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Studies of Bentonite Added Protein-Tannic Acid Multilayer Thin Films

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Abstract. Bentonite added in bovine serum albumin (BSA) increases the thickness of the multilayer film. BSA-TA multilayer thin films are built on silicon surface. The growths of the multilayer thin films are investigated by ellipsometry. The surface morphologies of the multilayer thin films are characterised with scanning electron microscopy (SEM). Both BSA-TA and BSA-Bentonite (BSAB)-TA layer-by-layer (LbL) assemblies exhibit linear growth behaviour. For all multilayer films studied here, a large amount of tannic acid molecules is eluted during washing, indicating weak binding and reversible adsorption on BSA surface. Oppositely, BSA absorb irreversibly on TA-terminated layer.

1. Introduction

Bentonite consists of two sheets of tetrahedral silicate and a sheet of octahedral aluminate exhibiting negative basal surface charge [1]. Through electrostatic interaction, bentonite adsorbs positively charged BSA in acidic pH, which is pH below BSA's isoelectric point [2]. A hybrid film can be fabricated using bentonite and biopolymers such as BSA and TA for biomedical [3, 4], pharmaceutical [5, 6] and environmental applications [7, 8].

This work investigates the adding of bentonite in BSA solution during LbL assembly with TA solution. There is an increment in BSA-TA film's thickness upon incorporation of bentonite into BSA aqueous solution. The thickness of the film has been compared with the film without incorporation of bentonite.

2. Materials and Method

2.1 Materials

BSA lyophilized powder ($\geq 96\%$), TA (ACS grade), hydrophilic bentonite and hydrochloric acid were purchased from Sigma-Aldrich. Ammonium hydroxide, 25% was purchased from Honeywell. Hydrogen peroxide (31%, 10 ppb grade) was purchased from MGC Pure Chemicals Singapore Pte. Ltd. Deionized (DI) water (specific resistivity higher than $18.2 \text{ M}\Omega \text{ cm}$) from Milli-Q plus 185 (Millipore) purification system was used to make all solutions. All chemicals above were used as received without further purification. Silicon substrates ($625 \text{ }\mu\text{m}$ thick) were purchased from Lotech Scientific Supply Pte. Ltd.



2.2. Method

LbL assembly of BSA and TA on flat silicon substrate (0.7 cm x 2.5 cm) was performed by a homemade, real time precision ellipsometry system as reported in previous study [9]. Prior to assembly steps, a silicon substrate was first boiled in a mixture of 2:1:1 by volume of deionized water, 25 % ammonium hydroxide and 31 % hydrogen peroxide) for 15 minutes, rinsed with deionized water and inserted in homemade cuvette fixed with a holder for measurement. As shown in figure 1, the components consisted of substrate and homemade cuvette were adjusted such that the incoming light was at near right-angle to the glass windows of the homemade cuvette, and the reflected light was perpendicular to the detector. Signal was maximized when driving frequency directed by a function generator matches the natural frequency of the modulator increasing the sensitivity. Output signals were recorded by computer. We performed alternate assemblies of BSA and TA by using 4 and 3 mg/ml water solutions, respectively. After that, we investigated the effect of bentonite on BSA-TA multilayer film by adding 0.2 % bentonite into the BSA aqueous solution, pH adjusted to pH 4 (denoted as BSAB) for LbL assemblies. The surface morphologies of the multilayer thin films are characterised with SEM.

