

# Surface Characterization and Thermodynamics of Adsorption of Congo Red-gelatin Layer-by-Layer Growth

Rosemary S. Turingan<sup>1</sup>, Ma. Cecilia P. Garcia<sup>1</sup>, Leon M. Payawan, Jr.<sup>1</sup>, and M. Cynthia Goh<sup>2</sup>

<sup>1</sup>*Advanced Physical Chemistry Research Laboratory*

*Institute of Chemistry*

*University of the Philippines*

*Diliman, Quezon City, PHILIPPINES*

<sup>2</sup>*Department of Chemistry*

*University of Toronto*

*Ontario, CANADA*

---

Gelatin, a denatured form of collagen was made cationic at a pH below its isoelectric point. With Congo Red as an anionic bolamphiphile, layer-by-layer assembly of dye-polyelectrolyte was made possible through electrostatic alternate adsorption. Effects of pH, concentration, and ionic strength were investigated to attain the optimum conditions for layer growth. Films fabricated were characterized using UV-Spectroscopy, Atomic Force Microscopy (AFM), and contact angle measurements. The extent of layer build-up was correlated with the dye's absorbance at  $\lambda = 497$  nm and it was found out that as more layers were deposited, a corresponding increase in absorbance was observed. Surface topology was studied through AFM and comparative contact angle measurements of the undeposited and deposited substrate were conducted. An increase in the wettability was associated with the adsorption of the hydrophilic layers on glass. Adsorption isotherms were constructed through the relation between amount adsorbed as a function of the bulk sample concentration.

---

**Keywords:** layer-by-layer growth; Congo Red; Gelatin; adsorption isotherm; contact angle; spectroscopy; Atomic Force Microscopy

## INTRODUCTION

Molecular self-assembly is one of the powerful approaches to create high ordered functional materials. During a self-assembling process, atoms, molecules, aggregates, and composites come together in an organized and spontaneous fashion, resulting in ordered functioning entities [1]. This interestingly simple process of nanofabrication allows the creation of materials, which are of superior properties. The process also eliminates the error and expense introduced by humans [2].

Multicomposites, or multicomponent nanostructures are of great importance in material science. Fabrication of these structures allows the combination of two or more desirable properties providing nanostructures with enhanced characteristics [2].

The fabrication of a multicomposite system from polymers can be carried out through molecular self-assembly [3]. The assembling process consists of a layer-by-layer adsorption of polyelectrolytes onto oppositely charged surfaces. The polyfunctionality of the polymers, combined with the stability of electrostatic attraction are the salient features of this type of fabrication mechanism.

Molecular self-assembly driven by electrostatics enables the creation of a nanoarchitecture with good positioning of individual layers. It is largely independent on the nature, size, and topology of the substrate. It also has the least steric demands, and is applicable to a very wide range of materials such as spolyelectrolytes [4, 5].

In this paper the multilayer build-up via layer-by-layer adsorption of gelatin and Congo Red dye on a glass substrate to form bilayers was investigated. Gelatin, a proteinaceous sub-

stance has already been used to fabricate self-assembled monolayers on mica [6]. From previous studies Congo Red, a negatively charged polyazo dye has also already been used to make dye-polyion molecular films [7]. The main driving force for the multilayer build-up is an electrostatic attraction between the two polyelectrolytes: the Congo-Red- gelatin polyelectrolyte pair. In the building-up process effects of pH, ionic strength, and dye concentration on the stability of the multilayered film was studied. The number of bilayers adsorbed was monitored by taking absorbance measurements every after each Congo Red-gelatin assembly. Absorbance was taken at the maximum absorbance wavelength of the dye (497 nm). Film thickness and layer growth were monitored, an increase in absorbance was observed as the number of bilayers deposited increased. Adherence to Beer's law, which requires a linear dependence of concentration with the absorbance, is almost always observed in adsorbing multilayer systems. This may be due to the fact that the working concentration range is quite low because the layers that are deposited are approximately just one molecule thick.

