

# Layer-by-layer self-assembly of polyelectrolyte and the divalent salt of fluorescein

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## Abstract

Smooth organic thin films have been obtained by repeated surface complexation between a polycation and fluorescein di-sodium, a divalent organic anion, in a layer-by-layer (LbL) fashion. UV-Vis and thickness measurements show a linear growth of the multilayer as a function of deposition cycles, and indicate that the dye is in a J-aggregate state in the multilayer. The dynamical properties of the multilayer, which are assessed by time-dependent UV spectroscopy, indicate the existence of two relaxation processes. The faster one is due to slow drying and thermal equilibration of the film. The slower process results from spatial rearrangement of dyes in the multilayer, leading to a lower aggregation rate.

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## 1. Introduction

A few years ago, Decher and coworkers developed a new technique of thin film assembly based on the alternate adsorption of oppositely charged polyelectrolytes [1]. This method has been extended to various organic or inorganic materials and substrates [2]. Among the large diversity of available compounds, bola-amphiphiles coupled to polyelectrolytes were used to build thin films [3,4].

Organic dyes were also tested as film components in many investigations [5–11]. Water soluble organic dyes are, generally, aromatic charged molecules. Ariga et al. studied a large variety of dyes bearing different numbers of charges [5]. Quartz crystal microbalance measurements revealed that the dye adsorption occurs as rapidly as the polyion adsorption. They found that well-packed monomolecular layers are formed and depend on size, number of charges and spatial orientation of dye molecules. Self-assembly of cyanine [7,8] and pyrene [9] dyes were investigated by UV-Vis spectroscopy. Inclusion of ruthenium complexes in multilayers

led to the elaboration of light emitting electrochemical devices [10,11].

Surprisingly, to our knowledge, no work deals with the self-assembly of fluorescein salts with polycation. Yet, this dye is one of the most common fluorophore and is extensively employed in the field of biochemistry [12]. In a previous work, we reported on the introduction of fluorescein as third component in multilayers made of a polycation and a polyanion [13]. Inward and outward diffusion of the dye in the films were studied. This work deals with the direct alternate assembly of the di-anion fluorescein disodium salt with two strong polycations. The multilayer growth is studied by UV-Vis spectroscopy and ellipsometry. The films structure is analyzed by X-ray reflectometry. A rapid investigation of the dynamical behavior of the multilayer is also conducted.

## 2. Experimental section

### 2.1. Materials

Fluorescein disodium salt dihydrate 90% was purchased from Acros Organics and was used without any further purification. As polycation, we used a poly(vinylbenzyl chloride) (PVBC) quaternized with *N,N*-dimethylethanolamine

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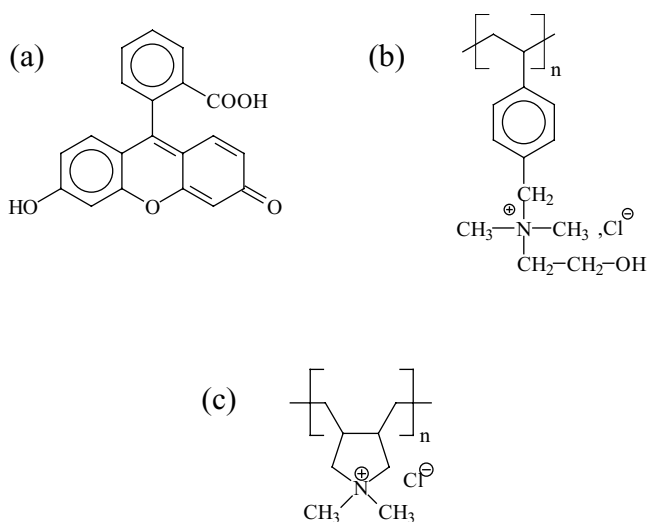


Fig. 1. Molecular structures of (a) fluorescein, (b) poly(vinylbenzyl chloride) quaternized with *N,N*-dimethylethanolamine (PVBAC), (c) poly(diallyldimethylammonium chloride) (PDADMAC).

(redistilled, 99.5+%, Aldrich), thereafter abbreviated as PVBAC (Fig. 1). The synthesis of this polymer is described below. 4-Vinylbenzylchloride (VBC, 98% of para-isomer, Aldrich, 90%, inhibited with *tert*-butylcatechol and nitroparaffin) were purified prior to polymerization by column filtration (aluminium oxide, activated, basic, 50–200 mesh, Acros Organics) to remove the inhibitors and stored at +4 °C in dark. The solvents were analytical grade and used as received. Azobisisobutyronitrile (AIBN) was recrystallized from ethanol just before use. Poly-diallyl-dimethyl-ammonium chloride (Aldrich) was also tested as alternate polycation.

