Full Length Article

Photophysical behavior of layer-by-layer electrostatic self-assembled film of azo dye Chromotrope-2R and a polycation

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This communication reports the fabrication of layer-by-layer electrostatic self-assembled films of an azo dye Chromotrope-2R (CH2R) and a Polycation poly(allylamine hydrochloride) (PAH) onto solid substrate. UV–vis absorption and steady state fluorescence emission spectroscopy successfully confirm the incorporation of dye molecules onto the PAH coated quartz substrate. The adsorption behavior of CH2R onto PAH backbone in LbL films highly depends upon the variation of the microenvironment namely pH of the dye solution from which the film was fabricated. PAH layer onto quartz substrate was able to swell sufficiently in the dye solution at very high pH. The Density functional theory was also utilized here to explain the origin of various spectral transitions from the ground electronic states for both in neutral and anionic form of CH2R. In LbL films the more closure association of dye molecules causes their aggregations which are reflected in their absorption and steady state fluorescence emission spectra when compared to those of pure dye solution. Atomic force microscopic images of LbL films assembled from CH2R aqueous solution at different pH clearly reveal the change in the surface morphology of the films and different degree of association of dye molecules in LbL films deposited at various pH of CH2R.

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1. Introduction

Organic thin films with confined structures for guest molecules and properties have drawn much attention in recent years for their potential applications in various fields namely, linear and non-linear optics, electronics, sensing, surface coating and in medical industries [1–7]. Among the different methods for fabrication of these ultrathin organic films, layer-by-layer (LbL) electrostatic self-assembly (ESA) technique based on spontaneous electrostatic interactions between the oppositely charged polyelectrolytes or species has become an elegant and versatile approach showing multifunctional properties [8]. This method was first developed by Decher and Co-workers to assemble oppositely charged polyelectrolytes [9] and later extended to many other interesting materials including nanocrystals and soft nanoarchitectures [10,11], biological macromolecules [12–14], semiconductor nanoparticles [15], dyes [16] etc. The basic mechanism of this method is the sequential and repeated electrostatic adsorption of positively and negatively charged molecules in a nanometer scale onto a suitable solid substrate from their aqueous solutions. This can create an arrangement of such materials in a molecular level to tailor the cooperative electronic and optical properties. The most attractive feature of the assembly procedure is the nanoscale control over their molecular organizations by varying several parameters such as pH of the solvent, polymer functionality, temperature, deposition time, thickness etc. [17–20]. This may be manifested in their physical and physicochemical properties showing variety of applications viz. ion-selective membrane, drug delivery systems etc. [21–23].

Although the LbL ESA method is based on electrostatic interactions between oppositely charged species, the primary driving force towards the formation of organized molecular assembly is the entropy, not enthalpy [24]. The entropy of the composite system may be increased due to electrostatic complexations of polyelectrolytes onto a charged surface which liberates undissociated low molar mass counterions. However, there may be an additional entropy gain due to the liberation of solvent molecules from the salvation shell of the specific ionic groups bound to the polymer. During each alternate adsorption step, the substrates are immersed into the aqueous
solution of oppositely charged polymers or any organic materials following rinsing the substrates between intermediate adsorption by deionized water or ethanol to remove any surplus ions loosely bound to the assembly. These washing steps may have several consequences especially materials loss during multilayer built up.

In recent times, much emphasis has been given for the assembling of nanostructured LbL films as a function of pH of the solvent used to prepare anionic or cationic solutions. There are many reports showing the dramatic changes of the photophysical or the physicochemical properties of multilayered LbL films of weak polyelectrolyte for slight changes in their solution pH. More precisely the adsorption mechanism is largely influenced by their local microenvironment. pH sensitive multilayered films have the potential applications in the area of controlled release of drugs, sensors, purification etc. [28–30]. Sukhorukov et al. [31] reported the formation of hollow microcapsules composed of at least one weak polyelectrolyte poly(allylamine)/poly(styrene sulfonate) (PAH/PSS) multilayered LbL films and can be made to be porous in nature depending on the local pH of the polyelectrolyte solutions. Open and closed conformations of the pores as a result of slight change in pH can facilitate loading and release of macromolecules from the capsule core. Rubner and Co-workers also showed the loading and release of small anionic molecules from multilayered polymeric films using pH dependent film swelling and local hydrophobicity in the films [32]. Apart from the change in local microenvironment, the multilayered growth of LbL film formations of organic molecules are not only found to be as linear [33] but also exponential [34] in many different cases and the growth mechanism of LbL films has been studied by the quartz crystal microbalance (QCM) technique [35,36]. Additionally for weak polyelectrolyte the acid-base dissociation behavior of the polymer films is found to be a major factor controlling the overall properties of the LbL films.