In addition to the fabrication process this paper also dealt with surface characterization of the multilayer film. Atomic force microscopy was used to elucidate surface topology. And contact angle measurements enabled the researchers to study the changes in wetting properties brought about by the adsorption process. Finally, thermodynamics of adsorption was investigated by constructing the adsorption isotherms from the absorbance data that represents adsorption process [8].

## METHODOLOGY

**Materials.** All reagents used in the experiment were analytical grade except for those indicated. Congo Red and gelatin were purchased from Sigma. Hydrochloric acid, sulfuric acid, and potassium hydroxide were purchased from Ajax. The ultra pure water was provided by the Analytical Science Laboratory of the Institute of Chemistry, University of the Philippines, Diliman. Ordinary glass slides were purchased from Romaro Chemical Traders.

### Experimental procedures

**Preliminaries.** Before multi-layer build-up was carried out, the glass slides having the dimensions, 6 mm × 22 mm were first cleaned in KOH-Ethanol (1:1) base solution for 24 h, then rinsed with ultra pure water and ethanol and dried in the oven at 110°C. Other glasswares such as storage glass vials, measuring and transfer pipets, beakers, graduated cylinders, spatulas used in the experiment were also subjected to the same cleaning process.

Polyelectrolyte solutions with varying concentrations and pH were prepared a day before build-up to allow complete dissolution and equilibration.

**Layer deposition proper.** Layer build-up was carried out by dip-coating with ten minutes dipping time for all the runs. The room temperature was kept within the range (22–25°C).

The glass slide substrate was immersed in the polycationic gelatin solution for 10 min. After 10 min, the glass slide was withdrawn from the solution and then washed three times with ultra pure water. After washing, the glass slide was dipped in polyanionic Congo Red solution. The slide was withdrawn at the end of 10 min and the film was washed with ultra pure water. At the end of this step, absorbance measurements were done at 497 nm using Shimadzu UV-3101 PC.

This procedure was repeated several times to allow multilayer build-up.

**Film characterization.** Surface topology characterization of the fabricated films was done by using Shimadzu SPM9500 AFM. The wetting property of the fabricated film was investigated using a contact angle measurement device designed by the Advanced Physical Chemistry Research Laboratory in UP at Diliman.

## RESULTS AND DISCUSSION

### Stability factors

**Effect of pH.** Gelatin has different charging behavior below and above its isoelectric point, pH = 5.5. Below the isoelectric point at pH = 3.0 and pH = 3.5, positive charges become dominant because of the undissociated proton in the N-terminal, C-terminal and in some of the R groups of the amino acid residues in gelatin which favor electrostatic attraction to the negatively charged dye. From the UV spectroscopic data shown in Figs. 1–3 below, more bilayers were deposited at these pH compared to that at the isoelectric point. An increase in the absorbance was observed as the number of bilayers increased. Greater layer stability was brought about by the strong electrostatic attraction between the negatively

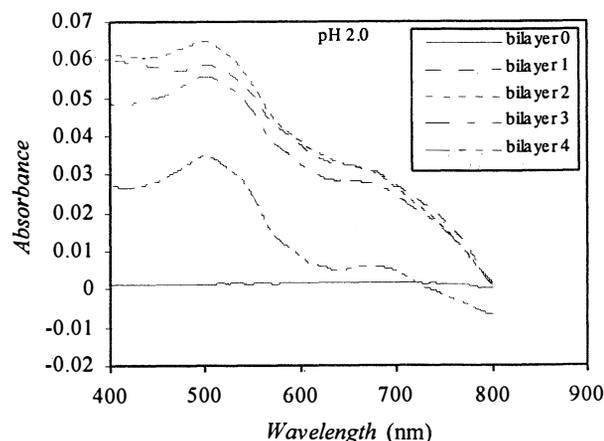


Fig. 1. Layer deposition using 1 mM Congo Red solution and 0.4 mg/mL, pH 2.0 gelatin solution.

