

## DRY PHASE DETECTION OF ULTRA THIN MULTILAYER POLY ELECTROLYTE FILMS USING SPECTRAL REFLECTANCE TECHNIQUE

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Surface modification with polyelectrolyte multilayer films appears as a versatile tool for immunoassay preparation in biosensing application. The technique of electrostatic self assembly (ESA), also known as poly electrolyte self assembly (PESA) or electrostatic layer by layer (LBL) deposition is based upon electrostatic interaction between polymers containing cation and anion groups. ESA method became extremely popular and within the last decade was adopted and further developed in many research laboratories. In present work, poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSS) were employed to build up multilayers on oxidized silicon substrates. An incubation period about 10 minutes is given to allow the binding take place before it was dried by nitrogen flow. The shift in reflectance from deposited polyelectrolyte layers is measured by spectral reflectance (SR) technique after each deposition. It was verified that this technique is able to measure down to angstrom level and suitable for label free optical detection for thin film in dry phase.

*Keywords:* Electrostatic self-assembly; Spectral reflectance; Layer by layer deposition; Dry phase detection; optical detection

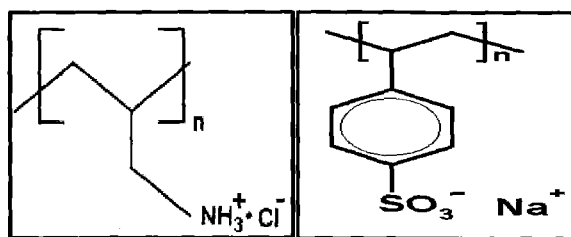
### 1. Introduction

Optical biosensors are contributing to an increasingly impact analytical technology for the biological and chemical species detection (Lechuga et al., 2006). The main advantage offered by optical biosensors is label-free detection, omitting the need for fluorescent, chemical, or radiolabeled tags. Additionally, biosensor technologies are relatively easy to use and offer real-time data collection, thus provide the possibility of monitoring different biochemical interactions (Daghestani and Day, 2010). Development of optical biosensor requires some considerations like surface immobilization chemistry, fluidics design, transduction signal generation, detection format and data analysis (Fan et al., 2008). Surface Plasmon Resonance (SPR) is one of the established methods in optical biosensing applications (Bañuls et al., 2006). First and most important step involves the immobilization of biomolecules onto the surface of solid substrates. Literature shows negative effect of bio molecules immobilization onto the surface of solid substrates as a change imposed in secondary structure preventing it to function properly (Nabok A., 2005). However, the method of polyelectrolyte self-assembly

(PESA) solved this problem due to its unique suitability for the formation of composite organic films containing other electrically charged compounds, such as, organic dyes, proteins, organic and inorganic nanoparticles(Nabok et al., 2002).The second thing that has a great importance in optical bio sensing technology is the sensing part of a biosensor.The sensor should be sensitive enough to detect the smaller dimension species. In practical, the species detected by a label free biosensor has dimension of few nanometers (Dey et al., 2011) while antigen concentration ranges from few  $\mu\text{g/ml}$  to  $\text{ng/ml}$ (Mustafa et al., 2010). In present work, multiple ultra-thinlayers of common polyelectrolytes, Poly (allyamine) Hydrochloride (PAH) and Poly (sterenesulphonate) sodium salt (PSS) were deposited on oxidized silicon substrate and detected through shift in reflectance spectra by commercially available Spectral Reflectance equipment. Sensitivity of spectral reflectance method was verified down to angstrom levels as a bilayer of PSS/PAH has a thickness of 1nm (Nabok et al., 2002). The application of the spectral reflectance to detect the binding of biomolecules opens a possibility for this method to be exploited in biosensing applications.

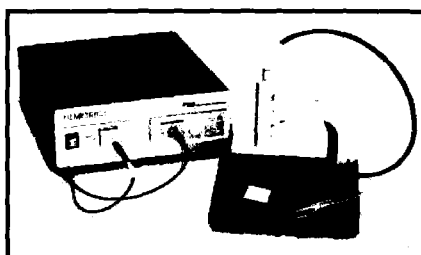
## 2. Experimental setup

The oxidized silicon was used as a substrate for polyelectrolyte layer by layer deposition in this experiment. Bare silicon wafer was provided by Micro and Nano Technology – Shamsuddin Research Center (MiNT-SRC), University Tun Hussein Onn Malaysia, oxidized by thermal oxidation method. Both polyanion and polycation employed in this work were purchased from Sigma Aldrich, USA. Fig.1 shows the chemical structures of polycation and polyanion used in this study.



