenching of RB, it can be used to determine proteins in serum. A physically adsorbed multilayered RB films exhibited interesting properties for molecular memory effect. RB and its derivatives have been well examined for excited state properties, including singlet oxygen formation, aggregation properties, photobleaching, photophysical probe, and others. RB and its derivatives have been extensively studied by several authors. However, detailed spectroscopic properties of RB in the restricted geometry of Layer-by-Layer (LbL) self assembled films was never been studied.

2. Experimental:
To obtain layer-by-layer self assembled films of RB, a weak polyelectrolyte, namely, poly (allyl amine hydrochloride) (PAH) (molecular weight = 70 000), was used as polycation. Both the dye (molecular weight 1017.64), purity 99% and the PAH were purchased from Aldrich Chemical Co. and were used without any further purification. Rose bengal and polymer (based on repeat unit)
solution (10^{-5}M) in triple distilled deionized milli-Q water (18.2 MΩ) were used as anionic and polycationic baths, respectively. Quartz substrates were first dipped into the PAH solution for 15 minute followed by rinsing in the milli-Q water baths for 2, 2 and 1 minutes respectively. The slides were then dipped into the anionic bath for 15 minutes followed by similar rising in a separate set of water baths to result one bi-layer of PAH-RB LbL film. LbL method utilizes the Vander-waals interactions between the quartz substrate and PAH as well as charge transfer (CT) interaction between PAH and the dye amaranth, which contains anionic groups and therefore used as anion. PAH was used as polycation for the fixation of the dye molecule to the substrate. The whole sequence was repeated to get desired number of PAH-RB LbL films.

Microcrystal film was prepared by spreading few drops of RB solution onto quartz substrate by a microsyringe and allowing them to spread throughout the substrate surface uniformly. After evaporation of the solvent, a cast film is formed, which actually contains the microcrystals of RB.

The UV-Vis absorption spectra were recorded using a Spectrophotometer (Lambda-25, Perkin Elmer). SEM photograph was recorded using a field-emission scanning electron microscope (FESEM, JEOL JSM-6700F).

3. Result and discussions:

Figure 1 shows the UV-Vis absorption spectra of aqueous solution of RB (10^{-5} M), microcrystal (MC) and one bi-layer PAH-RB LbL film. The pure RB solution absorption spectrum shows distinct and prominent band systems in the range between 450 and 600 nm and is characterized by a maximum at 549 nm along with a weak shoulder having peak at around 511 nm which are in well agreement with the reported results. The relative intensity of the shoulder to the peak maximum is usually used as the measure of the aggregation of RB. The absorption spectrum of RB microcrystal shows almost similar band pattern to that of solution spectrum. But a red shift of band positions with respect to the solution spectrum are observed having peaks at around 570 nm and 530 nm. Also the relative intensity of the shoulder increases. The RB-PAH LbL film possess almost identical spectral profile to that of microcrystal with respect to band position and shape.

It is interesting to mention in this context that O. Vlades-Aguilera et. al. studied the spectroscopic characteristics of RB in different solution. They observed red shift along with increase in relative intensity of the shoulder peak and explained to be due to the partial aggregation of the dye and formation of RB dimmers in salt solutions. P. Fini et. al reported small bathochromic shift of the RB absorption band due to the addition of cyclodextrins to aqueous
The formation of inclusion complexes between RB and cyclodextrins.

In the present case the observed red shift of the absorption peaks along with an increase in the relative intensity of the shoulder peak in films with respect to the solution spectrum may be due to the aggregation of dye molecules and presence of greater amount of RB dimmer compared to the solution.

A schematic representation of the interaction scheme between PAH and RB molecules is shown in figure 2. A closer look at the interaction scheme reveals that the anionic part of RB molecule interacts with the cationic part of the PAH molecule. Thus in the complex species, RB molecules get closer side by side and closer association of RB molecules takes place resulting a favorable condition for aggregation and consequent dimmer formation of RB molecules.

To confirm the aggregation of RB in the PAH-RB LbL films in figure 3 we have shown the Scanning Electron Micrograph (SEM) picture of 10 bi-layer LbL film. Though the film appears homogeneous in optical microscope, the SEM picture reveals a clear heterogeneous morphology with distinct crystalline domains. Thus the SEM photograph provides compelling visual evidence of aggregation of RB molecules in LbL films.