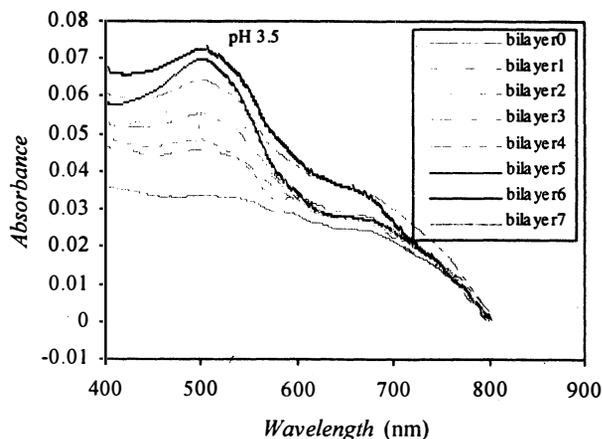


Fig. 2. Layer deposition using 1 mM, Congo Red solution and 0.4 mg/mL, pH 3.5 gelatin solution.

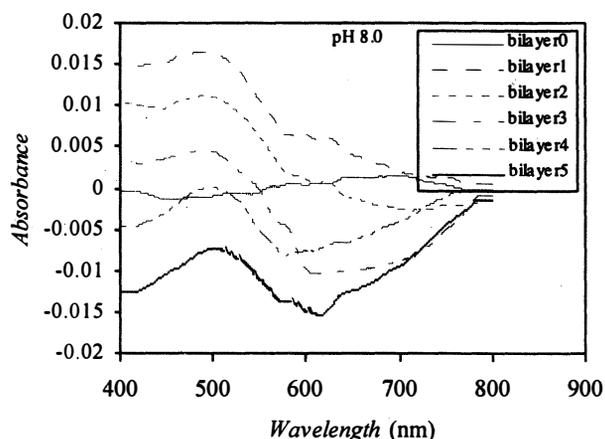


Fig. 4. Layer deposition using 1 mM, Congo Red solution and 0.4 mg/mL, pH 8.0 gelatin solution.

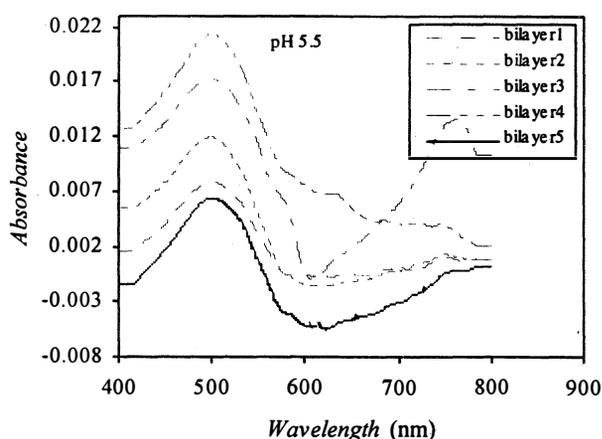


Fig. 3. Layer deposition using 1 mM, Congo Red solution and 0.4 mg/mL, pH 5.5 gelatin solution

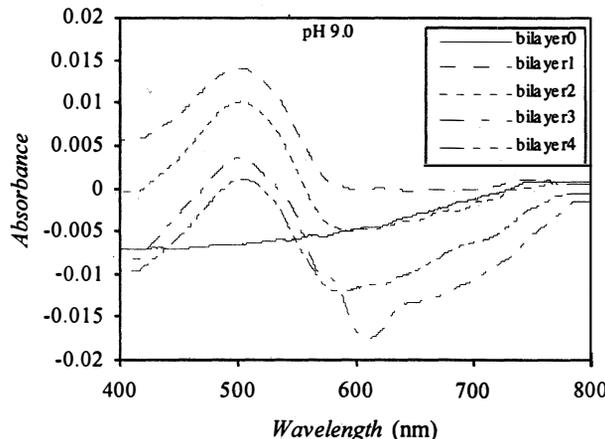


Fig. 5. Layer deposition using 1 mM, Congo Red solution and 0.4 mg/mL, pH 5.0 gelatin solution