(a) (b)  
**Fig. 1:** Chemical structure of (a) PAH and (b) PSS

The measurement of optical properties is done by thin film analyzer (Filmetrics, USA) based on the spectral reflectance (SR) technique. The equipment is shown in Fig 2.



**Fig. 2:** Thin film analyzer (Filmetrics F20, USA).

## 2.1 Layer by layer deposition of polyelectrolyte thin films

The chemicals involve in this experiments are;

- i) 2mg/ml PAH
- ii) 2mg/ml PSS
- iii) DI water

Since SiO<sub>2</sub> has negatively charge surface (Hau.W.L., 2003), the experiment was started by dipping the substrate into 2mg/ml PAH for 10 minutes incubation time. After that it was rinsed under running stream of DI water (Millipore, USA) for at least 1 minute and then dried by allowing gentle nitrogen flow on substrate. Gas flow and control was crucial at this stage because high pressure could remove the molecules bind on surface. The thickness and optical properties of the substrate was measured before and after each deposition. The process was repeated for multiple layers of PAH and PSS.

## 2.2 Spectral Reflectance Measurements

Spectral reflectance method is based on measurement of beam reflected by the sample understudy. For single interface, separates medium 1 and 2, with respective indices of refraction  $N_1$  and  $N_2$  and angle of incidence and angle of reflection  $\phi_1$  and  $\phi_2$  (related to Snell's law). This reflectance "R" has a relation with Fresnel reflection co-efficient "r" as follows, and it is different for *p*-waves and *s*-waves.

$$R^p = r^p_{12} = \frac{N_2 \cos \phi_1 - N_1 \cos \phi_2}{N_2 \cos \phi_1 + N_1 \cos \phi_2} \quad (1)$$

$$R^s = r^s_{12} = \frac{N_1 \cos \phi_1 - N_2 \cos \phi_2}{N_1 \cos \phi_1 + N_2 \cos \phi_2} \quad (2)$$

The complex index of refraction  $N$  is combination of a real and imaginary number and is designated as ;

$$N = n - jk \quad (3)$$

Where  $n$  = index of refraction and  $k$  = extinction co-efficient (decrease in intensity of light passing through a material). For dielectric surface, having zero extinction coefficient, (3) becomes,

$$N = n \quad (4)$$

For, normal angle of incidence and angle of reflection i.e.  $\phi_1 = \phi_2 = 90^\circ$ , and by (4), the relation between  $R$  and  $r$  becomes,

$$R_p = R_s = r^p_{12} = r^s_{12} = \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2$$

A detailed analysis of above equations can be found in (Tompkins and McGahan, 1999, Salik E., 2012) A commercial software calculates the thickness according to the optical properties as a function of wavelength and fit the calculated and measured spectra using well known Cauchy's theorem. However the thickness of PESA films is too thin to detect by this equipment as number of reflections depend on the thickness of deposited layer therefore oxidized silicon is used as a substrate to deposit PESA films so multiple number of reflections are generated and instead of measuring thickness, the shift in reflectance spectra was investigated.

### 3. Results and discussions

At neutral PH, silicon oxide possesses negative charge due to presence of silanol groups ( $\equiv\text{Si}-\text{O}-$ ) at the surface. When these surfaces come in contact with a solution containing ions, positive ions will be attracted to the surface (Hau.W.L., 2003). Therefore we started with the polycation, PAH as first layer. The initial result of shifted reflectance of PAH is shown in Fig. 3. The shifted of reflectance spectra means successful adsorption of polycation layer onto the oxidized substrate.