In this present communication, we have addressed the preparation of LbL self-assembled films of an azo dye Chromotrope-2R abbreviated as CH2R and a polycation poly(allylamine hydrochloride) abbreviated as PAH onto solid substrates via electrostatic adsorption mechanism. The chemical structures of both the dye and polycation are shown in Fig. 1a and b respectively. The film growth kinetics and pH dependent adsorption properties of CH2R onto PAH layer and their detail photophysical properties have been studied in the light of UV–vis absorption and steady-state fluorescence emission techniques aided by Density functional theory (DFT). CH2R is a class of monoazo dye CH2R (–N=N–) disulfonated organic hydrophilic dye which has potential applications in industry such as textiles, papers, gasoline etc. [37–40]. CH2R can be applied to for plasma staining and counterstaining in animal histology [41]. The most interesting observation in this work is that, adsorption of CH2R molecules to the cationic backbone of PAH layer assembled onto quartz substrate is largely influenced by the pH of dye solution from which it was assembled. Photophysical investigations of this LbL films also reveal the interaction and aggregation behaviors of CH2R dye molecules in LbL films. The morphology of PAH/CH2R LbL films were closed packed and homogeneous which have been confirmed by Atomic Force Microscopy (AFM).

2. Materials and methods

2.1. Materials

The anionic dye Chromotrope-2R (CH2R) (MW = 468.37 g/mol, dye content ~75%) and cationic polyelectrolyte poly(allylamine hydrochloride) (PAH) (MW = 70,000 g/mol), purity > 99%, were purchased from Aldrich Chemical Company, USA and were used without further purification. The solubility of CH2R in the studied aqueous phases was found to be 1 mM. For the fabrication of LbL films, aqueous solution of PAH was prepared using triple distilled deionized water (Milli-Q, resistivity of 18.2 MΩ cm, Millipore) and the polyelectrolyte deposition bath was made using aqueous solution of PAH having concentration of 1 mM (based on monomer molecular weight). Aqueous solution of CH2R was also prepared for a concentration of 10 μM using triple distilled deionized Milli-Q water and the solution pH was adjusted with HCl and NaOH. Fluorescence grade quartz slide, Fisher brand microscopic glass slide and polished silicon wafers (100) were used as substrates to deposit LbL films for various characterizations. These substrates were cleaned in a 3:1 mixture of H2SO4/H2O2 for 30 min, followed by subsequent rinsing three times with Milli-Q water and then dried in Nitrogen air prior to use [42,43]. This type of cleaning has the advantage to remove very small amount of organic contaminants from the substrates and render them hydrophilic (by adding OH-groups) in a highly oxidative acid bath. These hydrophilic substrates are then suitable to adsorb the PAH from its aqueous solution onto their surface.

2.2. Fabrication of PAH/CH2R LbL self-assembled films

LbL ESA film fabrication by dipping the above suitable substrates alternately in CH2R and PAH aqueous solutions with the aid of commercially available automatic dip coater (Model: SDC-2007C, Make: Apex Instruments Co Ltd, India). This method of film fabrication utilizes both van der Walls interaction between PAH and substrates and electrostatic interaction between oppositely charged PAH and CH2R moieties. PAH was used here to fix and organize the small hydrophilic dye molecules (CH2R) onto solid substrates. Thoroughly cleaned substrates (quartz, glass, Si wafer etc.) as already described above were then immersed into PAH solution for 15 min followed by rinsing with deionized water and dried in a nitrogen air flow for 1 h. The purpose of rinsing basically is to wash out the surplus cations attached to the substrate. The dried substrate containing one layer of PAH was then immersed into CH2R solution having desired concentration for 15 min. followed by same rinsing procedure and finally dried in Nitrogen air flow for 1 h. PAH/CH2R LbL self-assembled film was thus prepared and the multilayered growth was monitored by recording the optical absorbance spectrophotometrically after each cycle of deposition. This process of film fabrication was carried out in a cyclic manner until the desired number of PAH/CH2R layer is deposited. The materials loss if any due to subsequent rinsing of LbL films with deionized water during multilayer build up was checked by monitoring the change in optical absorbance of the LbL film spectrophotometrically before and after each rinsing steps followed by drying the film in N2 air.