### 2.1.1. Synthesis of the polycation (PVBAC)

PVBC was synthesized according to a published procedure [14]. PVBC was characterized by size exclusion chromatography ( $M_w = 46300 \text{ g mol}^{-1}$ ,  $M_w/M_n = 5.2$ ). To prepare PVBAC from PVBC, 3 g of PVBC ( $1.96 \times 10^{-2}$  mol of monomer units) are dissolved in 30 ml of DMF, 5.26 g of *N,N*-dimethylethanolamine ( $5.9 \times 10^{-2}$  mol) are added and the solution is stirred at 65 °C for 48 h. The precipitate is filtered and washed with DMF. The polymer is dissolved in a small amount of deionized water, precipitated in THF, washed with THF, redissolved in water and freeze-dried. The degree of quaternization was estimated by elemental analysis to be 97%, as confirmed by  $^1\text{H}$  NMR.

### 2.1.2. Substrate preparation

Fused silica SUPRASIL<sup>®</sup> slides and (100) one-side polished silicon wafers were purchased from Hellma and ACF, respectively. Silicon and fused silica substrates were dipped for 30 min in a “piranha” acid bath (50 vol.% of concentrated  $\text{H}_2\text{SO}_4$ /50 vol.% of  $\text{H}_2\text{O}_2$  (30%). *Caution: pirhana solutions are strongly oxidizing solutions and should not be stored in closed containers.*), then rinsed five times with deionized water and dried with warm air before use.

### 2.1.3. Assembly of the films

Films were built by alternated deposition of polycation and fluorescein onto the substrates. Concentrations of polyelectrolyte solutions were  $10^{-2}$  M (concentration in monomer units) in deionized water (pH = 6). If not specified, the substrates were first immersed for 8 min in the polycation solution, rinsed with a continuous flow of deionized water for 60 sec and dried with warm air for 3 min. They were then immersed for 15 min in the fluorescein solution ( $10^{-2}$  M), rinsed and dried in the same fashion. The process was repeated using an automated dipper (Riegler and Kirstein, Germany), to obtain multilayers. A deposition cycle is defined in the following as a (polycation/fluorescein) bilayer.

## 2.2. Characterization techniques absorption measurements

UV/visible spectra of dyes-doped multilayers were taken using a Cary 50 concentration UV-Vis spectrophotometer (Varian).

### 2.2.1. Fluorescence measurements

Excitation and emission fluorescence spectra were obtained with a Fluorolog-2 fluorimeter (Jobin Yvon–Horiba). The detector was placed at 22.5° from the pathway of the light source.

### 2.2.2. X-ray reflectometry measurements

The experimental setup is based on a Siemens D5000 2-circles goniometer. X-rays of 1.5418 Å wavelength (Cu  $K\alpha$ ) were obtained from a rotating anode operated at 12 kW. Monochromatization was achieved with the help of a secondary graphite monochromator, complemented with pulse height discrimination (scintillation counter). Proper collimation of the beam was obtained by using slits adjustable with micrometer precision. The corrected intensity is reported versus  $K_{z0}$ , the component perpendicular to the interface of the wavevector in a vacuum of the incident photons (i.e.,  $K_{z0} = (2\pi/\lambda) \sin \theta$ , where  $\lambda$  is the X-ray wavelength and  $\theta$  is half the angle between the incident and the reflected beams). Data analysis was performed either by Fourier transforming the data after proper normalization to obtain a Patterson function or by fitting the data by standard routines as described previously [15].

## 3. Results and discussion

Multilayers were grown by alternate adsorption of polycation and fluorescein disodium in deionized water. Fig. 2 depicts the UV spectra measured at each half-cycle (a whole cycle corresponding to a bilayer). The inset shows the absorbance of the peaks at 225 and 500–520 nm (characteristic absorption peak of the dye). The visible absorbance of the fluorescein signal increases almost monotonically indicating the growth of the multilayer. However, the absorption