To study the effect of pH of the solution on aggregation we have recorded absorption spectra of RB solution at different pHs (figure 4). It was observed that the intensity of absorption bands is maximum when the pH of the solution is 5. However, the absorbance intensity decreases for low as well as high pHs. This may be due to the ionic nature of the solution at low and high pHs. However, the ratios of the intensities of the bands at 530 and 570 nm remained the same (inset of figure 4). This shows that the band at 570 nm cannot be due to any kind of intermolecular charge transfer. The band position did not change with pH, which suggests that the nature and size of the aggregated species do not depend on the pH of the RB solution.
Figure 5 shows the UV-Vis absorption spectra of different layered (1 – 16 bi-layered) PAH-RB self assembled LbL films. It is interesting to note that absorption spectra of different layered LbL films show almost similar band pattern irrespective of layer number except an increase in intensity and have distinct similarity and identical band position with that of the RB microcrystal absorption spectrum. This is a clear indication that closer association of RB molecules occurred in the PAH-RB LbL self assembled films due to the interaction of the RB molecules with the PAH molecules resulting aggregation and consequent dimmer formation.

The inset figure 5 shows the plot of the intensity of 570 nm absorption band as a function of layer number. The linear increase in intensity of the absorption band (570 nm) with the layer number indicates the accumulation of large number of molecules with increasing layer number and confirms the successful incorporation of RB molecules in the PAH-RB LbL films.

The absorption spectra of PAH-RB 1-bilayer LbL films with different dipping time are shown in figure 6. Here in all the cases the polycation (PAH) deposition time was kept fixed at 15 minutes but the dye (RB) deposition times were varied from 1 minute to 40 minute. From the figure it is observed that the intensity of the absorption spectra increases for the films with dye deposition time up to 15 minute and remained almost constant for the dye deposition time greater than 15 minutes. This is also evidenced from the plot of the intensity of the absorption maxima versus time (inset of figure 6). This indicates that the interaction of RB molecule with the PAH layer is completed within 15 minutes and after 15 minutes no PAH molecule remained free within the film for further interaction with the RB molecule.

To monitor the growth of the films as well as to check whether there is any material loss during film deposition, the absorption spectra were recorded after deposition of each layer (either RB or PAH). Figure 7 (top) shows the intensity of absorption maxima for 570 nm band as a function of number of deposited layers. The first absorption spectrum was recorded after the deposition of RB layer and then the absorption spectra were recorded after deposition of each layer either the polycation (PAH) or the dye (RB). The deposition was started with the polycation deposition and ended up with the dye deposition. Figure 7 (top) represents 31 layer or 16 bilayer of self assembled PAH-RB LbL films. A closer look at the figure reveals that the intensities of the absorption maxima increase after deposition of each RB layer and decrease after each PAH layer deposition showing a zigzag pattern. This confirms that few RB molecules come out of the film during PAH deposition. To minimize the material loss we have tried to vary the electrostatic adsorption process using PAH-RB combination (as polycation) and Poly acrylic acid (PAA) (as polyanion) for LbL film deposition 27. Here the RB molecules are anchored to the PAH chain.
The molar concentration of RB and PAH were chosen in such a way that the PAH-RB combination as a whole acts as the polycation. Here the intensity of the absorption maximum increases monotonically with the number of deposited layer (figure 7, bottom). In this case the absorption intensity after either polyanion or polycation deposition did not show any decrease at regular interval. This shows that the RB molecules did not come out of the films during any layer deposition. Thus the material loss can be minimized if the RB molecules are anchored to a polycation (PAH) prior to deposition onto solid substrate.

4. Conclusion:

In conclusion our results show that LbL films of rose bengal (RB) could be fabricated onto quartz substrate by the alternative adsorption of poly cation PAH and RB. The UV-Vis absorption spectra reveal that RB molecules form aggregates and dimmer in the PAH-RB LbL films. SEM photograph confirms the aggregation RB molecules in LbL films. Although the absorbance intensity of RB solution depends on the pH of the solution. However, the nature and size of the aggregated species do not depend on the pH of the RB solution. Almost 15 minute was required to complete the interaction between RB and PAH molecules in the 1-bilayer LbL film. Few molecules of the dye (RB) were found to come out of the film during the subsequent polycation deposition. As an alternative approach when RB was anchored to the polycation PAH via physi-adsorption and controlling the concentration, the combination of RB and PAH was used as polycation and poly (acrylic acid) (PAA) as poly anion for film deposition. The absorption spectra after each deposition showed that there was no material loss during the layer deposition via second method.

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