2.3. Characterizations

Various analytical techniques have been employed to characterize the CH2R aqueous solution and PAH/CH2R LbL self-assembled films deposited onto solid substrates. UV–vis absorption spectroscopy (Lambda-25, Perkin Elmer, USA), steady state fluorescence emission spectroscopic (LS-55, Perkin Elmer, USA) methods were used to understand the photophysical properties of PAH/CH2R self-assembled LbL films deposited onto quartz substrates. The detailed surface morphology of the films deposited onto

![Fig. 1. Molecular structure of (a) CH2R (b) PAH.](image-url)
3. Theoretical calculation

The theoretical calculations were carried out using Gaussian-09 suite of software [44]. Various forms of the CH2R molecule have been optimized by Density functional theory (DFT). Becke’s three parameter hybrid exchange (B3) [45] and Lee–Yang–Parr correlation functional (LYP) [46] were used for the DFT calculations. The optimized geometries and their respective SCF energies were obtained from the B3LYP/6-311 g(d,p) level of theory. Theoretical absorption spectrum along with the oscillator strengths (f) of the various forms of the molecule at the ground state was also estimated at TDDFT/ B3LYP/ 6-311g(d,p) level of theory. The solvent effects on the structure of the molecule were incorporated through the integral equation formalism polarized continuum model (IEFPCM) [47]. During the process of geometry optimization for the fully relaxed method, the absence of imaginary values in the wave numbers along with the convergence of all of the calculations confirmed the attainment of local minima on the potential energy surface. The pKₐ value and the variations in the population of various forms of the CH2R molecular species with pH were envisaged using the Marvin 5.1.0 software [48].

4. Results and discussions

The optimized structure of the neutral and the anionic forms of the CH2R molecule in the electronic ground (S₀) state is shown in Fig. 2a and b respectively. The molecular structure belongs to C₁ point group symmetry. Fig. 3a and b show the experimentally observed UV-vis absorption and steady state normalized fluorescence emission spectra of pure CH2R solution (concentration of 10 µM), one bilayer PAH/CH2R LbL self-assembled film, PAH/CH2R mixed aqueous solution (1:1 volume ratio) and CH2R bulk microcrystal respectively. For fluorescence emission, the excitations of the samples were done at wavelength of 490 nm. The CH2R solution absorption spectrum shows distinct and intense band system in the region of 200–600 nm having a broad structured band having its maximum located at around 508 nm along with a vibronic shoulder centered at around 540 nm. Two bands observed in the ultraviolet region centered at around 305 nm and 368 nm which are due to the benzene and naphthalene rings of CH2R and are assigned to a π–π* transition and partly forbidden n–π* transition respectively [49]. The more intense and broad band observed at 508 nm may be attributed to the presence of two forms of the dye molecules in solution and stems from an allowed π–π* transition. In fact, CH2R molecule is subjected to intramolecular hydrogen bonding tautomeric interaction (N₁₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋_-
with its maximum at around 534 nm and another broad band at around 579 nm with respect to its solution absorption spectrum. Actually in the solid state, dye molecules are close enough for intermolecular dipole interactions and certainly they form aggregates which are reflected as the absorption band at 579 nm. However, it should be noted that the difference of the aggregation of dye molecules in the CH2R/PAH mixed solution as well as in microcrystal is definitely due to the different microenvironment in both the cases as experienced by the dye molecules. Dye molecules are in much more restricted geometry in the solid state when compared to its pure solution. The emergence of new band at higher wavelengths both in CH2R/PAH mixed solution as well as in Lbl films is due to the side by side stacking geometries of dye molecules resulting their molecular J-aggregates. According to the exciton coupling theory this corresponds to a lower electronic energy state which is manifested as the red shift of the absorption spectrum of CH2R bulk microcrystal, PAH/CH2R Lbl films and PAH/CH2R mixed solution when compared to that of pure dye in aqueous solution.

