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# APPENDICES

# APPENDIX A

**The average thickness of multilayers**

**Table A-1**. The average thickness of PSS-CHI bilayer calculated from ellipsometric data of Si-$NH_2$-$(PSS\text{-}CHI)_5$ assemblies as a function of chitosan concentration in the presence of 0.25 M NaCl.

| Chitosan concentration ( mg $mL^{-1}$) | Average thickness of bilayer (Å) |
|---|---|
| 0.25 | 30.32 ± 3.85 |
| 0.5 | 29.9 ± 6.66 |
| 0.75 | 29.4 ± 2.45 |
| 1 | 31.32 ± 4.21 |

**Table A-2.** The average thickness of PSS-CHI bilayer calculated from ellipsometric data of Si-$NH_2$-$(PSS\text{-}CHI)_{10}$ assemblies as a function of NaCl concentration.

| Concentration of NaCl (M) | Average thickness of bilayer (Å) |
|---|---|
| 0 | 16.28 ± 6.85 |
| 0.25 | 32.18 ± 8.23 |
| 0.5 | 35.76 ± 6.30 |
| 1 | 44.68 ± 7.99 |

**Table A-3**. Ellipsometric thickness of Si-$NH_2$-(PSS-CHI)$_{10}$ assemblies as a function of the number of layer.

| Number of layers | Average Thickness (Å) | | | |
|---|---|---|---|---|
| | **Without NaCl** | **0.25 M NaCl in CHI solution** | **0.25 M NaCl in PSS solution** | **0.25 M NaCl in both CHI and PSS solutions** |
| 5 | 56.60 ± 1.52 | 64.80 ±5.97 | 89.80 ±2.56 | 119.20±6.57 |
| 6 | 71.20 ± 7.12 | 87.00 ±5.83 | 91.00 ±3.42 | 145.00±5.00 |
| 9 | 77.20 ± 4.04 | 131.80 ±7.26 | 157.40 ±4.57 | 246.80±4.55 |
| 10 | 88.80 ± 5.54 | 149.50 ±6.66 | 185.20 ±3.56 | 297.80±6.42 |
| 15 | 126.00 ± 6.12 | 228.60 ±3.27 | - | - |
| 16 | 140.40 ± 5.41 | 248.50 ±6.14 | - | - |
| 19 | 150.80 ± 2.86 | 303.60 ±3.40 | - | - |
| 20 | 162.75 ± 6.85 | 321.80 ±8.23 | - | - |

# APPENDIX B

**Stratification of multilayered film**

**Table B-1.** Zeta-potential of Si-$NH_2$-(PSS-CHI)$_n$ assemblies, 0.25 M NaCl was added to chitosan solution.

| Top layer | Number of layer | Zeta-potential (mV) |
|---|---|---|
| Si-$NH_2$ | 0 | 10.15 |
| Poly(styrene sulfonate) | 1 | -18.27 |
| Chitosan | 2 | 0.06 |
| Poly(styrene sulfonate) | 5 | -5.57 |
| Chitosan | 6 | 8.07 |
| Poly(styrene sulfonate) | 9 | -16.09 |
| Chitosan | 10 | 7.84 |
| Poly(styrene sulfonate) | 15 | -16.51 |
| Chitosan | 16 | 11.53 |

**Table B-2** Water contact angle of Si-$NH_2$-(PSS-CHI)$_n$ assemblies, 0.25 M NaCl was added to chitosan solution

| **Top layer** | **Number of layer** | **Water contact angle (°)** |
|---|---|---|
| Si-$NH_2$ | 0 | 70±1.52 |
| Poly(styrene sulfonate) | 5 | 78±1.71 |
| Chitosan | 6 | 83±0.82 |
| Poly(styrene sulfonate) | 9 | 77±1.71 |
| Chitosan | 10 | 83±1.25 |
| Poly(styrene sulfonate) | 15 | 76±0.85 |
| Chitosan | 16 | 80±1.71 |
| Poly(styrene sulfonate) | 19 | 77±1.46 |
| Chitosan | 20 | 82±1.25 |

# APPENDIX C

**Surface topography of multilayered films**

**Table C-1.** The surface roughness of Si-$NH_2$-(PSS-CHI)$_n$ prepared in the absence of NaCl and in the presence of 1 M NaCl.