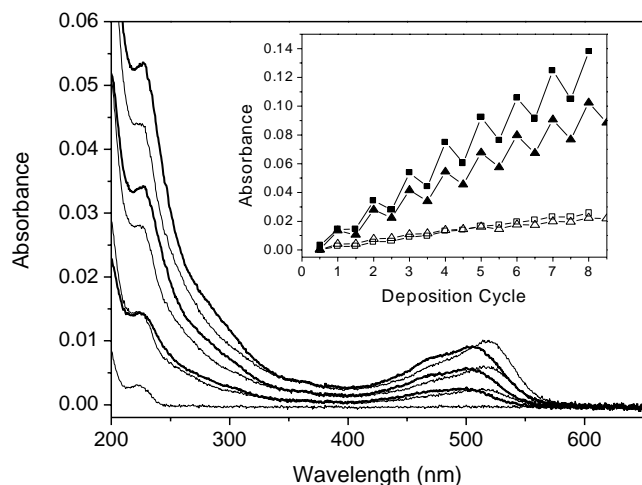


Fig. 2. UV spectra of PVBAC-fluorescein alternate assemblies on quartz plates. Lines are thin when PVBAC is the outermost layer and bold when fluorescein is the outermost layer. The inset shows the absorbance maxima of peaks at 225 nm (filled symbols) and 500 nm (open symbols) for PVBAC-fluorescein (squares) and PDADMAC-fluorescein (triangles) multilayers. Half-cycles correspond to the adsorption of the polycation.

wavelength varies between 500 nm and 518 nm depending on whether polycation or fluorescein is the outermost layer (Fig. 3). The red shift observed in comparison with a soluble polycation (PVBAC)/fluorescein complex in water ( $\lambda_{\max} = 501$  nm) indicates the formation of fluorescein J-aggregates in the films. The aggregation rate increases as the multilayer grows and tends to a plateau. This plateau is reached more rapidly when PDADMAC is used as a polycation instead of PVBAC, which could be due to favorable interactions between the aromatic residues of PVBAC and the aromatic dye. The larger shift displayed when the polycation is the top layer can be attributed to a higher degree of aggregation

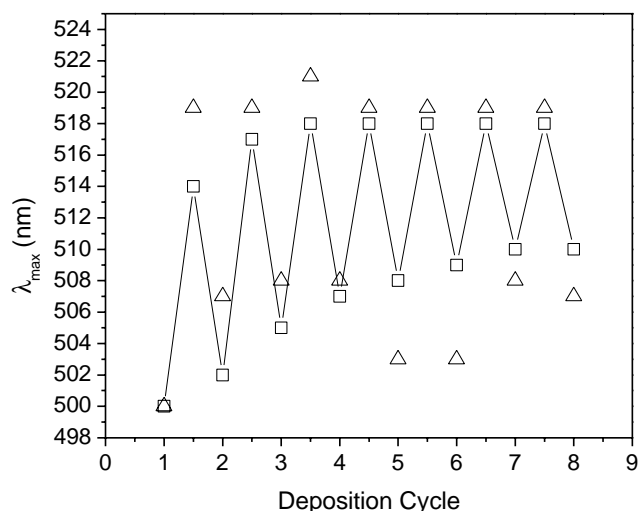


Fig. 3. Maximum absorption wavelength of fluorescein as a function of deposition cycles for PVBAC-fluorescein (squares) and PDADMAC-fluorescein (triangles) multilayers. Half-cycles correspond to the adsorption of the polycation.

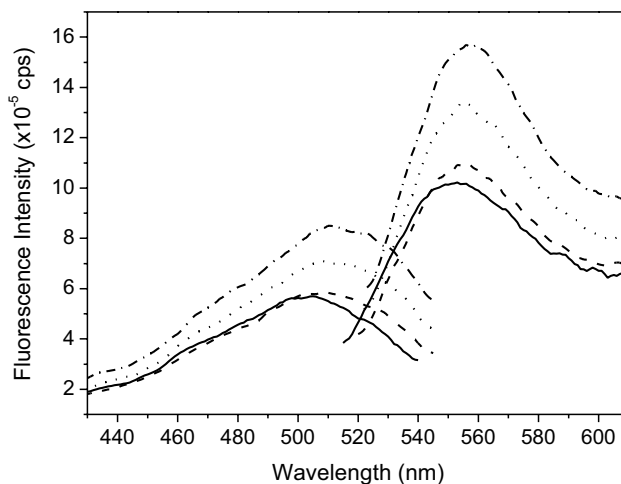


Fig. 4. Emission and excitation fluorescence spectra of PVBAC/fluorescein multilayers grown on glass substrates. Number of layers are respectively two (continuous), four (dashed), six (dotted) and eight (dash-dotted) bi-layers.