The steady state normalized fluorescence spectrum of pure CH2R solution as shown in Fig. 3b gives distinct bands systems within the region of 500–675 nm. With increase in concentration of CH2R in aqueous solution, the fluorescence intensity of the 0–0 band centered at 567 nm increases. However, the spectrum becomes diffused and is sufficiently broadened at the concentration of 10×10^{-4} M of CH2R. This may possibly be due to the aggregation of dye molecules and thereby overlapping of several vibrational bands of the electronic states at such very high molar concentration [53,54]. Interestingly, fluorescence spectrum of CH2R/PAH mixed aqueous solution shows the intense 0–0 band of CH2R at around 536 nm along with a new broad structureless band in the longer wavelength region with peak centered at around 626 nm. This shifting of 0–0 band of CH2R as well as the formation of higher wavelength broad structureless band clearly indicate the interactions of CH2R molecules with PAH backbone in solution and as a consequence the formation of dye aggregates. On the other hand, in case of PAH/CH2R Lbl films, there is shift of the
Fig. 5. UV-vis absorption spectra of (a) different bi-layered PAH/CH2R LbL film deposited onto quartz substrates. Deposition time for both PAH and CH2R were 15 min. Inset shows the absorbance of 510 nm band of LbL film with layer number (b) one bi-layered PAH/CH2R LbL film deposited onto quartz substrates at various deposition time for CH2R. Inset shows the absorbance of 510 nm band of LbL film with deposition time of dye.

0–0 band (c.a. 573 nm) of about 6 nm only with very low intensity when compared to the fluorescence spectrum of pure CH2R solution. Moreover the lower energy broad band at around 616 nm in LbL films also may be due to the formation of dye aggregates and the intermolecular vibrational coupling between neighboring CH2R molecules adsorbed within PAH chains in LbL films. This vibrational coupling can produce some deformations of the electronic states of dye molecules confined in LbL films thereby causing a change in their dipole–dipole interactions [55] which may facilitate their edge-on orientation (J-aggregates) [56,57]. The formation of dye aggregates and the intermolecular vibrational coupling may also correspond to some intersystem crossing which in turns facilitates the reduction of the intensity of 0–0 band. The spectral profile is also similar to the fluorescence spectrum of CH2R bulk microcrystal deposited onto quartz substrate as shown in Fig. 3b. That is in the solid state CH2R molecules come closure enough so that there is some overlapping of several vibrational states of each electronic energy state of the excited CH2R molecules. Therefore the shifting of the 0–0 band along with the generation of new higher wavelength band in the fluorescence spectra of both PAH/CH2R mixed solution and PAH/CH2R LbL films is definitely owing to the closer association of dye chromophores while binding with the positively charged PAH chain and thereby subsequent formation of molecular aggregates.

Fig. 5a shows the UV–vis absorption spectra of different bilayered (1–14 bilayers) PAH/CH2R LbL films deposited onto quartz substrate. In each layer of deposition the emission time of the substrate to PAH or CH2R solution was 15 min. pH of both the solutions were set at 7.0. There was no significant material loss due to rinsing the LbL films by deionized water during multilayer build up as observed from the UV–vis absorption spectra (Figure not shown) of LbL film monitored before and after each rinsing steps. It is interesting to observe from Fig. 5a that the absorption spectral profile after deposition of each bilayer (PAH/CH2R) in LbL film shows almost similar pattern except the sequential increase in absorption intensity. The multilayer growth in the LbL films was almost linear up to ten bilayers as is evidenced from the plot of absorbance intensity at 510 nm band with layer number (inset of Fig. 5a). This linear increase of absorbance is due to the surface charge overcompensation after each layer of deposition onto solid substrate. The distribution of CH2R molecules in this way might be homogeneous throughout the LbL film. More precisely, this increase in absorbance intensity with increase in layer number definitely indicates the growth of PAH/CH2R complex molecular assemblies with increase in film thickness and also confirms the successful charge alternation after each layer of deposition in LbL films. However, at higher layer numbers i.e. > 10 bilayers, the change in absorbance intensity at 510 nm was not linear rather zigzag pattern. This is because of the fact that roughness of the film might have increased at such higher layer number and therefore the electrostatic interaction between PAH and CH2R does not work as well causing the inhomogeneous distribution of CH2R molecules in LbL films. This type of observation was also reported in our earlier works [19].