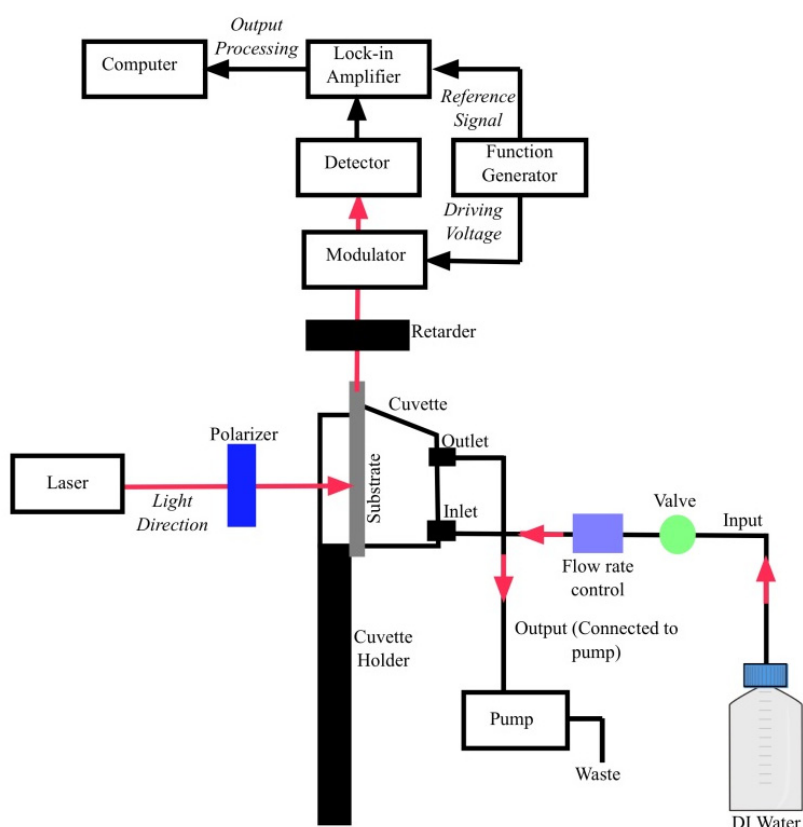


Fig. 1. Scheme of experimental set up for LbL assembly.

3. Results and discussions

LbL assembly process of (BSA-TA)₂ and (BSAB-TA)₂, respectively is shown in figure 2. The total thickness of (BSA-TA)₂ film is 6.93 nm. When bentonite is incorporated into BSA at pH 4, the total thickness of (BSAB-TA)₂ film increases more than three folds to become 21.38 nm. In acidic pH, BSA is positively charge and can interact with negatively basal charge bentonite. The first BSA layer's thickness is 9.67 nm, 2.8 folds higher than BSAB's layer, 3.47 nm. This shows that BSA penetrates

the bentonite's interlayer [10]. For all the thin films, a large amount of tannic acid molecules is eluted during washing, indicating weak binding and reversible adsorption on BSA surface [11]. Oppositely, BSA absorbs irreversibly on TA-terminated layer [12]. The assemblies of $(\text{BSA-TA})_2$ and $(\text{BSAB-TA})_2$ exhibit linear growth pattern, as shown in figure 3.

Evidence of incorporation of bentonite can be seen in SEM image, figure 4 (b). Without bentonite, the surface of $(\text{BSA-TA})_2$ appears to be smoother than with addition of bentonite.

