

# Study of Hysteresis during pH and Temperature Changes of Acriflavine: A Gateway to Optrode

**SOMA BANIK, DIBYENDU DEY, D. BHATTACHARJEE and SYED ARSHAD HUSSAIN\***

*Department of Physics, Tripura University*

*Suryamaninagar-799 022 (Tripura)*

*\*E-mail: sa\_h153@hotmail.com*

## **Abstract**

*Optrodes are basically synonyms of fluorescence sensors. Sensing of pH and temperature has wide applications in analytical, biomedical and clinical chemistry. The present work reports the investigations on the properties of a cationic laser dye Acriflavine (Acf) in solution and in Layer-By-Layer self assembled films to explore its possible application as pH sensor and temperature sensor. It has been observed that 35 minute is required to complete the adsorption process of Acf on to LBL films. The fluorescence spectra of Acf in solution and in LBL films were pH sensitive over a wide pH range 2.5-13. Fluorescence intensity shows hysteresis during increase and decrease of pH in both solution and LBL films. Both UV-Vis absorption and fluorescence spectra were found to be temperature sensitive.*

**Key words :** Optrodes, fluorescence sensors, chromophore, layer-by-layer.

## **I. Introduction**

Now a days, optrodes (fluorescence sensors) have attracted a real attention in the detection of various analytes [1-13]. The introduction of optrodes has shown a lot of technological advantages over the conventional devices because of their microsize, remote sensing and independence of electrical dependence [1-13]. The presence of an analyte can change the properties of the optrode and so that the analyte can be determined [2]. An optrode consists of a fluorescent molecule adsorbed on to a supporting sensing matrix (polymer, porous glass etc.) [2]. Sensing of pH and temperature has lot of potential applications in analytical, biomedical and clinical chemistry [3, 4]. V.Misra et al. constructed a pH sensor by studying the fluorescence resonance energy transfer between Acriflavine and Rhodamine 6G in solution [1] and in Nafion matrix [5]. In one of our work we have also demonstrated a pH sensor which can be used to sense the pH over a wide pH range of 3-12 [6]. Nguyen et al. has demonstrated a photochemical

pH sensor by immobilizing carboxy-fluorescein-encapsulating liposomes in sol-gel thin films for a range of pH 6-7.5 with sensitivity 0.01 [7]. Using different dye molecules immobilized in different supporting matrices, viz. cellulose, polystyrene, sol-gel etc, Wolfbeis and co-workers have demonstrated various types of optrodes [8-13]. A pH sensor based on excited state proton transfer in a cellulose support has been discussed, which can sense a pH range from 1-3 [12]. There are few polymers where the variation of temperature can shrink the chain associations of those polymers [14, 15]. He Cheng et al. explained this kind of property of PNIPAM and its technological applications [16]. The behavior of such kind of polymers can be used for the sensing of temperature over a large range [17].

Layer-By-Layer (LBL) method is an effective and experimentally convenient method for the fabrication of ultra thin films of water soluble molecules on to solid substrate [18]. A few fascinating features of this technology are, ease of fabrication, environmental friendliness and

conformability. Originally this technique was invented for the adsorption of oppositely charged polyelectrolytes on to solid substrate. But it has been observed by the recent studies that the process can also be used for the manipulation of many different types of molecules including polymers, light emitting materials, nonlinear optical polymers, inorganic nano particles, bio-materials, dyes and various other systems [19]. Therefore the LBL process can be used to manipulate materials at a molecular level to produce cooperative electronics and control the optical properties. Among many possible applications of LBL technique, sensing is one of the most promising due to the ability of LBL films to assemble materials at nanoscale dimensions and their sensitivity to various chemical environments [20]. LBL films are particularly well studied for sensing in aqueous and humid environment due to their natural selectivity to water. But as the method is used in applications such as organic thin film transistors [21] and lithographic contact printings [22], the film hydration state has shown to be an important consideration.

In the present communication the variation of spectral characteristics of a cationic dye Acriflavine (Acf) in both solution and LBL films have been studied with respect to the variation of pH and temperature and the hysteresis was found. The hysteresis has been found in various phenomena such

as magnetic properties of various materials, chromatic properties of crystals and contact angles of solid surfaces. Hysteresis related with bi-stability and orientation of the chromophores has attracted much attention from the view point of applications to switching and memory devices. The hysteresis phenomenon in LBL films has huge significance in relation to the development of the magnetic and elastic materials, and there has also been interests developed at nano scale in such systems with nano electronics [23-24] and drug delivery systems [25-26]. Therefore, it is interesting to study the properties of Acf at different pH and temperature to explore their possible application as pH and temperature sensor.