In the present work, we have also examined the effects of deposition time on the adsorption behavior of dye molecules onto the PAH layer in LbL film. Fig. 5b shows the UV–vis absorption spectra of one bilayer PAH/CH2R LbL films for various dye deposition time. The deposition time for PAH in all the cases was 15 min. whereas CH2R deposition time was varied from 1 to 60 min. From the figure it is observed that the absorbance at 510 nm of PAH/CH2R LbL films increases almost linearly up to 15 min. and then decreases abruptly and continue to irregular or zigzag pattern as shown in inset of Fig. 5b. This observation reveals that the electrostatic interaction between PAH and CH2R was completed up to 15 min. However, after this time the rapid decrease in the intensity of the absorbance band (510 nm) may be due to some enhanced dye–dye repulsive interaction between CH2R molecules in their aqueous solution and that present in LbL films. Also the PAH layer deposited onto quartz substrate became sufficiently hydrated for prolonged exposure to CH2R aqueous solution. This can cause certain release of dye from the already adsorbed LbL films due to enhanced dye–dye interaction between film and the solution. Furthermore, there were no available free cationic binding sites of PAH in LbL films to adsorb further CH2R molecules after the saturation time of 15 min. That is why deposition time of 15 min. was considered for the CH2R deposition throughout the experiment.

4.1. Effect of pH on dye loading in the LbL films

The effective loading behavior of the small molecules azobenzene derivative dye CH2R onto the PAH layer in LbL films may also depend upon variety of factors such as hydrophilic/hydrophobic balance of PAH, pKa, charge density of dyes, size, local electrostatic environment etc. In the present work efforts are also given to explore the pH dependence of the adsorption of CH2R molecules onto PAH coated quartz substrate from a fundamental point of view. The theoretically estimated populations of neutral form (pH ≤ 7.0) of the CH2R
molecule and its anionic form with pH (pH > 9.5) in aqueous solution are shown in Fig. 6. The pK\textsubscript{a} value for the HS\textsuperscript{−}H functional group of the molecule has been estimated to be 7.56. Thus, depending upon the pH of the medium, molecule exists in neutral and in anionic forms.

The population of the neutral form of the molecule is monotonically increases with increase in pH value at the pH value and vanishes at alkaline pH. The population of the anionic species of the CH\textsubscript{2}R molecule is almost zero at lower pH but increases with increasing pH and maximum population of the anionic form of the molecule exhibits at pH value ~ 9.43. The anionic form of the molecule exhibits maximum population at alkaline pH medium.

