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Photophysical characterization of layer-by-layer self-assembled films of deoxyribonucleic acid

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Abstract. This communication reports the photophysical characterization of self-assembled layer-by-layer (LbL) films of DNA (deoxyribonucleic acid) fabricated at different temperatures by electrostatic interaction with a polycation, poly(allylamine hydrochloride). It was observed that there was a successful incorporation of DNA molecules in DNA–PAH LbL films at room temperature as well as after melting temperature. An abrupt increase in intensity was observed in the absorption spectra of the films fabricated at high temperature which is an indication of the immobilization of unzipped DNA after melting of DNA. The films were observed to remain unaffected even after 250 h of film fabrication. The total electrostatic interaction time between DNA and PAH is about 15 min, that is, no PAH binding site is free.

Keywords. Layer-by-layer; deoxyribonucleic acid; interaction; immobilization.

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1. Introduction

In recent years, ultra-thin films of biomolecules are receiving technological importance due to their vivid potential applications in the field of optical and electronic devices [1], biochips, biomaterials [2], sensors [3], molecular electronic devices [4,5] etc. The layer-by-layer electrostatic self-assembly technique is a novel method [6] for the fabrication of films of water soluble molecules. This method provides a means to build multilayer structures with considerably less sophisticated experimental procedures as they are based on the spontaneous adsorption of a material of interest on a substrate or on an already deposited layer which is electrostatically opposite to it.

Deoxyribonucleic acid (DNA) is a very interesting and important biological material that controls the heredity of life. It is also an interesting anionic polyelectrolyte having unique double helix structure [7]. Thin organic films with a supramolecular architecture in which DNA is oriented or embedded are of special interest, mainly in the development of biological sensors [8,9] including work on nanoparticles [10].

In this communication we report successful incorporation and immobilization of DNA at high temperature into layer-by-layer films along with poly(allylamine

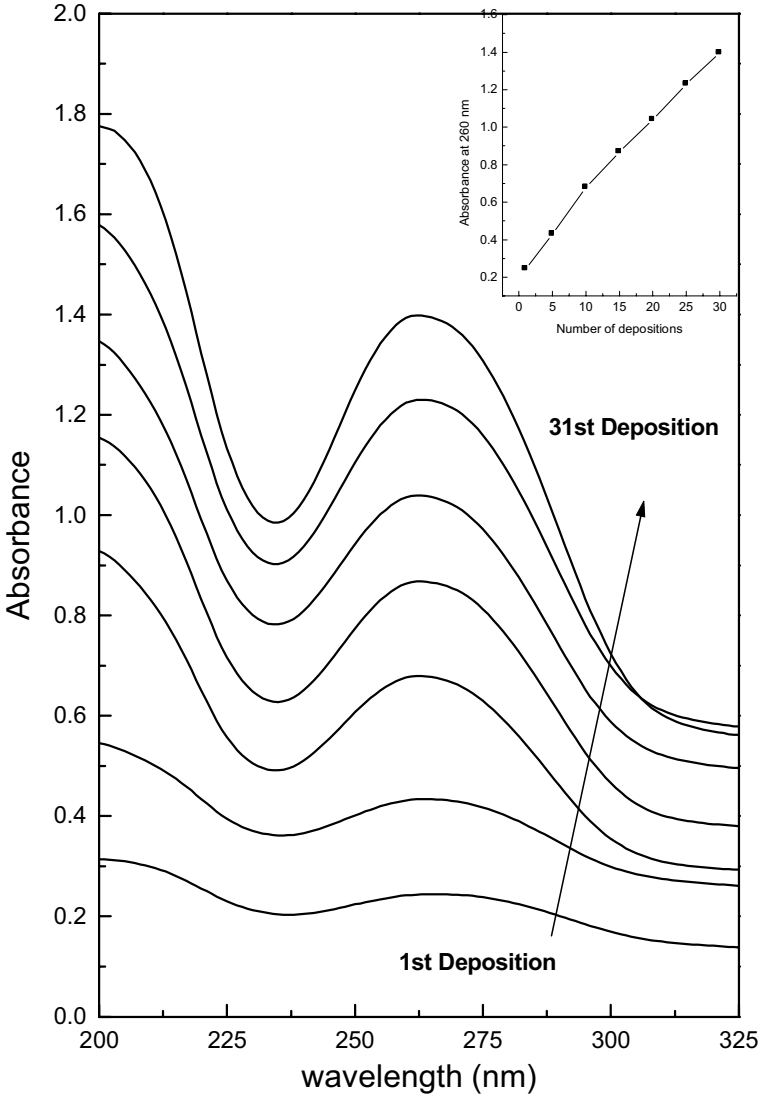

Figure 1. The UV–Vis absorption spectra of different layered (1–30 deposition) PAH–DNA self-assembled LbL films. Inset of the figure shows the plot of maximum intensity as a function of deposition number.

hydrochloride) (PAH). It is interesting to observe that films were made in this process without any material loss and at high temperature. UV–Vis absorption spectra showed almost four times increase in intensity which is a clear indication of immobilization of unzipped DNA at high temperature.