## 2. Experimental

### 2.1 Materials

The laser dye Acf was purchased from Sigma Chemical Co., USA and used as received. Molecular structure of the dye is shown in the inset of Fig. 1(a). Triple distilled deionized (resistivity 18.2 MΩ-cm) Millipore water was used as solvent. Poly (acrylic acid) (PAA) was used as polyanion during LBL self assembled film preparation. PAA was purchased from Aldrich Chemical Co., USA and was used without further purification.

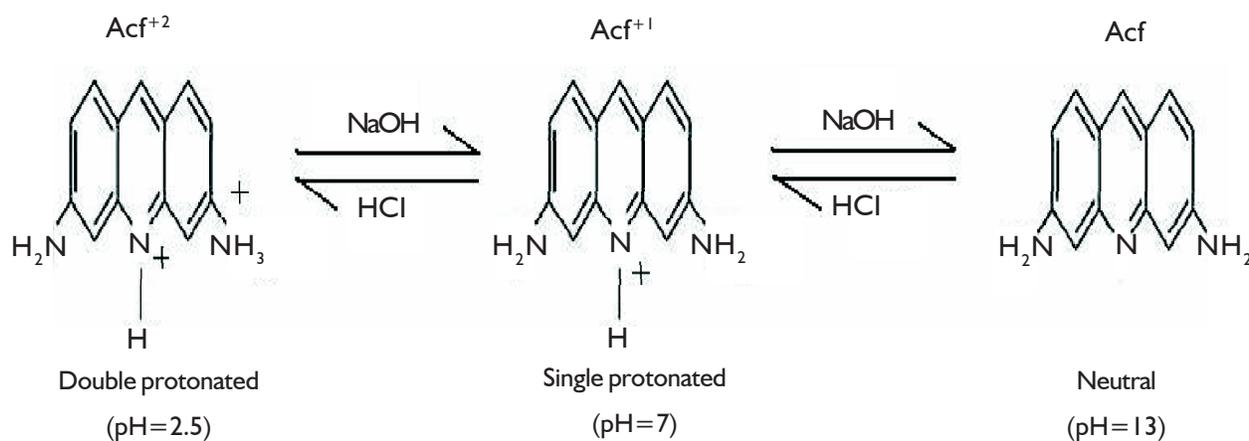


Fig. 1. The structural changes in Acf in different pH solution

## 2.2 Film Preparation

Electrolytic deposition bath of cationic dye Acf was prepared with  $10^{-4}$ M aqueous solution using triple distilled deionized (resistivity 18.2 M $\Omega$ -cm) Millipore water. The anionic electrolytic bath of PAA was prepared also with triple distilled deionized Millipore water (0.25 mg/mL). LBL self assembled films were obtained by dipping thoroughly clean fluorescence grade quartz substrate alternately in solutions of anionic PAA and oppositely charged Acf dye solution. Automatic computer controlled dip coater unit (Apex- SDC 2007C) has been used for LBL film formation. This method utilizes the Vander Waals interactions between the quartz slide and PAA as well as charge transfer interaction between PAA and cationic dye [27]. The quartz slide was dipped in the aqueous solution of PAA for 30 mins. Then it was taken out and sufficient time was allowed for drying and then rinsing in water bath for 2 minutes so that the surplus anion attached to the surface washed off. The dried substrate was then immersed in cationic dye Acf followed by same rinsing procedure. Deposition of PAA and Acf layers resulted in one bi-layer of self assembled film.

## 2.3 UV-Vis Absorption and Fluorescence Spectra Measurement

UV-Vis absorption and steady state fluorescence spectra were recorded by a Perkin Elmer UV-Vis Spectrophotometer (Lambda-25) and a Perkin Elmer Fluorescence Spectrophotometer (LS-55) respectively. The fluorescence light was collected from the sample surface at an angle of 45° (front face geometry) and the excitation wavelength was 420 nm.