The UV–vis absorption spectra of CH\textsubscript{2}R molecule in aqueous solution at various pH of the medium are shown in Fig. 7a. The absorption band maxima along with the shape of the absorption curves are almost same for pH of 3, 5 and 7. The absorption maximum was observed at 504 nm for all these pH values. However, on further increase in pH of the medium from 7 i.e. at pH of 9.5, 11 and 12, the band centered at 368 nm and 540 nm have been disappearing. Furthermore, the absorption band observed at ~ 510 nm is considerably broadened, red shifted and structureless as well as diffused pattern when compared to the absorption spectrum of CH\textsubscript{2}R solution at pH higher than 7 as described. The appearance of red shifted band at ~ 510 nm in the absorption spectra may primarily signify the preferential existence of the anionic form of the CH\textsubscript{2}R molecule at alkaline pH of the medium. Fig. 7b shows the theoretically simulated absorption spectrum of the modeled anionic form of the molecule which is in harmony with the experimental observation. The corresponding absorption parameters of CH\textsubscript{2}R at some higher pH namely, 12 are summarized in Table 1. The simulated absorption spectrum at this pH (as obtained from DFT study) is presented here for comparison and to explore the origin of the new red-shifted band observed at 510 nm of CH\textsubscript{2}R experimental solution absorption spectrum at such high alkaline pH when compared to that at pH of 5. Additionally at this high pH, the population of the neutral form of the dye molecules vanishes (as predicted from Fig. 6) and the optical absorption profile of CH\textsubscript{2}R molecules assembled onto the PAH backbone in LbL films fabricated from the dye solution at lower pH (namely at 3) when compared to that assembled at neutral pH (i.e. 7). This increase in absorption intensity is basically due to the fact that CH\textsubscript{2}R molecules which contain two sulfonated groups prefer to be adsorbed within the PAH matrix at lower pH because of strong electrostatic attractive forces between fully charged free amine groups of PAH chains and dye molecules. More precisely, surfaces of the PAH layer onto quartz substrate are highly protonated when exposed to CH\textsubscript{2}R solution at low pH namely 3 due to the presence of excess H\textsuperscript{+} ions. Interestingly, at pH of 5, the absorbance (at 510 nm) is maximum and on further decrease in pH of dye solution, the intensity of the main absorption band decreases along with red shift and spectral broadening. As the electrostatic repulsive forces between positive charges of PAH chain are greatest at lower pH, this in turn allows less number of CH\textsubscript{2}R molecules adsorbed onto positive ionic sites of PAH in LbL film rather it enhances more direct interaction between CH\textsubscript{2}R molecules in LbL film. This essentially results more closer association of dye molecules which can avoid any ionic cross-linking between dye and PAH in LbL films thereby forming their molecular aggregates. The red shift accompanied with spectral broadening as well as the development of new band at higher wavelength (at 620 nm) may be due to the formation of J-type aggregates of dye molecules as this is consistent with the exciton splitting theory [58]. In this case the CH\textsubscript{2}R molecules are arranged in one side-by-side due to the arrangement of the aromatic rings on neighboring molecules [51]. So, in the lower pH region i.e. 3 and 5, the red shift of the absorption band is basically
due to the formation of aggregate but not just due to the incorporation of CH2R molecules in the LbL films.

Inset of Fig. 8a also reveals that the absorbance at 510 nm band of LbL films deposited at lower pH initially increases and reaches maximum at pH of 5 because PAH and CH2R became fully charged at this pH and this corresponds to the highest ionic cross-link between polymer and dyes in LbL film. On further increase in pH, the absorbance at 510 nm rapidly decreases and became minimum at pH of 11. In fact the concentration of CH2R in LbL films rapidly decreases when the pH of CH2R solution is going towards more basic condition (i.e. 11) because of reduced charged density of the free amine (NH2−) group of PAH (as the pKs value of PAH ~ 10.8) when exposed to an aqueous environment of CH2R molecules at pH of 11. At this condition, the increased repulsion between free SO3− groups of CH2R also responsible for their reduced adsorption onto the LbL film. Additionally at very high pH namely, 11, the relative population of anionic form of CH2R is much higher compared to that at neutral pH, thereby causing the minimum absorbance at 510 nm. It is well known that PAH layer assembled onto a substrate starts to swell when exposed very high basic medium [59] above its pKs value. As a result some of the dye molecules might have directly occupied the free space in between the polyelectrolyte chain under this circumstance. The adsorption scheme at pH 5 and 11 is schematically shown in Fig. 8b. Also, the attractions of counterions by PAH layer in LbL film may increase leaving very few positive binding sites of PAH onto the substrate for sufficient adsorption of CH2R molecules. Hence, the absorbance at 510 nm band is less for LbL film fabricated from very low pH of CH2R solution.