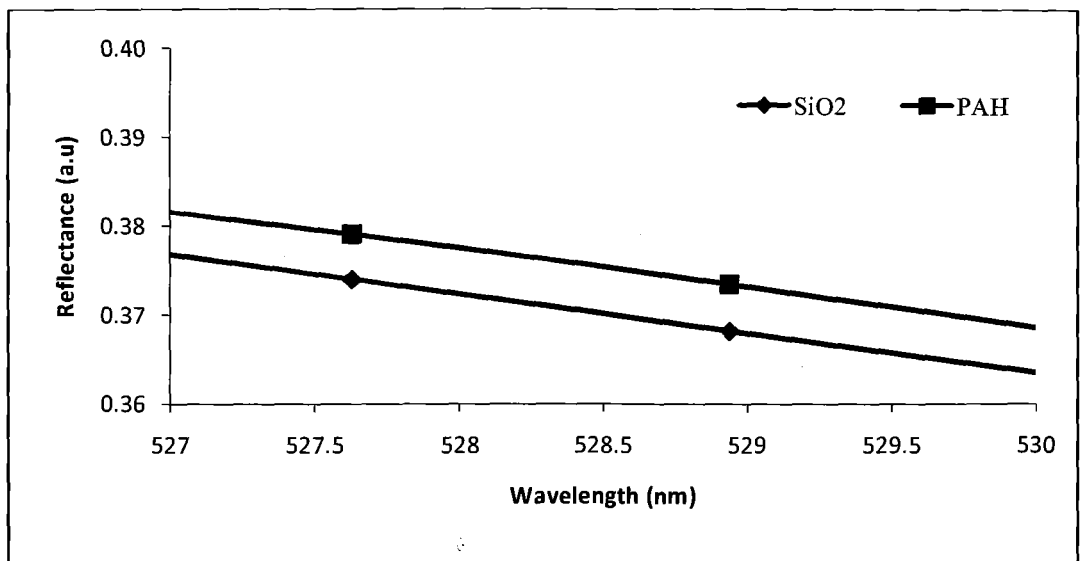


Fig.3: Adsorption of first PAH layer on silicon oxide substrate.

Following the deposition routine as describe earlier, multiple layers of PAH and PSS were deposited and analyzed. Figure 4 show significant shift of the spectra for each layer.

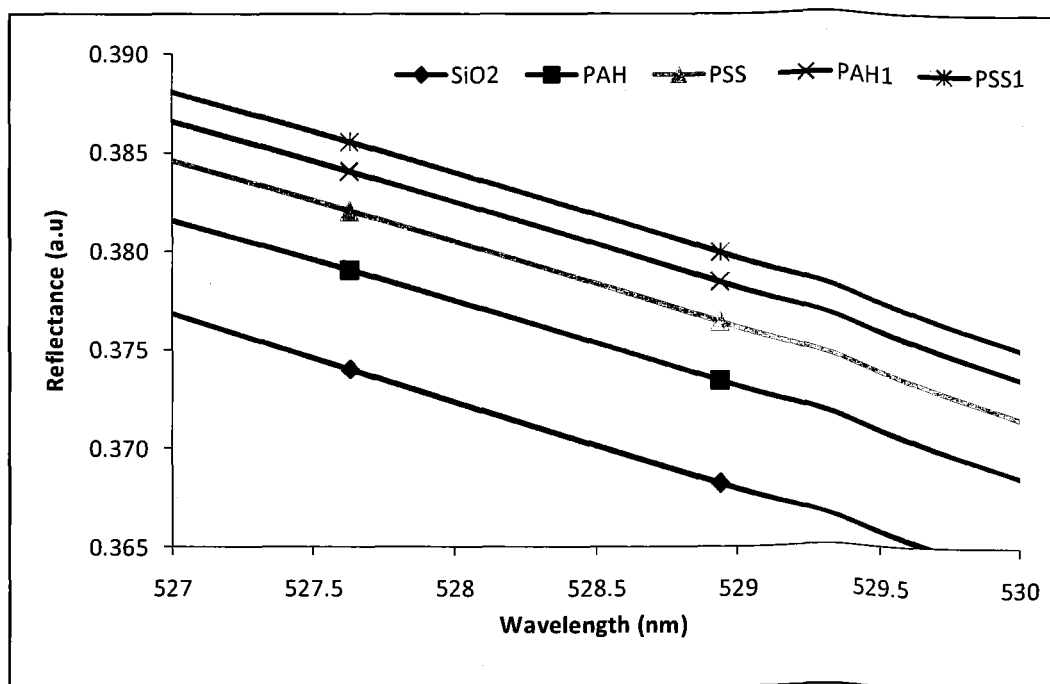


Fig. 4: Adsorption of two bilayers of PAH and PSS.