charged Congo Red dye and the positively charged gelatin [7]. Lowering the pH increases the number of positive residues in gelatin, which in turn provides more attachment points for the bolamphiphillic Congo Red. The layer becomes sufficiently negative for the deposition of the next gelatin layer, finally leading to bilayer growth. The greatest number of stable bilayers was formed using gelatin at pH 3.5 as shown in Fig. 2. At pH = 7.0 and 8.0, gelatin is negatively charged and adsorption is electrostatically unfavorable. Figures 4 and 5 indicate that no layer build-up was observed as there was a decrease in absorbance. Destabilization occurred at these pH ranges because of the electronic repulsion between the negatively charged Congo Red, and now negative gelatin [6].

From the above results, it can be deduced that gelatin at pH 3.5 was optimum for bilayer growth. At this pH which is below the isoelectric point of gelatin, gelatin exhibits a positive overall charge. At this pH, the electrostatic attraction between the positively charged gelatin and Congo Red is most stable, thus allowing six bilayers to build-up. On the other

hand, at pH 2 only to three stable bilayers were formed. Destabilization may be due to initial acid hydrolysis of the peptide bonds in gelatin.

**Effect of concentration of Congo Red dye.** Using the optimum pH and concentration of gelatin (pH 3.5 and 0.4 mg/mL solution), the extent of bilayer growth was again investigated this time using varying concentrations of the Congo Red solutions. The concentrations of Congo Red solutions used were 0.01mM, 0.05 mM, 0.25 mM, and 0.75 mM. The absorbance data indicate that the number of bilayers formed was independent of the Congo Red concentration at a concentration range (0.01 mM to 0.75 mM). Figures 6–9 show that there was a relatively uniform number of layers formed despite the varying Congo Red concentrations. The above results mean that at the minimum concentration of 0.01 mM Congo Red the binding sites for layer build-up was already more than sufficiently saturated.

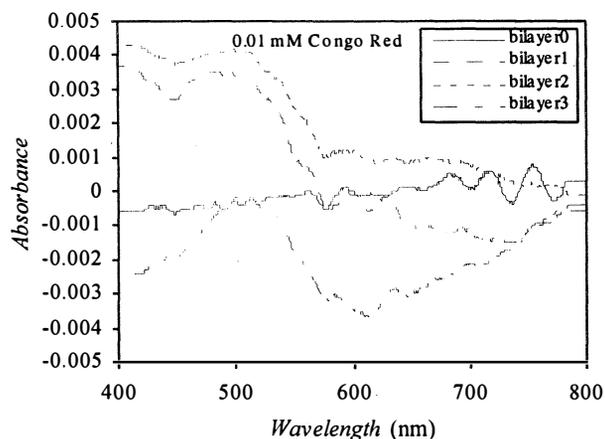


Fig. 6. Layer deposition using 0.01 mM, Congo Red solution and 0.4 mg/mL, pH 3.5 gelatin solution.

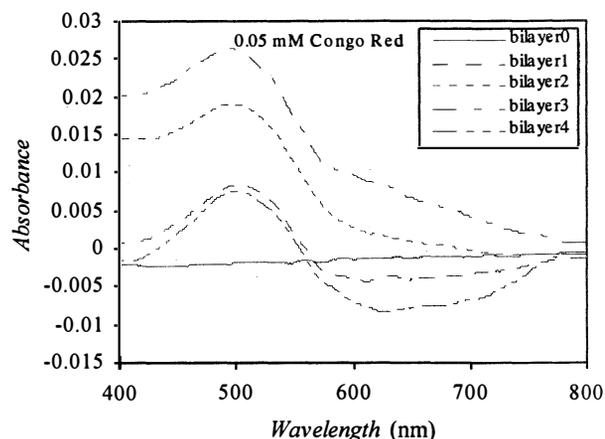


Fig. 7. Layer deposition using 0.05 mM, Congo Red solution and 0.4 mg/mL, pH 3.5 gelatin solution.