However, the most interesting observation is that the number of CH2R molecules in LbL films is unexpectedly increased on further increase of pH of dye solution after 11. This is reflected as the increased absorbance (at 510 nm) as shown in the inset of Fig. 8a. Furthermore, the spectrum becomes sufficiently broadened along with the higher wavelength band centered at 620 nm. This spectral broadening and the observed lower energy band are due to the aggregation of dye molecules in LbL films as also discussed earlier. The increase in absorbance of 510 nm band along with the emergence of aggregation band possibly due to the increased dye molecular density in LbL films fabricated at higher pH namely, 12. One possible reason is that when the PAH coated quartz substrate exposed to dye solution at high pH (i.e. 12), the PAH layer loose most of the positive charges and therefore increases its swelling ability [59,60] due to unbalanced electric charges while immersed into the dye solution; that is the polyelectrolyte layer in LbL films become sufficiently hydrated. As a consequence, this increased swelling ability creates more free space to accommodate the dye molecules between the polymer chains onto the quartz substrate but not above the PAH layer. However, the swelling ability may depend upon many factors like local electrostatic environment of the hydrated films, hydrophilic/hydrophobic balance of the polymer chain etc. Some authors [25,61] suggest that polyelectrolyte multilayers films also able to swell when exposed to very high pH aqueous environment because of the variation in charge density of the polymer chain onto solid substrate as influenced by the change in pH of the medium in which they are emerged for dye adsorption. Additionally, the increased free space in the LbL films along with enhanced hydrophobic interactions between polycation and dye essentially provides more mobility to the dye molecules to overcome any undesirable electrostatic forces. As a result dye molecules find suitable sites for more favorable van der Waal or hydrogen bonding interactions. However, the rate of swelling of PAH layer at higher pH should be much faster than the time required to adsorb the dye molecules to the LbL films. The swelling behavior of polyelectrolytic multilayers films has been rigorously studied by Tenchak and Barrett [62]. Their mobility and increasing number of dye molecules in LbL films fabricated at very high pH also favour their aggregation which is already reflected as the appearance of 620 nm band in their UV–vis absorption spectra.

The detailed surface morphology of PAH/CH2R LbL self-assembled films deposited onto smooth Si substrate from two different pH of CH2R solution has been studied by tapping mode Atomic Force Microscopy (AFM). Fig. 9 shows the AFM topographic images along with height profile and histogram analysis for PAH/CH2R LbL films deposited from CH2R solution at pH of 5 and 11 respectively. The dye molecules as well as their aggregates are clearly observed in AFM images which also confirm the formation of nano-dimensional dye/polycation self-assembled LbL film deposited onto solid substrate for the dye solution at two different pH namely, 5 and 11. However, if we further look at the height profile of the images, we immediately observe that the average height as well as the RMS roughness of the PAH/CH2R assembly onto solid substrate deposited at pH of 5 is much higher than that fabricated at higher pH namely 11. The average surface roughness of the LbL films deposited at pH 5 and 11 are found to be 6.2567 nm and 2.8505 nm respectively. The other important morphological parameters of LbL films are illustrated in Table 2. The decrease in average height as well as average surface roughness of LbL films onto the solid substrate assembled at higher pH (11) definitely confirms that the dye molecules did not attached onto the top of PAH rather dye molecules eventually occupied in the free space between the PAH chain onto the substrate at such higher pH. Furthermore, PAH layer lose the charge density of NH2− groups at pH of 11 causing very less number of CH2R molecules adsorbed in the backbone of PAH in LbL film because of unfavorable electrostatic interaction between PAH and CH2R molecules. This is also consistent with the lowest absorbance value at 510 nm band of LbL film as observed from UV–vis absorption spectroscopic results (Inset of Fig. 8a). It is already mentioned earlier that when PAH layer assembled onto the substrate exposed to CH2R solution at lower pH namely, 5, the PAH layer becomes highly protonated causing strong electrostatic interaction between fully charged NH2− groups of polymer chain and SO3− group of CH2R. As a result more number

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**Fig. 8.** (a) UV–vis absorption spectra of one bi-layered PAH/CH2R LbL film deposited onto quartz substrate from CH2R solution at various pH values. (b) Schematic presentation of the adsorption scheme for the formation of PAH/CH2R LbL films fabricated at pH of 5 and 11 of dye solution at ambient temperature.
of CH2R molecules adsorbed onto the top of highly charged PAH layer in the solid substrate. Therefore, the average height, peak-to-peak height as well as RMS roughness of the LbL films fabricated at pH of 5 were much higher than prepared at higher pH i.e. 11. Additionally, strong electrostatic interaction between PAH and CH2R at pH of 5 facilitates more closer association of dye molecules in LbL films thereby forming large number of dye aggregates onto the top of PAH moieties and this is also responsible for the increased RMS roughness as observed from AFM images and corresponding height profile analysis (Fig. 9a). Therefore, from the AFM analysis, it is also concluded that the loading of CH2R molecules and the degree of their aggregations in LbL films also depend upon the pH of dye solution from which they were fabricated.