## 3. Results and Discussion

### 3.1 Spectroscopic Characteristics and Reaction Kinetics

UV-Vis absorption and steady state fluorescence spectra of pure Acf in aqueous solution and in LBL film are shown in Fig. 2 (a) and (b), respectively. Both absorption and fluorescence spectra are characteristics of the presence of monomers [28].

The fluorescence spectra were recorded by exciting close to the absorption maxima of single protonated Acf (420 nm). The absorption and emission maxima of Acf solution are centered at 449 and 502 nm respectively which are assigned due to the Acf monomers [28]. On the other hand Acf absorption spectrum of LBL film possesses prominent intense 0-0 band at 449 nm along with a weak hump at 470 nm which is assigned due to the 0-1 vibronic transition. The single protonated Acf monomer absorption band within 444 - 453 nm depending on the concentration of Acf<sup>+</sup> has been reported [29]. For Acf dimer it has been reported that instead of a single monomer band two bands at around 437 and 470 nm are observed with the intensity of the blue band higher than the other [29]. In LBL film Acf shows strong fluorescence with monomer band at 515 nm, which is red shifted with respect to aqueous solution. This shift in the excited state may be due to the change in microenvironment when Acf molecules are incorporated into the polymer (PAA) backbone in the restricted geometry of LBL film.

To observe the maximum time needed for the interaction between PAA and Acf, we studied the absorption and fluorescence spectra of 1 bi-layer Acf-PAA LBL films prepared with different deposition time in Acf deposition bath, whereas for all the films the time of deposition in PAA was same. The inset of Fig. 2 (b) is the plot between the maximum intensity of absorption and emission spectra of the LBL films prepared by varying dye deposition time starting from five minutes to fifty five minutes. It is observed that the maximum intensity of both absorption and emission spectra of Acf increases initially with the deposition time of Acf, but after around 35 minutes the intensity becomes almost constant and remains the same for all the films prepared with dye deposition time greater than 35 minutes. From this it is evident that the interaction of Acf with PAA molecules takes about 35 minutes and no PAA molecule is left in the film for further interaction. So the ideal film deposition time is estimated to be 35 minutes and the deposition time for all the films was 35 minutes.