| Top layer | Number of layer | 0 M NaCl | | 1 M NaCl | |
|---|---|---|---|---|---|
| | | $R_a$ (nm) | $R_Z$ (nm) | $R_a$ (nm) | $R_Z$ (nm) |
| Si-$NH_2$ | 0 | 0.5 | 4.9 | 0.5 | 4.9 |
| Poly(styrene sulfonate) | 1 | 1.2 | 24.3 | 9.1 | 80.8 |
| Chitosan | 2 | 0.9 | 36.1 | 9.1 | 40.1 |
| Chitosan | 10 | 7.2 | 148.1 | 11 | 127.3 |
| Poly(styrene sulfonate) | 15 | 3.0 | 22.8 | 9.9 | 46.2 |
| Chitosan | 16 | 7.5 | 104.7 | 10.1 | 47.2 |
| Chitosan | 20 | 9.3 | 109.3 | 15.7 | 158.0 |

$R_a$ : The arithmetic average of the deviations from the center plan.

$R_z$ : Standard deviation of the z values.

# APPENDIX D

**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 $Na_2$tartrate, $Na_2CO_3$, $NaHCO_3$ in 0.2 M NaOH, pH 11.25. Reagent B is 4% (W/V) bicinchoninic acid solution, pH 8.5. Reagent C is 4% $CuSO_4 \cdot 5H_2O$ 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 D-1 shows complexation between bicinchoninic acid and $Cu^{1+}$.

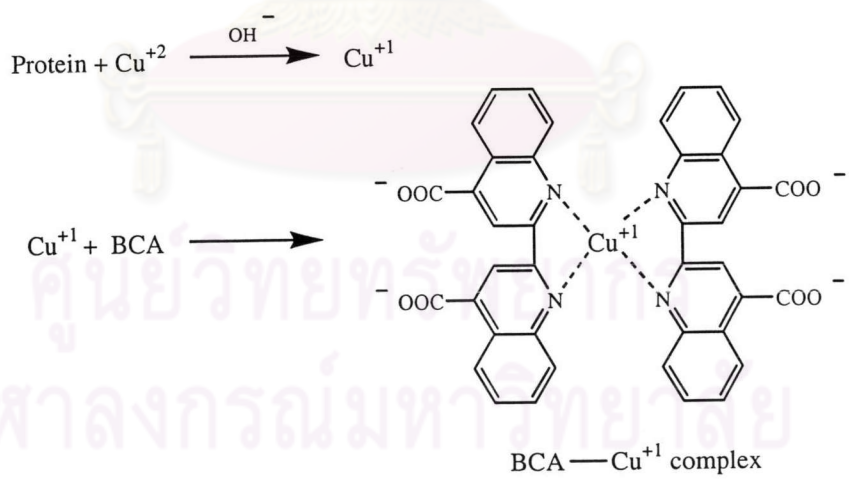


**Figure D-1.** Formation of purple complex between BCA and cuprous ion generated from the biuret reaction.

**Table D-1.** The amount of plasma protein adsorption per surface area ($\mu g/cm^2$) of Si- $NH_2$-$(PSS\text{-}CHI)_n$ assemblies prepared in the absence of NaCl and presence of 1 M NaCl.

| **Top layer** | **Number of layer** | **The amount of plasma protein adsorption ($\mu g/cm^2$)** | |
|---|---|---|---|
| | | **0 M NaCl** | **1 M NaCl** |
| Si-$NH_2$ | 0 | 4.828±4.100 | 1.494±0.424 |
| Poly(styrene sulfonate) | 1 | 3.908±0.999 | 2.567±0.663 |
| Chitosan | 2 | 7.893±0.531 | 3.142±1.154 |
| Poly(styrene sulfonate) | 5 | 6.475±1.153 | 3.908±0.791 |
| Chitosan | 6 | 8.046±0.844 | 11.954±2.304 |
| Poly(styrene sulfonate) | 9 | 6.475±2.839 | 7.126±0.633 |
| Chitosan | 10 | 7.165±3.309 | 12.299±1.416 |
| Poly(styrene sulfonate) | 15 | 4.866±1.564 | 15.172±0.973 |
| Chitosan | 16 | 5.862±2.110 | 18.797±5.886 |
| Poly(styrene sulfonate) | 19 | 9.234±0.948 | 27.510±10.078 |
| Chitosan | 20 | 8.391±0.633 | 29.080±15.667 |

# VITAE

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