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APPENDICES

APPENDIX A

Confirmation of Multilayer Formation

Table A-1 Frequency shift, ΔF (Hz) of QCM of three pairs of multilayer film in the presence of 1M NaCl.

Number of Layers	Frequency Shift, ΔF (Hz)		
	(chitosan-PSS)	(PAH-SFC)	(HTACC-PAA)
1	53.1	29.5	32.4
2	72.7	106.5	41.6
3	117.9	189.3	80.4
4	137.9	377.5	92.7
5	180.2	543.9	116.4

Stratification of multilayered film

Table A-2 Water contact angle of treated PET-(chitosan-PSS)$_n$ assemblies, 1.0 M NaCl was added to both polyelectrolyte solutions

Top layer	Number of layer	Water contact angle (°)
Treated PET	0	53.5±2.44
Poly(styrene sulfonate)	2	43.6±2.07
Chitosan	3	67.9±1.89
Poly(styrene sulfonate)	6	43.7±2.31
Chitosan	7	67.7±3.68
Chitosan	9	66.8±2.15
Poly(styrene sulfonate)	10	53.9±4.95

Table A-3 Water contact angle of treated PET-(PAH-SFC)$_n$ assemblies, 1.0 M NaCl was added to both polyelectrolyte solutions

Top layer	Number of layer	Water contact angle (°)
Treated PET	0	53.5±2.44
SFC	2	55.5±4.79
PAH	3	85.9±1.91
SFC	6	68.4±3.13
PAH	7	86.0±2.26
PAH	9	90.3±2.07
SFC	10	74.1±2.76

Table A-4 Water contact angle of treated PET-(HTACC-PAA)$_n$ assemblies, 1.0 M NaCl was added to both polyelectrolyte solutions

Top layer	Number of layer	Water contact angle (°)
Treated PET	0	53.5±2.44
PAA	2	57.4±2.07
HTACC	3	65.4±1.24
PAA	6	56.0±3.58
HTACC	7	71.5±1.92
HTACC	9	68.3±0.58
PAA	10	55.0±2.65

APPENDIX B

Bicinchoninic acid assay

Bicinchoninic acid assay is a method used for determination of the amount of proteins. The standard reagents used in this method are reagent A, reagent B and reagent C. Reagent A consists of an aqueous solution of Na_2tartrate, Na_2CO_3, $NaHCO_3$ in 0.2 M NaOH, pH 11.25. Reagent B is 4% (W/V) bicinchoninic acid solution, pH 8.5. Reagent C is 4% $CuSO_4$ $5H_2O$ in deionized water.

The principle of the bicinchoninic assay (BCA) relies on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to protein present. It has been shown that the peptide bond is able to reduce Cu^{2+} to Cu^{1+}. BCA forms a purple-blue complex with Cu^{1+} in alkaline environments, thus providing a basis to monitor the reduction of alkaline Cu^{2+} by proteins.[30] Figure B-1 shows complexation between bicinchoninic acid and Cu^{1+}.

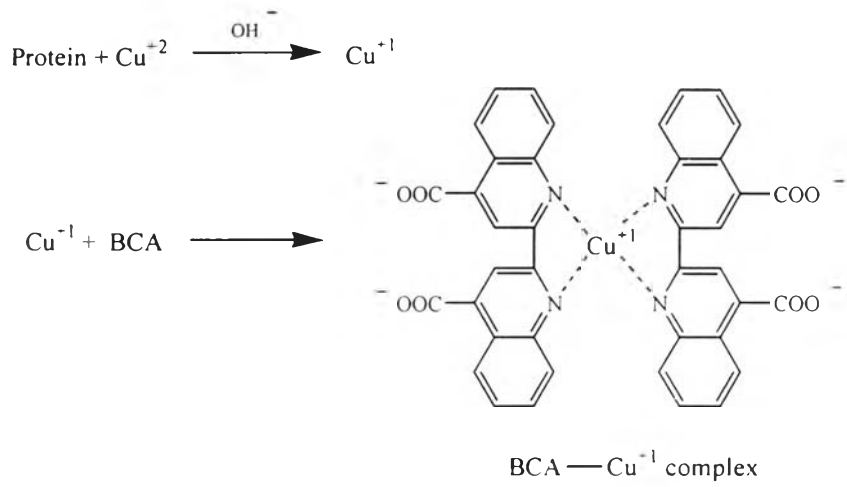

Figure B-1. Formation of purple complex between BCA and cuprous ion generated from the biuret reaction.