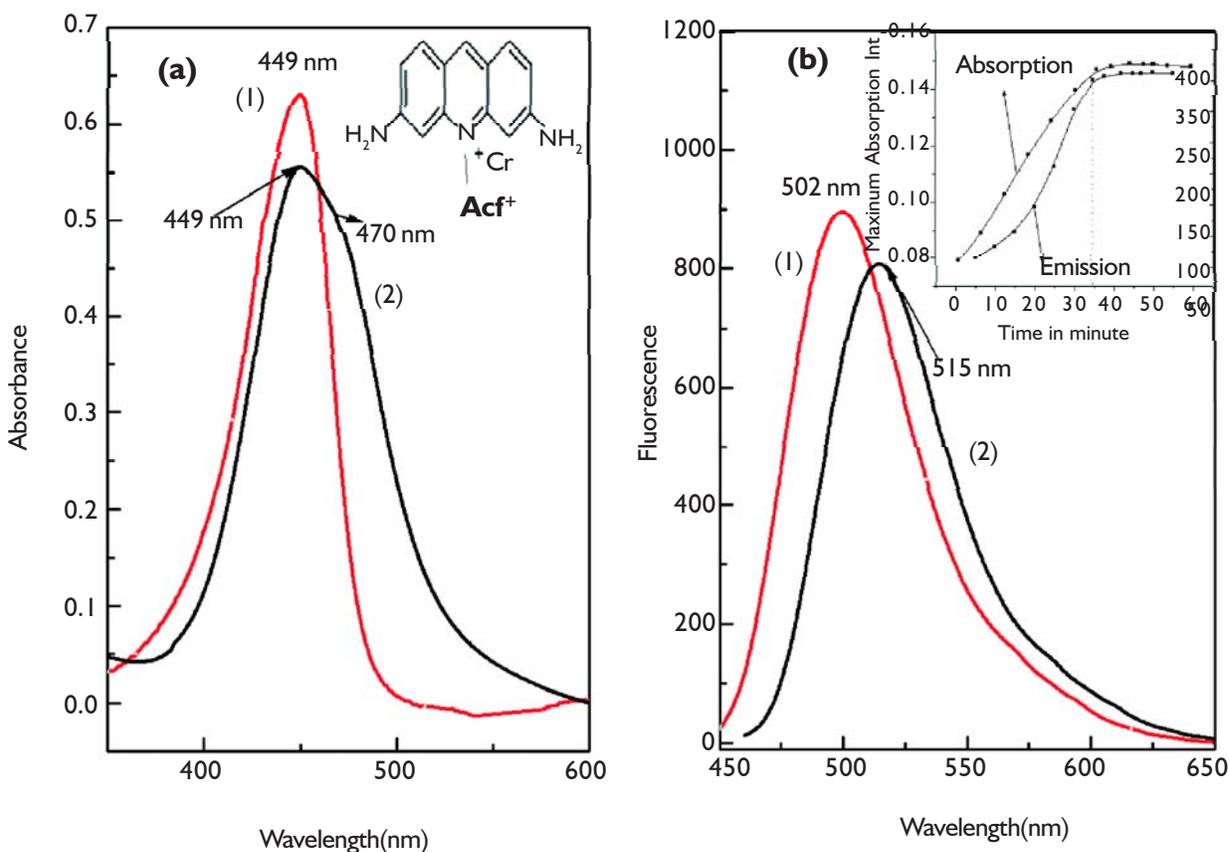


Fig. 2(a). Absorption spectra of Acriflavine solution (1) and LBL film (2). Inset showing the structure of cationic Acriflavine (Acf<sup>+</sup>). (b) Fluorescence spectra of Acriflavine solution (1) and LBL film (2). Inset showing the reaction kinetics of the LBL film for both Absorption and Emission. Concentration of the dye was  $10^{-6}$ M for solution and  $10^{-4}$ M for LBL film

### 3.2 pH Effect

The effect of pH on the Acf in aqueous solution and in Acf-PAA LBL films has been investigated. The molecule (Acf<sup>+</sup>) under current investigation is pH sensitive because of its basic nature of the central nitrogen atom [30]. Figure 3(a) and 3(b) represents the wavelengths corresponding to the absorption and emission maxima respectively in both solution and LBL film with an increasing pH (2.5 to 13). It is observed from the figures that the position of the absorption maxima is independent of pH of Acf solution and in LBL film. On the other hand the fluorescence spectra of Acf are affected with change in pH. It was observed that the Acf fluorescence was red shifted with decrease in pH. At lower pH Acf molecules mainly remain as double protonated form (Fig. 1) due to the increase in local proton

concentration with decreasing pH. Accordingly the dipole moment of the excited state of double protonated Acf has been changed. This change in dipole moments may be responsible for the observed large stoke shift or red shift of the Acf fluorescence. The electron donor type functional group of Acf becomes more acidic with increasing pH in the excited state, consequently the fluorescence spectra shifts towards shorter wavelength. The change in dipole moments of the Acf molecules are mainly in excited state, which results in no change of the absorption maxima of the Acf molecules in the ground state. From Fig. 3(b) it is observed that the stoke shift of emission maxima in LBL film is more sensitive (518 nm-570 nm) in compare to solution (506 nm-531 nm). The large distribution of the pKa values in solid matrix could be responsible for this larger red shift with