Further investigations were carried out to deposit more layers of polyelectrolyte as shown in Fig. 5. The binding of multiple layer of polyelectrolyte is due to strong electrostatic attraction. Result from this work open the possibility that this method could be employed for interaction of antibody-antigen for biosensing applications. As the detection protocol for antigen requires layer PAH-Protein-Antibodies, this initial result looks promising and further work need to be done for the detection in liquid environment.

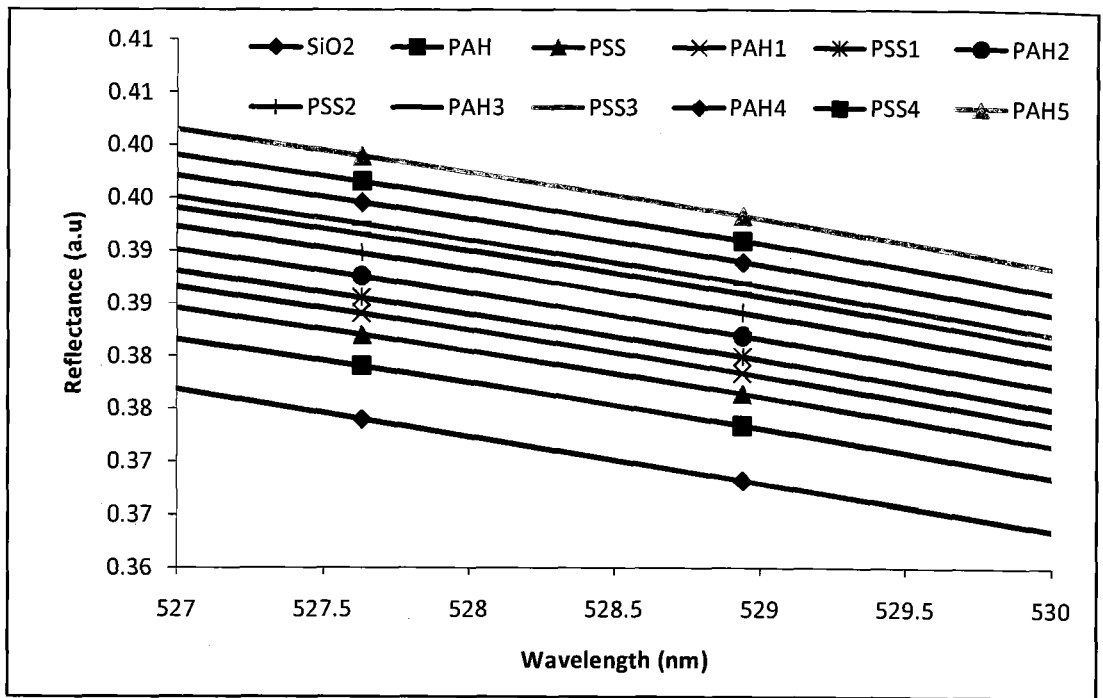


Fig. 5: Adsorption of four bilayers of PAH and PASS and a final PAH layer.

#### 4. Conclusion

In the present work, we showed the successful detection of ultrathin ( $< 1$  nm) polyelectrolyte thin films by investigating the shift in reflectance spectra in dry phase. The method manage to measure up to four bilayers of PAH and PSS. Ideally, to be used in biosensing applications, the detection of biochemicals must be in liquid environment. Further ongoing work is to develop a liquid cell for biosensing applications using spectral reflectance method. By exploiting this equipment which is typically available in microelectronics lab for biosensing application will save cost and optimized the usage.

#### Acknowledgments

This work was supported by Fundamental Research Grant Scheme (FRGS) vot 1048. The authors wish to thank the Universiti Tun Hussein Onn Malaysia (UTHM) and Ministry of Education, Malaysia.

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