2. Experimental

The polycation used in this work is poly(allylamine hydrochloride) (PAH) (molecular weight = 70,000, purity > 99%), which was purchased from Aldrich Chemical Co. and was used without any further purification. The DNA used is Herring sperm sheared DNA, purchased from SRL India and was used as-received. The purity of DNA was checked by UV–Vis absorption and fluorescence spectroscopy before use. The electrolytic bath was prepared with 0.2 mg/ml DNA and 10^{-3} M (based on the repeat units of polyion) PAH aqueous solution using triple distilled demonized (18.2 MΩ) millipore water.

Electrostatic self-assembled layer-by-layer films were fabricated by immersing a thoroughly cleaned quartz substrate in PAH solution for 15 min followed by rinsing in water in order to remove off the surplus cations attached to surface and then in the solution of DNA for 15 min followed by the same rinsing procedure. A sufficient time was allowed to dry up the film. The UV–Vis absorption spectra were recorded by UV–Vis absorption spectrometer (Lambda 25, Perkin Elmer).

3. Results and discussions

The UV–Vis absorption spectra of different layered (1–30 deposition) PAH–DNA self-assembled LbL films are shown in figure 1. It is very interesting to note that the absorption spectra of different layered electrostatic self-assembled LbL films show

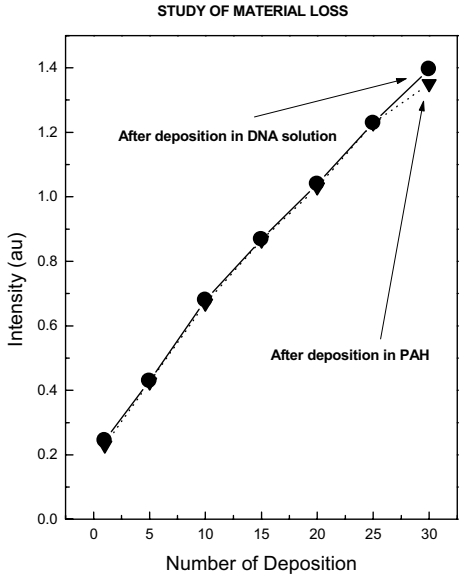

Figure 2. The intensity of absorption maximum as a function of the number of depositions after dipping in DNA solution (solid lines) and PAH solution (dotted lines).

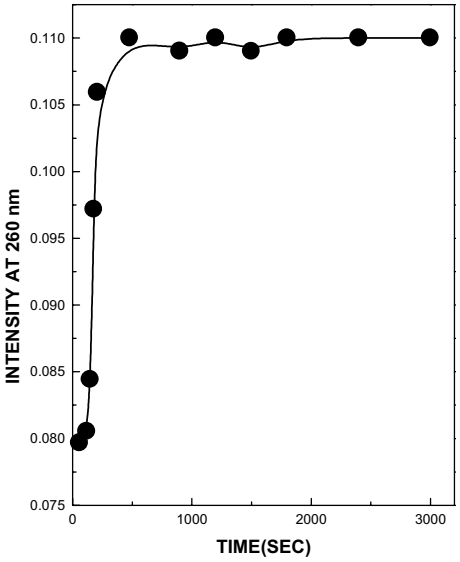

Figure 3. Plot between the maximum intensity of absorption spectra of the LbL films deposited at different times starting from 30 s to 3000 s.

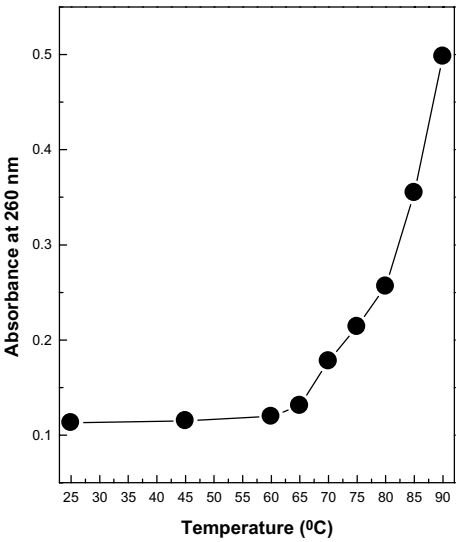

Figure 4. The UV–Vis absorption spectra of LbL self-assembled films fabricated at different temperatures.

almost similar band pattern irrespective of the layer number except an increase in intensity and have distinct similarity and identical band position. This observation is a clear indication of closer association of DNA molecules in DNA–PAH LbL films due to interaction with the PAH molecules. Inset of figure 1 shows the plot of maximum intensity as a function of the number of depositions. From the figure, it is observed that maximum intensity increases systematically with the increase in the number of depositions, which is an indication of the formation of DNA–PAH complex with the increase in film thickness and that definitely confirms the successful incorporation of DNA–PAH molecules in the self-assembled LbL films.