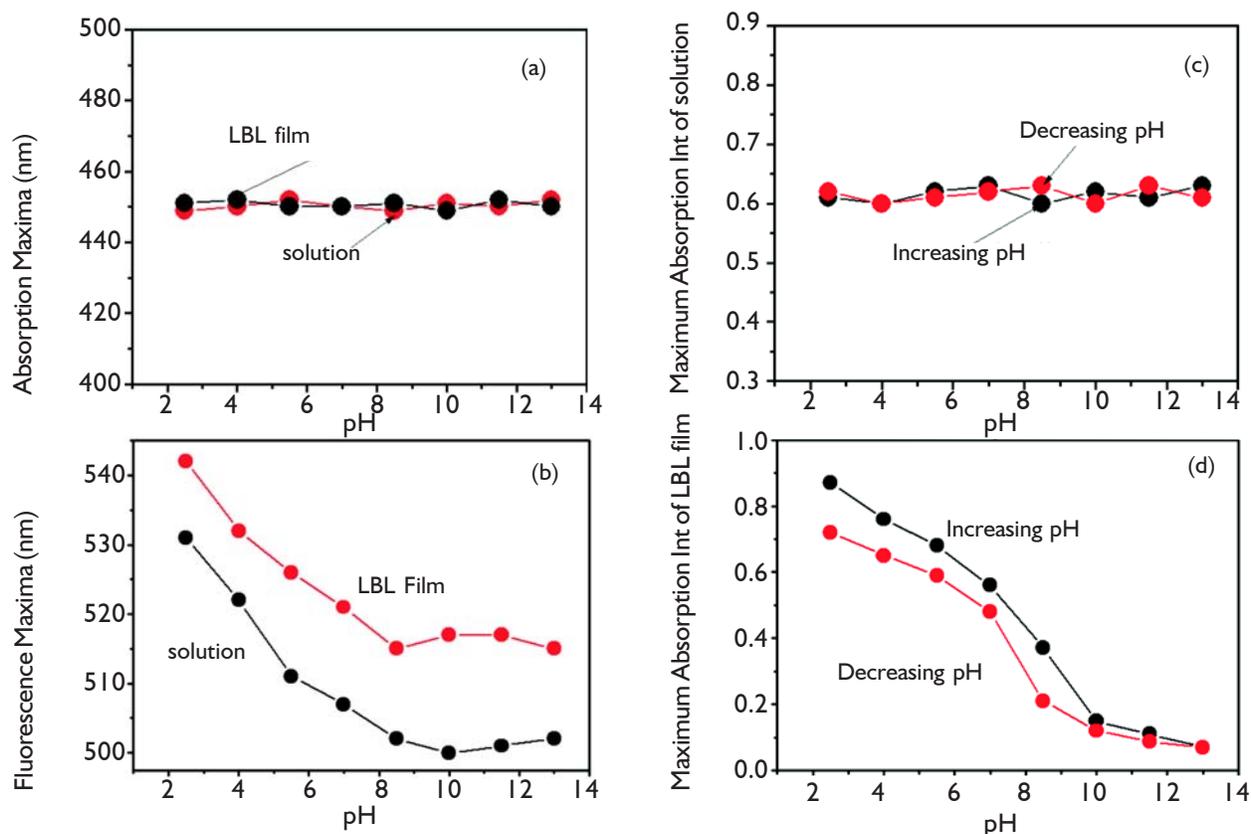


Fig. 3. The band positions of Absorption maxima (a) and Fluorescence maxima (b) of Acriflavine in both solution and LBL film with varying pH. Maximum Absorption intensity of Acriflavine in both solutions (c) and LBL films (d) with increasing and decreasing pH. Concentration of the dye was  $10^{-6}$ M for solution and  $10^{-4}$ M for LBL film

decreasing pH. A similar kind of larger red shift of Acf fluorescence in Nafion has been observed and explained due to change in the dipole moments in the excited state of the double protonated Acf [31] and due to the broad distribution of pKa in nafion matrix [1].

Figure 3(c) and 3(d) represents the absorbance of Acf solution and LBL film respectively with increasing and decreasing pH (2.5-13). It is observed from Fig. 3(c) that the absorption intensity of Acf solution almost remains same with increasing as well as decreasing pH, indicating the independence of Acf absorption intensity with pH in solution. On the other hand the absorption intensity in LBL film is decreased with increasing pH. The three forms of Acriflavine are shown in Fig. 1. To execute the experiment, we start with double protonated Acf

molecules. As it is having the most cationic format, it results in a larger interaction with anionic PAA molecules observed as high absorption intensity in the LBL film. Now adding NaOH to the solution we convert double protonated Acf in to single protonated Acf and single protonated Acf in to neutral Acf (Fig. 1) and consequently the small amount of cation in Acf (single protonated and neutral) molecules provide a less interaction with PAA. On the other hand the presence of  $\text{Na}^+$  in the solution also occupy few space on the matrix by attraction towards PAA, since  $\text{Na}^+$  ion is lighter than the single protonated Acf or neutral Acf molecules. That could be another reason for the less intensity of absorption by single protonated Acf and neutral Acf molecules. The reaction is shown as forward reaction as step 1 and step 2. Now to reverse the pH from 13 to 2.5 we add HCl with the Acf solution of pH 13 and the

reaction is shown as reverse reaction by step 3 and step 4. From Fig. 2(d) it is observed that for the same value of pH the intensity is slightly less in the reverse direction resulting in a hysteresis. It is observed from the reverse reaction, at the same pH of single protonated Acf and neutral Acf solutions the number of  $\text{Na}^+$  ions in the reverse reaction is double, compare to forward reaction. As a result some more space on the PAA matrix is occupied by the  $\text{Na}^+$  ions resulting in a less interaction of single protonated Acf or double protonated Acf molecules with PAA observed as a less absorption intensity in the reverse direction.