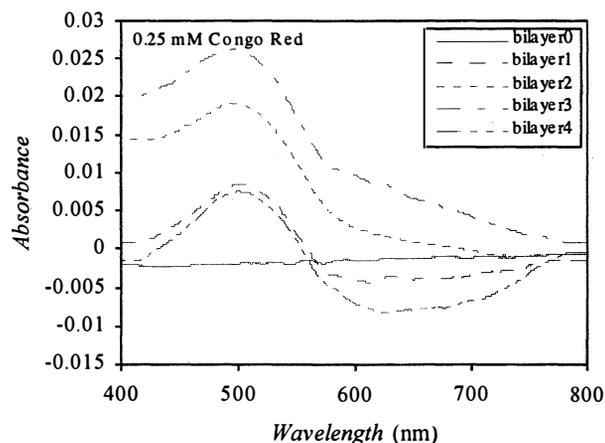


Fig. 8. Layer deposition using 0.25 mM, Congo Red solution and 0.4 mg/mL, pH 3.5 gelatin solution.

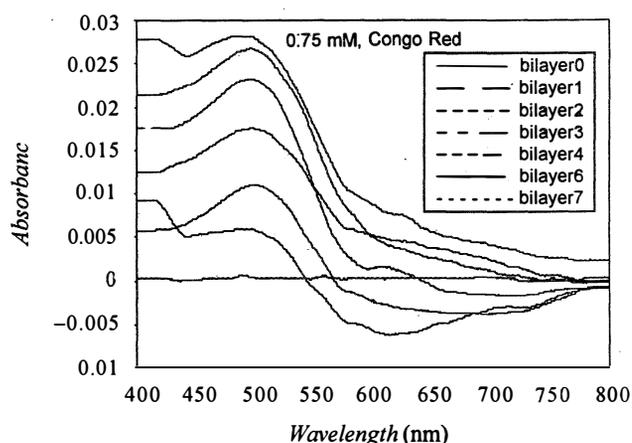


Fig. 9. Layer deposition using 0.75 mM, Congo Red solution and 0.4 mg/mL, pH 3.5 gelatin solution.

#### Effect of added electrolyte and the ionic strength of solution.

At a pH of 3.0 and a dye concentration of 1.0 mM, the effect of an added electrolyte  $\text{KNO}_3$  (0.01 M, 0.05 M, 0.10 M) was investigated. The observed effect was almost the same for the three different concentrations.

A decrease in absorbance took place, indicating a bilayer destabilization. When electrolyte was added, the net result was to slightly increase the steric repulsion range (*i.e.*, to decrease the electrostatic attraction between oppositely charged molecules). More bilayers are formed when there is no added electrolyte. With added electrolytes, the charges of the deposited layer are being shielded. This shielding effect decreases the electrostatic interaction between bilayers, and thus causing destabilization. Increase in ionic strength also decreases the intramolecular repulsion in gelatin, hence, causes the gelatin to fold up, and be more compact [2]. Results show that increased intramolecular attraction in gelatin renders bilayer destabilization.

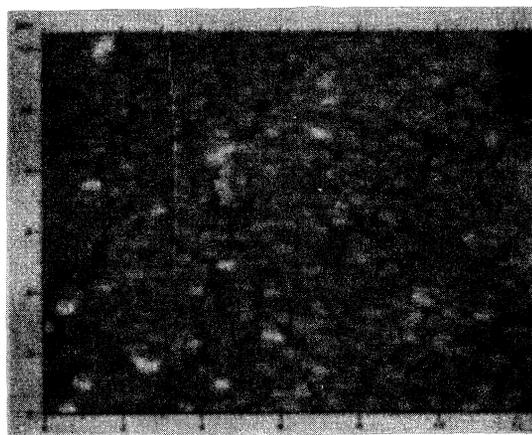


Fig. 10. AFM micrograph of the glass deposited with Congo Red.