of the dye. Within the experimental error, the aggregation rate is independent on the nature of the polycation. Fluorescence spectra of PVBAC/fluorescein multilayers shown in Fig. 4 also indicate the aggregation of dyes. The excitation and emission wavelengths are respectively shifted from 503 to 515 nm and from 552 to 557 nm as the film grows. Such aggregates of fluorescein moieties have already been observed and described in Langmuir, Langmuir–Schaefer [16] and Langmuir–Blodgett films [17]. It must be pointed out that both the maximum absorption wavelength and the extinction coefficient increase with the degree of aggregation. The monotonical evolution of the visible absorbance is, thus, an apparent behavior only. The UV absorbance at 225 nm shows a signal decrease when the polycation is deposited on the top layer. This observation indicates partial desorption of the dye, as already proposed in the literature for similar systems [5,6,9]. The amount of fluorescein released during the polycation adsorption is very difficult to quantify, since the extinction coefficients may not be taken as constant. A coloration of the polycation solution appears after a few immersion cycles. On the basis of UV-Vis measurements only, it is also difficult to estimate the amount of material deposited in the layers. However, it seems that more fluorescein is adsorbed when PVBAC is used as a polycation. Once again, this may be due to favorable interactions between the aromatic residues of PVBAC and the aromatic dye.

X-ray reflectograms of PVBAC/fluorescein and PDADMAC/fluorescein multilayers grown, respectively, on fused silica and silicon substrates are shown in Fig. 5. For this range of film thickness, the number of Kiessig fringes is typical of films of low surface roughness (around 10 Å whatever the polycation), when fluorescein is the outermost layer. Thickness measurements show a linear growth of the multilayers when built on silicon substrates (Fig. 6).

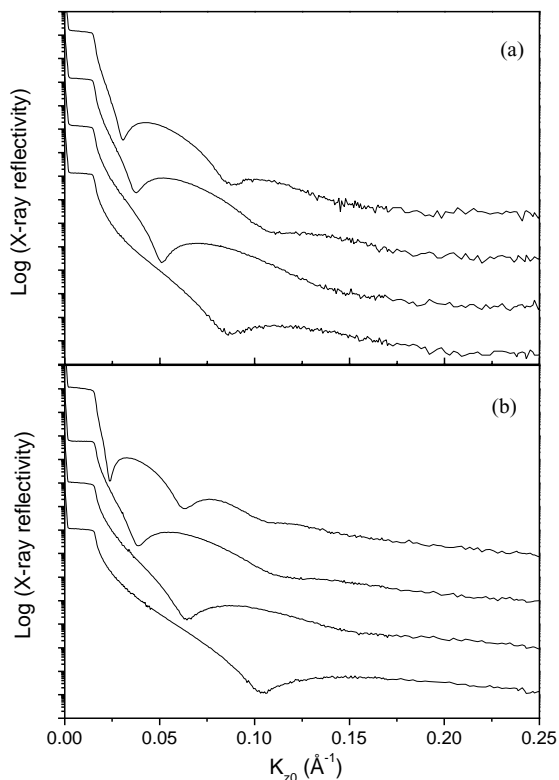


Fig. 5. X-ray reflectivity of polycation/fluorescein multilayers. (a) PVBAC/fluorescein deposited on fused silica substrates. From bottom to top, deposition cycles are two, four, six and eight. (b) PDADMAC/fluorescein deposited on silicon substrates. From bottom to top, deposition cycles are 2, 4, 8, 16. For clarity, reflectograms are vertically shifted by a multiplicative factor 1,  $10^2$ ,  $10^4$  and  $10^6$ , respectively.

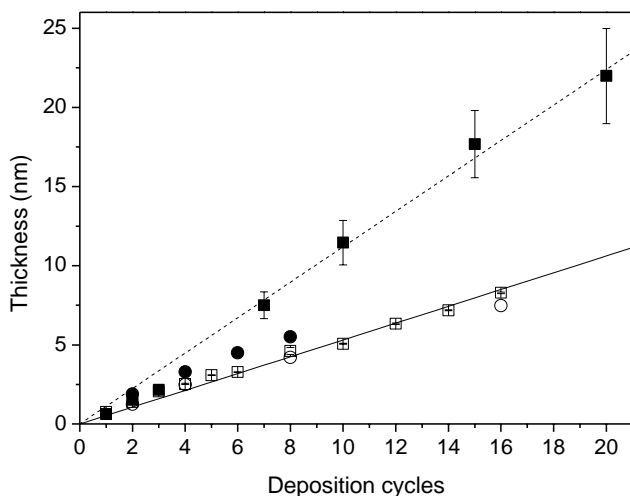


Fig. 6. Thickness of films versus number of deposition cycles for PVBAC/fluorescein (filled symbols) and PDADMAC/fluorescein (open symbols) multilayers. Measurements are obtained by ellipsometry (squares) and X-ray reflectivity (circles).