It is interesting to mention in this context that there are several reports on pH sensors. Egami et al has introduced a fiber optic pH sensor, using polymer doped with either congo red (pH range 3 to 5) or methyl red (pH range from 5 to 7) [32]. pH sensor based on the measurement of absorption of phenol red has also been reported [33], which can sense a pH range of 7-7.4. In the present system of pH measurement using the change in emission maxima and absorption intensity with pH is capable of measuring over a wide range of pH 2.5 to 13.0. This is one advantage with respect to previous systems.

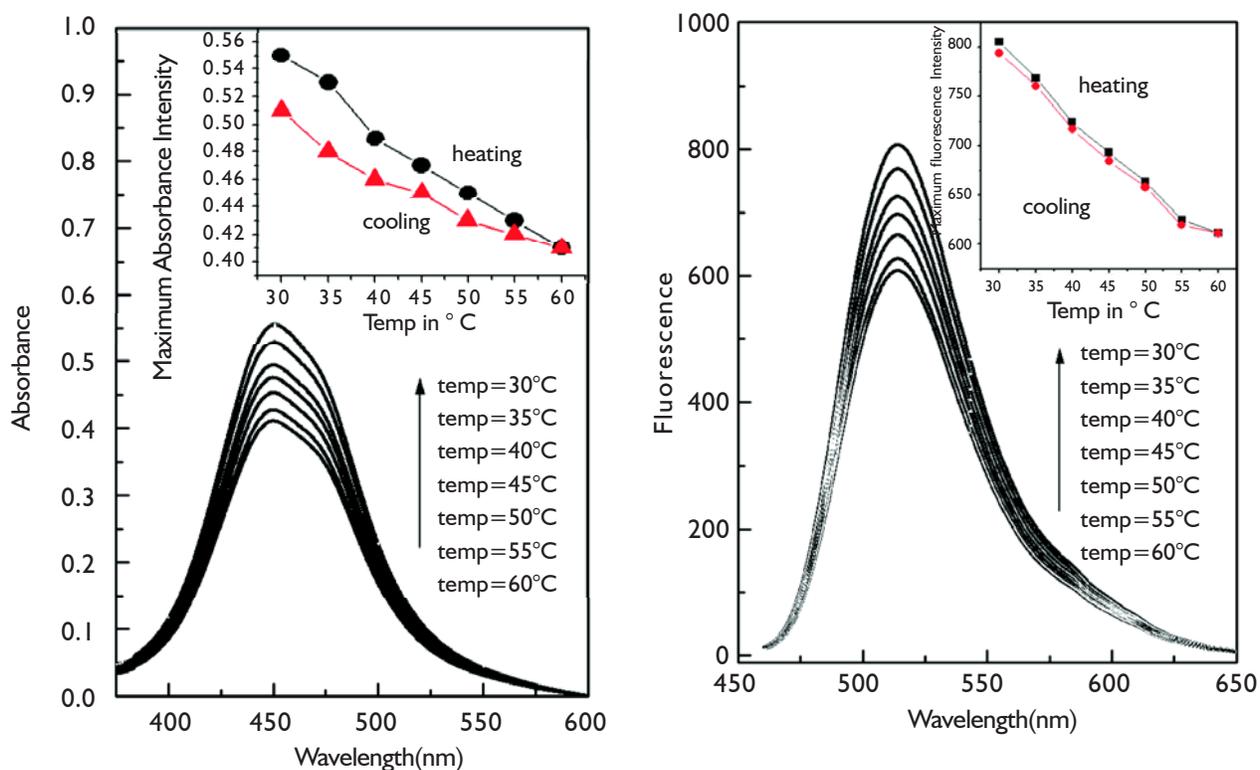
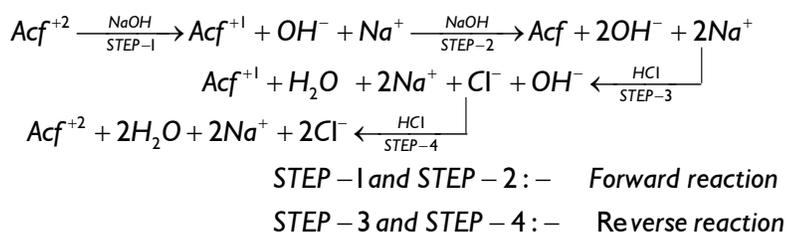