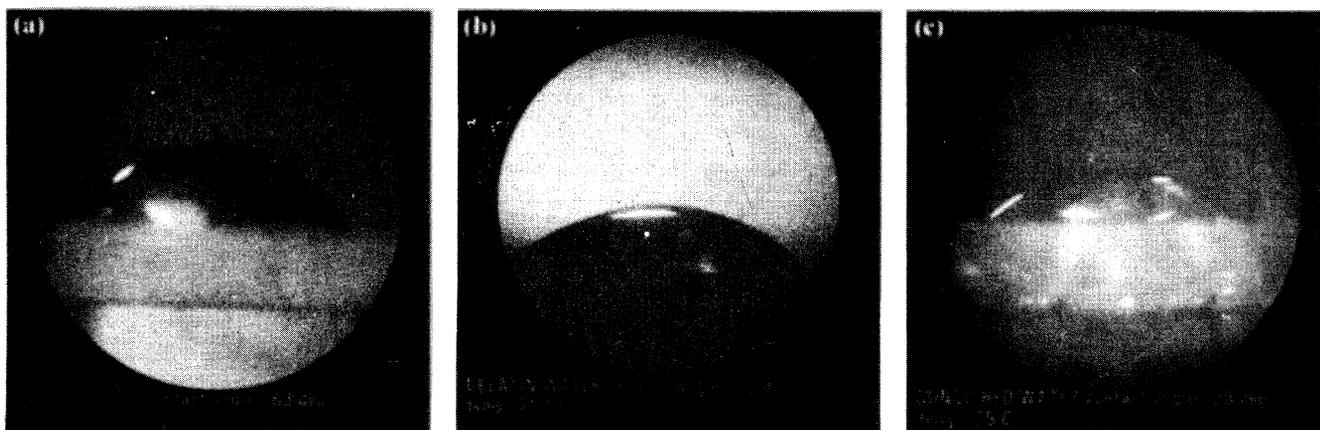


Fig. 11. (a) Contact angle measurement of water on undeposited glass substrate; (b) Contact angle measurement of water on glass substrate deposited with gelatin solution; (c) Contact angle measurement of water on glass substrate deposited with Congo Red solution.

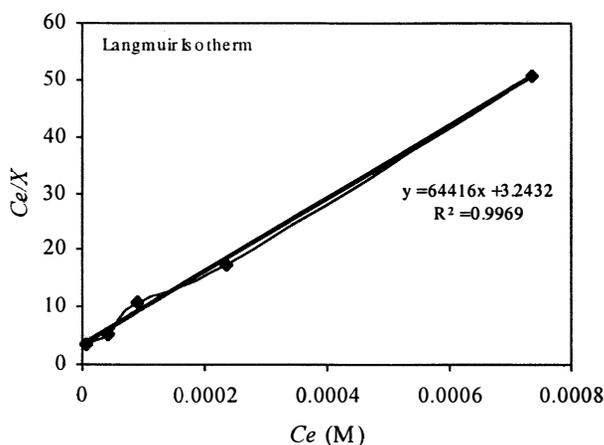


Fig. 12. Linear plot of the Langmuir isotherm.

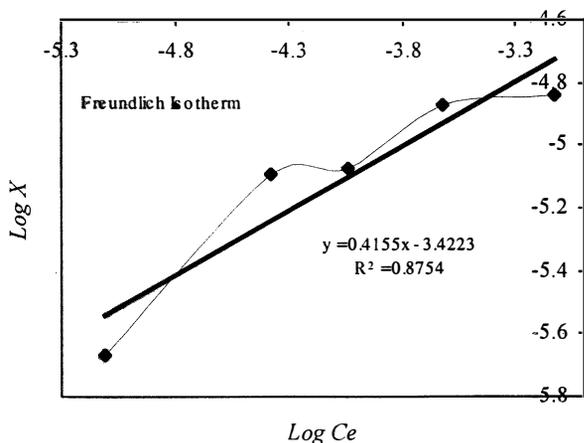


Fig. 13. Linear plot of the Freundlich isotherm.