The average thickness per deposition cycle was estimated to be  $11.8 \text{ \AA}$  for  $(\text{PVBAC/fluorescein})_n$ , and  $5.3 \text{ \AA}$  for  $(\text{PDADMAC/fluorescein})_n$ . This result confirms the trend observed by UV-Vis measurements. Thin films of PVBAC/fluorescein measured by X-ray reflectometry exhibit a non-linear growth contrary to other results. This discrepancy can be due to the fused silica substrate used for this sample: The higher surface roughness of the substrate may perturb film growth.

Polyelectrolyte multilayers, as other macromolecular materials, are dynamical structures. The motions of polyelectrolytes and ions in the multilayers were shown by dielectric spectroscopy [18]. Lateral diffusion of lipids [19] and proteins [20] was also observed by fluorescence recovery techniques. Self-assembly of PVBAC and fluorescein brings another illustration of motions occurring in thin films. Optical properties of fluorophores are strongly dependent on their environment and their degree of aggregation. The time dependence of the optical properties of the dyes embedded in the multilayer is shown in Fig. 7. The film made of 10.5 PVBAC/fluorescein bilayers (the polycation is the outer layer) was built using the procedure described above and, straightaway, placed in the UV-visible spectrometer at  $20^\circ\text{C}$ . Spectra were measured every 30 min during 50 h. A decrease in visible absorbance and in the wavelength of maximum absorption was seen to occur in two steps, and could be described by a double exponential decay. The solid line in Fig. 6 is a fit to the absorbance data according to the following equation:

$$A = A_\infty + a \exp\left(-\frac{t}{\tau_1}\right) + b \exp\left(-\frac{t}{\tau_2}\right) \quad (1)$$

where  $A_\infty$  is the absorbance at the equilibrium,  $\tau_1$  and  $\tau_2$  are the two characteristic times, and  $a$  and  $b$  are prefactors.

The fastest relaxation process ( $\tau_1 = 0.63 \text{ h}$ ) can be probably attributed to a polarity change of the medium due to water evaporation and to some thermal equilibration. The

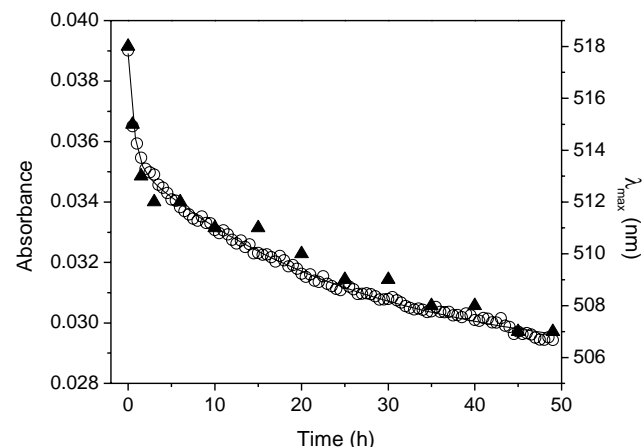


Fig. 7. Time dependence of the visible absorbance (open circles) and the wavelength (filled triangles) of 10.5 PVBAC/fluorescein bilayers (PVBAC is the outermost layer). The solid line is a fit the absorbance data according to Eq. (1).

slowest process ( $\tau_2 = 25.5$  h) corresponds to a spatial reorganization of the dye molecules in the layer. The maximum absorbance wavelength decrease indicates a diminution of the aggregation rate. This wavelength does not tend to the value of non-aggregated molecules complexed with PVBAC in solution (501 nm) but the asymptotic value ( $\lambda_{\text{max}} \rightarrow 505$  nm) obtained owing to the fit shows a low degree of aggregation when the equilibrium is reached. This phenomenon could be also accompanied by an orientation change of the fluorescein molecules which also affect the optical properties.

#### 4. Conclusion

It has been shown that a bivalent organic ion can be self-assembled with a polycation to build smooth thin films. UV-Vis and thickness measurements show a linear growth of the multilayer as a function of deposition cycles. As already observed in the literature, a release of dye molecules occurs when the film is dipped in the polycation solution. Fluorescein is found to be in a J-aggregate state in the multilayer.

Time dependent UV-Vis measurements display the dynamical properties of the multilayer. Two relaxation processes have been observed. The faster one can be attributed to drying and thermal equilibration of the film. The slower is due to spatial rearrangement of dyes in the multilayer, leading to a lower aggregation rate. This suggests that the mobility of polyelectrolyte multilayers may be quite larger than originally thought.

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