In order to check the material loss and the film growth during successive deposition, absorption spectra were recorded after deposition of each layer. Figure 2 shows the intensity of absorption maximum as a function of the number of depositions after dipping in DNA solution (solid lines) and PAH solution (dotted lines). It is seen that the intensity remained almost the same for both the cases, that is, there is no loss of DNA molecules during the successive deposition and thus the films are fabricated without any material loss.

To determine the time for maximum interaction between PAH and DNA we studied the adsorption kinetics of film deposition. Figure 3 is the plot between the maximum intensity of absorption spectra of the LbL films deposited at different times starting from 30 s to 3000 s. From the figure it is observed that the maximum intensity of absorption spectra increases initially with the deposition time of DNA but after 900 s the intensity becomes almost constant and remains the same for all the films deposited above 900 s. From this, it is evident that the interaction

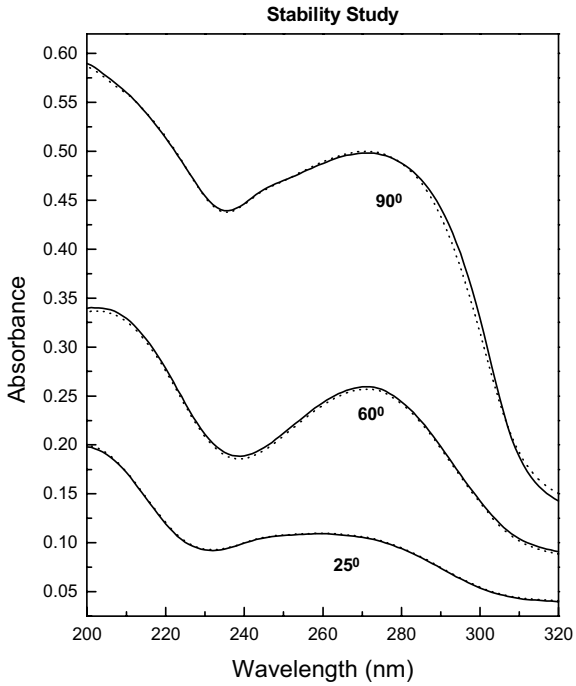

Figure 5. The absorption spectra of the films fabricated at different temperatures (solid line) and after 250 h (dotted line).

of DNA with PAH molecules takes about 900 s and no PAH molecules are further available in the film for interaction. So the ideal film deposition time is estimated to be 900 s and the deposition time for all the films was 900 s.

The effect of temperature of DNA films was found to be remarkable. DNA monolayer was deposited on a PAH layer at different temperatures starting from 25°C (room temperature) to 90°C. The UV–Vis absorption spectra of LbL self-assembled films fabricated at these temperatures are shown in figure 4. From the figure it is observed that the band pattern remains almost similar for all the cases but the intensity of absorption increases remarkably, and it is seen that the absorption intensity of films fabricated at 90°C is almost four times that of the films fabricated at 50°C and at room temperature. A possible explanation for the above observations can be that the DNA remains in its double helix structure before melting temperature and remains under twisted condition. The dipole moment of 260 nm absorption band is directed along the short axis of horizontal base pair and so the intensity is substantially less below the melting temperature. With the increase of temperature the length of DNA strands increases dramatically and there is an increase in length of the dipole moment vector [11] and the absorption intensity increases thereby.

Figure 5 is the absorption spectra of the films fabricated at different temperatures (solid) and after 250 h (dotted line). It is observed that there is no significant change in intensity which implies the formation of stable films of DNA at room

temperature as well as at high temperature, that is, the molecular organization remains unaffected even after 250 h.

4. Conclusion

This communication reports the fabrication of self-assembled LbL films of DNA at room temperature and also at higher temperatures. The layer effect studies of the DNA–PAH films surely confirm the successful incorporation of DNA molecules in the films. The adsorption kinetics of the films also reveals the fact that total time of interaction of PAH and DNA is about 15 min. The immobilization of denatured DNA strands at high temperature is an important result of our observations. Moreover, after 250 h of fabrication, the films remained unaffected which indicate the formation of stable films.

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