Fig. 4. Absorption spectra of Acriflavine with increasing temperature (inset showing the hysteresis in maximum absorption intensity with a heating-cooling cycle). Concentration of the dye was  $10^{-6}\text{M}$  for solution and  $10^{-4}\text{M}$  for LBL film

### 3.3. Temperature Effect

To study the effect of heating on the UV-Vis absorption and fluorescence spectra, we performed a heating cooling cycle in the range of 30-60°C on the Acf-PAA LBL films. A decrease in absorbance as well as fluorescence intensities are observed in Fig. 4a and 4b, respectively which indicates that the structure of the polyelectrolyte layers has changed. The changes in the polyelectrolyte layers could be due to the formation of aggregates among the PAA molecules with increasing heat. The aggregation among the polymer molecules is responsible for the less interaction of the cationic dye molecules (Acf) with PAA, resulting in a decrease in the binding energy between Acf and PAA. A similar kind of observation is done by He Cheng and co-workers where the chain association and dissolution of the polymer PNIPAM in water during one heating-cooling cycle is explained [16]. Thus it can be concluded from the above results that the polyelectrolyte layers are thermally unstable and there is a less interaction between the Acf polycations and PAA polyanions. Therefore, temperature dependence of the laser dye Acf under investigation makes the system a suitable candidate for sensing of temperature over a range of 30° to 60°C.

In the heating cooling cycle there was no change of absorption band positions on reversible process with cooling but a hysteresis in the absorbance intensity occurs which is shown in the inset of Fig. 4a. This suggests the change in possible preferential orientations of the chromophore in the monolayer during the process of cooling. A similar kind of observation for an azo dye Chicago Sky Blue has been observed in one of our previous work during the heating-cooling cycle of 30° to 90°C [34].

### 4. Conclusion

In the present communication we have successfully demonstrated the formation of LBL films of a cationic laser dye Acf. Also the effect of temperature and pH on the spectral characteristics of Acf in solution and LBL films has been demonstrated. It has been observed that almost 35

min is required to complete up the absorption process of Acf onto LBL films. The fluorescence spectra of Acf were pH sensitive both in solution and in LBL films. Fluorescence maxima were found to be red shifted with decreasing pH and blue shifted with increasing pH for both solution and in LBL films. On the other hand both the absorbance and fluorescence intensities were found to be temperature dependent. These results indicate that with proper calibration it is possible to use the present system as pH sensor and temperature sensor.

### References

- [1] V. Mishra, H. Mishra, H.C. Josh and T.C. Pant, *Sens. Actutators: B Chem.*, **63** (2000) 18.
- [2] J.J. Peterson and G.G. Vurek, *Science*, **224** (1984) 123.
- [3] O.S. Wolfbeis (Ed.), *Fibre Optic Chemical Sensors and Biosensors* (CRC Press), (1991).
- [4] J.R. Lakowicz (Ed.), *Topics in fluorescence spectroscopy, Probe Design and Chemical Sensor* (Plenum Press), (1996).
- [5] V. Mishra, H. Mishra, H.C. Joshi and T.C. Pant, *Sens. Actutators: B Chem.*, **82** (2002) 133.
- [6] D. Dey, D. Bhattacharjee, S. Chakraborty and S.A. Hussain, *J. Photochem. Photobiol.*, **A 252** (2013) 174.
- [7] T. Nguyen, K.P. McNamara and Z. Rosenzweig, *Anal. Chim. Acta*, **400** (1999) 45.
- [8] H. Offenbacher, O.S. Wolfbeis and E. Furlinger, *Sens. Actutators: B Chem.*, **9** (1983) 73.
- [9] W. Trettnak, M.J.P. Leiner and O.S. Wolfbeis, *Biosensor*, **4** (1988) 15.
- [10] H.E. Poach, M.J.P. Leiner, O.S. Wolfbeis and Fresenius, *J. Anal. Chem.*, **334** (1989) 162.
- [11] O.S. Wolfbeis, T. Werner, N.V. Rodriguey and M. Kessla, *Microchim. Acta*, **108** (1992) 133.
- [12] T. Werner, O.S. Wolfbeis and Fresenius, *J. Anal. Chem.*, **346** (1993) 564.
- [13] B.H. Weigi, A. Holober, W. Trettnak, I. Kilmant, H. Kraus, P.O. Leory and O.S. Wolfbeis, *J. Biotech.*, **32** (1994) 127.
- [14] S.G. Schulaman, Sh.X. Chen. F. Bai, M.J.P. Loner, L. Weis and O.S. Wolfbeis, *Anal. Chim. Acta.*, **304** (1995) 165.