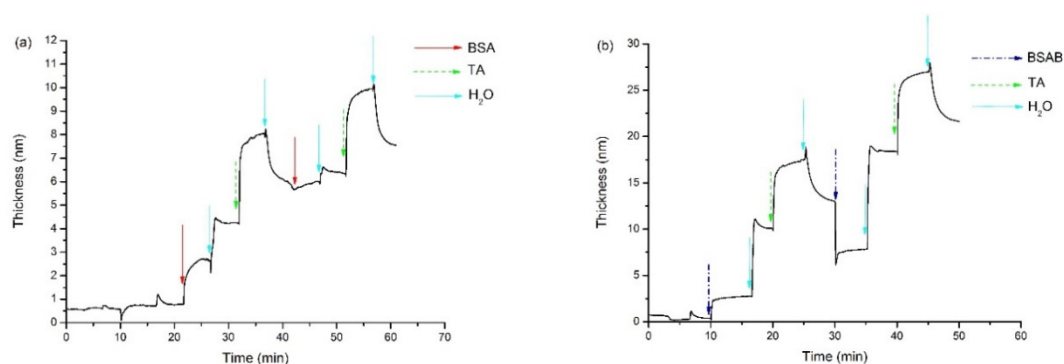


Fig.2. LbL assembly of (a) $(\text{BSA-TA})_2$ and (b) $(\text{BSAB-TA})_2$

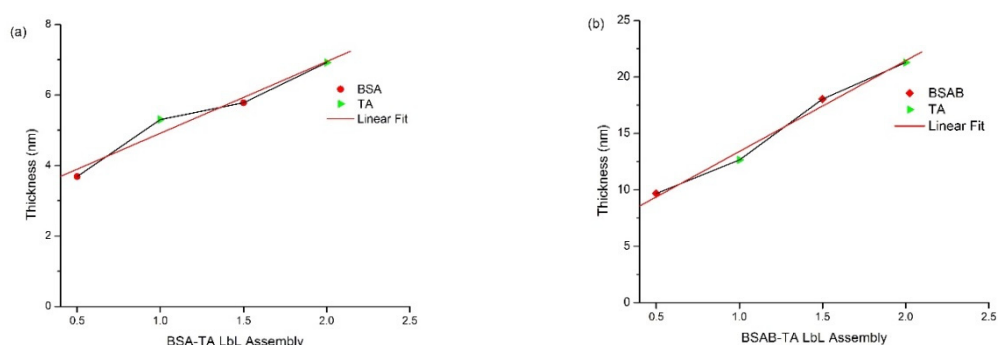


Fig. 3. Each layer build-up of (a) $(\text{BSA-TA})_2$ and (b) $(\text{BSAB-TA})_2$

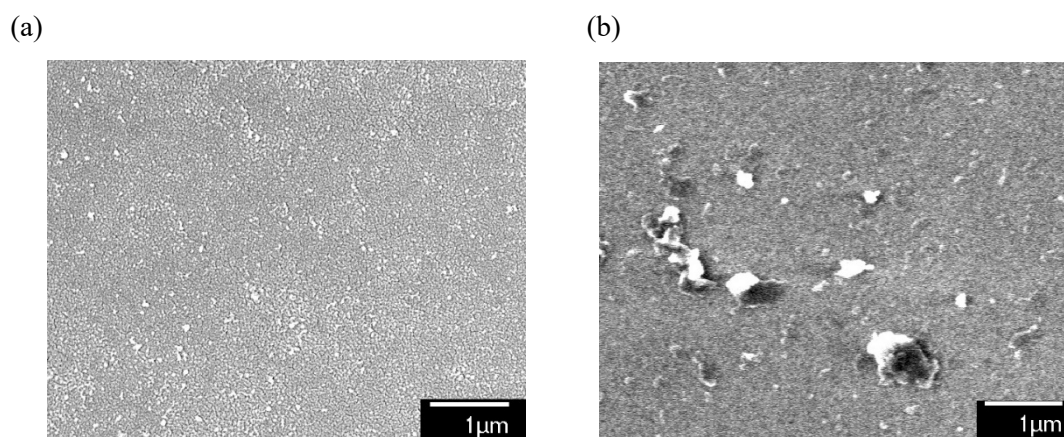


Fig. 4. SEM images of (a) $(\text{BSA-TA})_2$ and (b) $(\text{BSAB-TA})_2$ films.

4. Conclusions

At pH below isoelectric point of BSA, pH 4, bentonite incorporates into (BSAB-TA)₂ multilayer film producing more than three folds of thickness of (BSA-TA)₂ multilayer film. The thickness of (BSAB-TA)₂ multilayer film can increase more than three folds of (BSA-TA)₂ by adding 0.2 % of bentonite into BSA solution at pH 4. Hence, the addition of bentonite into BSA-TA multilayer thin film can reduce the number of bilayers need to be built which is more efficient and productive LbL assembly approach.

Acknowledgement

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