5. Conclusions

In summary, our experimental observations reveal that the absorption spectrum of pure CH2R solution comprises the band system in the region of 200–600 nm with a maximum at around 504 nm and is responsible for an allowed π−π* transition. DFT studies successfully predicted the origin of these electronic spectral transitions of CH2R molecules both in neutral and anionic states and are consistent with the experimental results. In the case of PAH/CH2R LbL films deposited onto quartz substrate, the absorption spectrum is slightly red shifted when compared to pure solution absorption spectrum of CH2R. The shift of the absorption curve of PAH/CH2R LbL films towards higher wavelengths side clearly reveals the formation of J-type aggregates. In fact, the sulfonic groups (SO3−) of CH2R were attached electrostatically to the positive binding sites (NH2+) of PAH chains in the LbL films and this facilitates the parallel orientation of their transition moments. Absorption spectrum of CH2R microcrystal is further red shifted. Therefore, the degree of interactions between dye molecules in all cases is different because of different microenvironments as experienced by dye molecules. Steady state fluorescence spectroscopy clearly confirms the shifting of 0–0 band of CH2R along with the generation of a lower energy band in LbL films when compared to that of pure CH2R solution and these definitely confirm the formation of dye aggregates in LbL films. The multilayer growth of PAH/CH2R LbL films was almost linear up to 10 bilayers and then becomes non-uniform. The adsorption behavior of CH2R onto PAH backbone in LbL films was found to be time dependent. The most interestingly, the adsorption of CH2R molecules onto LbL films depends upon pH of CH2R solution from which it was assembled. At lower pH value namely 3 the red shift and spectral broadening was due to the formation of dye aggregates because of much closer association of dye molecules in the LbL films at this pH. However, at pH of 5, the absorption intensity at 510 nm band was maximum suggesting more favorable electrostatic environment between dye and PAH in the LbL films. At higher pH namely, 11, the adsorption of CH2R onto LbL films was minimum because of reduced charge density of the polyelectrolyte (PAH) at this pH and PAH layer was just able to swell within the dye solution. On further increase of pH

Table 2
Morphological parameters of PAH/CH2R LbL films as obtained by Atomic Force Microscopy.

<table>
<thead>
<tr>
<th>Systems</th>
<th>RMS roughness (nm)</th>
<th>Peak to peak height (nm)</th>
<th>Average roughness (nm)</th>
<th>Average height (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH/CH2R LbL films fabricated from dye solution at pH of 5</td>
<td>1.8337</td>
<td>20.8374</td>
<td>1.4290</td>
<td>6.2567</td>
</tr>
<tr>
<td>PAH/CH2R LbL films fabricated from dye solution at pH of 11</td>
<td>1.2492</td>
<td>13.2380</td>
<td>0.8917</td>
<td>2.8505</td>
</tr>
</tbody>
</table>

Fig. 9. AFM images of one bi-layered PAH/CH2R LbL film deposited onto smooth Si substrate for (a) pH of 5 and (b) pH of 11. Concentration of CH2R was 10−5 M, deposition time for CH2R was 15 min.
absorption intensity of CH2R increases because of larger availability of free space to dye solution due to increased swelling of PAH layer when exposed to CH2R solution at very high pH. Atomic Force Microscopy confirms the visual evidence of the surface morphology of LbL films deposited at pH 5 and 11. The surface roughness of the film was much higher when fabricated at low pH namely, 5 than that fabricated at higher pH namely, 11. This also reveals that the adsorption and association of dye molecules in the LbL films highly depends on the microenvironment of dye solution from which it was assembled.

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