Calculation of Protein Adsorption

Table B-1 Standard BSA solution, for the calibration curve.

Standard	Solution (mL)	SDS (mL)	BSA conc (μg/mL)
S_1	0.5 of BSA (1000 (μg/mL)[a]	4.5	100
S_2	4.0 of S_1	4.0	5.0
S_3	4.0 of S_2	4.0	25
S_4	4.0 of S_3	6.0	10
S_5	4.0 of S_4	4.0	5
S_6	4.0 of S_5	4.0	2.5
S_7	4.0 of S_6	6.0	1.0
S_8	4.0 of S_7	4.0	0.5

a : standard BSA was pipette from 1 mg/mL ampule

After reading the UV absorbance of the samples and standard BSA solution at λ = 562 nm. , the result was then calculated for the net absorbance by substracting the absorbance of the blank (SDS).

$$\textbf{Net } A_{562} = \textbf{recorded } A_{562} - A_{562} \textbf{ (blank)} \qquad \text{B-1}$$

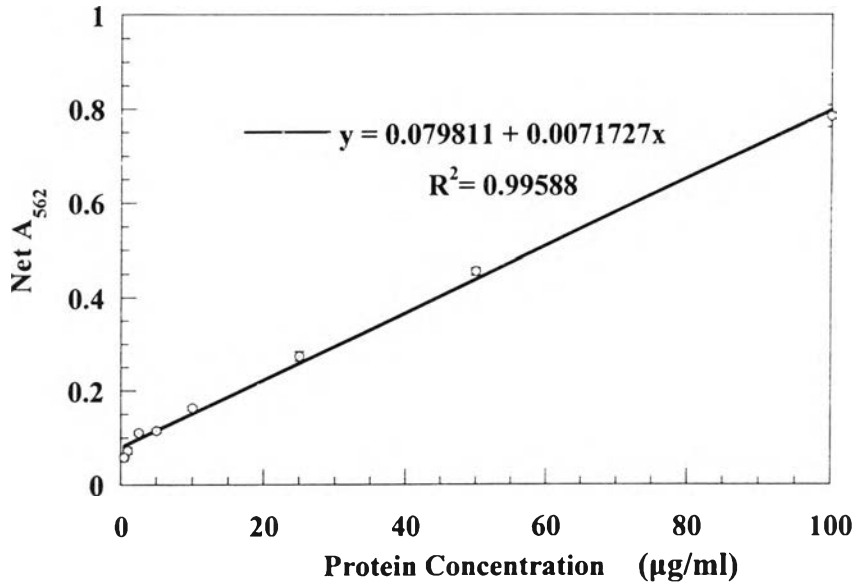

Figure B-2 A calibration curve of the amount of albumin adsorbed and the absorbance obtained from BCA microassay.

The protein concentration (C; µg/mL) in each well was determined from the calibration curve. The total amount of protein (P) in the original solution (2 mL) was calculated from the sampling sample (100 µL) + BCA working solution (100 µL)

$$\textbf{Total amount of protein (P)} = \frac{\text{C (µg/mL) x 200 (µL)}}{\text{1000 (µL/mL)}} \times \frac{\text{2000 (µL)}}{\text{100 (µL)}} \qquad \text{B-2}$$

$$\textbf{Adsorbed protein/surface area } P_{ads} = \textbf{P/surface area (2 sides) (µg/cm}^2\textbf{)} \qquad \text{B-3}$$

VITAE

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