### Surface characterization

**Atomic Force Microscopy (AFM).** Figure 10 shows the AFM image of the glass deposited with Congo Red. The Congo Red molecules appeared as bead-like structures and the sur-

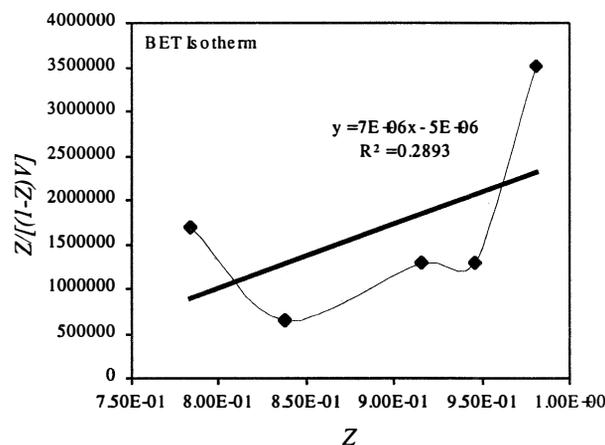


Fig. 14. Linear plot of the BET isotherm.

face topology of the fabricated film was relatively uniform. The AFM micrograph confirmed that there was indeed a dye-polyion deposition on glass [7].

**Comparative contact angle measurements.** Figure 11 shows the changes in the contact angle of water on a glass substrate with and without deposits of gelatin and Congo Red on the surface. The contact angle were greatly reduced when the glass surface was coated with gelatin and Congo Red. These coatings impart their ionic character to the surface and render it more hydrophilic, resulting in the spreading of the water droplet. This behavior indicates that there is indeed a layer assembly of gelatin and Congo Red molecules on the glass substrate.

**Thermodynamics of adsorption.** Three isotherms, namely-Langmuir, Freundlich and  $BET^{ref}$ , were constructed to see the behavior of the adsorption process (Figs. 12–14). The Congo Red-gelatin adsorption is shown to follow the Langmuir model. Hence, the empirical assumptions behind the isotherm could be applied to the dye-polyion layer growth. The process cannot proceed beyond monolayer coverage, that all sites are

equivalent and the surface is uniform and that the ability of a molecule to adsorb at a given site is independent of the occupation of the neighboring sites [9].

The monolayer coverage by the polyelectrolytes allowed uniform saturation of the binding sites. This in return resulted to stable bilayers held by electrostatic interactions.

## REFERENCES

1. Ulman, A. *Chem. Rev.* 96, 1533 (1996).
2. Van de Steeg, H., Cohen Stuart, M., de Keizer, A., and Bijsterbosch, B. *Langmuir*. 8, 2538 (1992).
3. Drecher, G. *Science*. 277, 1232 (1997).
4. Bohmer, M. and Koopal, L. *Langmuir*. 8, 2660 (1992).
5. Berndt, P., Kurihara, K., and Kunitake, T. *Langmuir*. 8, 2486 (1992).
6. Kawanishi, N., Christenson, H, and Ninham B. J. *Phys. Chem.* 94, 4611 (1990).
7. Ariga, K., Lvov, Y., and Kunitake, T. *J. Am. Chem. Soc.* 119, 2224 (1997).
8. Lu, X., Laroniec, M., and Madey, R. *Langmuir*. 7, 173 (1991).
9. Atkins, P. W. *Physical Chemistry*, 6<sup>th</sup> ed. (Oxford: Oxford University Press, 1998).