- [15] O.S. Wolfbeis, E. Furlinger, H. Kroneis, H. Marsoner and Fresenius, *J. Anal. Chem.*, **314** (1983) 119.
- [16] F. Bignotti, M. Penco, L. Sartore, I. Peroni, R. Mendichi, M. Casolaro and A.D. Amore, *Polymer*, **41** (2000) 8247.
- [17] T.V. Burova, N.V. Ginberg, V.Y. Grinberg, E. V. Kalinina, V.I. Lozinsky, V.O. Aseyev, S. Holappa, H. Tenhu and A.R. Khokhlov, *Macromolecules*, **38** (2005) 1292.
- [18] He Cheng, Lei Shen and Chi Wu, *Macromolecules*, **39** (2006) 2325.
- [19] K. Otake, H. Inomata, M. Konno and S. Saito, *Macromolecules*, **23** (1990) 283.
- [20] G. Decher, *Science*, **277** (1997) 1232.
- [21] Z. Tang, N.A. Kotov, S. Magonov and B. Ozturk, *Nat. Mater.*, **2** (2003) 413.
- [22] J.B. Schlenoff, *Langmuir*, **25** (2009) 14007.
- [23] J.T. Stricker, A.D. Gudmundsdottir, A.P. Smith, B.E. Taylor and M.F. Durstock, *J. Phys. Chem.*, **B 111** (2007) 6322.
- [24] X. Gong, J. Yang, L. Han and C. Gao, *Langmuir*, **24** (2008) 13925.
- [25] T.H. Kim, E.Y. Jang, N.J. Lee, D.J. Choi, K. J. Lee, J.T. Jang, J.S. Choi, S.H. Moon and J. Cheon, *Nano. Lett.*, **9** (2009) 2229.
- [26] W.L. Leong, P.S. Lee, A. Lohani, Y.M. Lam, T. Chen, S. Zhang, A. Dodabalapur and S.G. Mhaisalkar, *Adv. Matter.*, **m 20** (2008) 2325.
- [27] J. Nam, N. Won, H. Jin, H. Chung and S. Kim, *J. Am. Chem. Soc.*, **131** (2009) 13639.
- [28] N. Tsapis, D. Bennett, B. Jackson, D.A. Weitz and D.A. Edwards, *Proc. Natl. Acad. Sci. U. S.A.*, **99** (2002) 12001.
- [29] D. Dey, S.A. Hussain, R.K. Nath and D. Bhattacharjee, *Spectrochim. Acta, Part A* **70** (2008) 307.
- [30] P.D. Sahare, V.K. Sharma, D. Mohan and A.A. Rupasov, *Spectrochim. Acta, Part A* **69** (2008) 1257.
- [31] J.L. Rosenberg and F.S. Humphries, *J. Phys. Chem.*, **71** (1967) 330.
- [32] V.K. Sharma, P.D. Sahare, R.C. Rastogi, S.K. Ghoshal and D. Mohan, *Spectrochim. Acta, Part A*, **59** (2003) 1799.
- [33] T. Ishiji, K. Kudo and M. Kaneko, *Sens. Actuators: B Chem.*, **22** (1994) 205.
- [34] C. Egami, Y. Suzuki, O. Sugihora, H. Fujimura and N. Okamoto, *Jpn. J. Appl. Phys.*, **36** (1997) 2902.
- [35] J.I. Peterson, S.R. Goldstein, R.V. Fitzgerald and D.K. Buckhold, *Anal. Chem.*, **52** (1980) 864.
- [36] D. Dey, M.N. Islam, S.A. Hussain and D. Bhattacharjee, *Chin. Phys. Lett.*, **10** (2008) 3732.