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APPENDICES

APPENDIX A

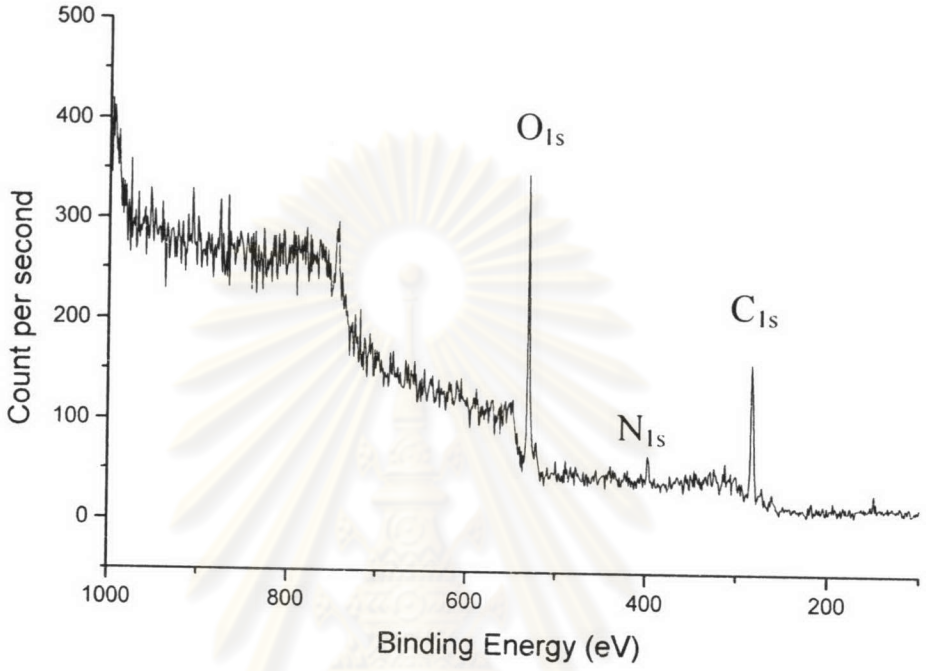

Figure A-1 XPS survey spectrum of chitosan film immerses in solvent combined NaI.

Table A-1 XPS atomic composition of modified and unmodified chitosan film

Surface type	Percent atomic compositon					
	C	O	N	I	S	Na
Chitosan-film	69.8	25.3	4.9	-	-	-
QAC-film	63.0	28.6	6.8	1.6	-	-
Chitosan film (QAC-control)	67.1	26.9	5.9	-	-	-
SFC-film	62.2	29.9	4.9	-	0.9	2.1

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% $CuSO_4 \cdot 5H_2O$ 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 B-1 shows complexation between bicinchoninic acid and Cu^{1+}.

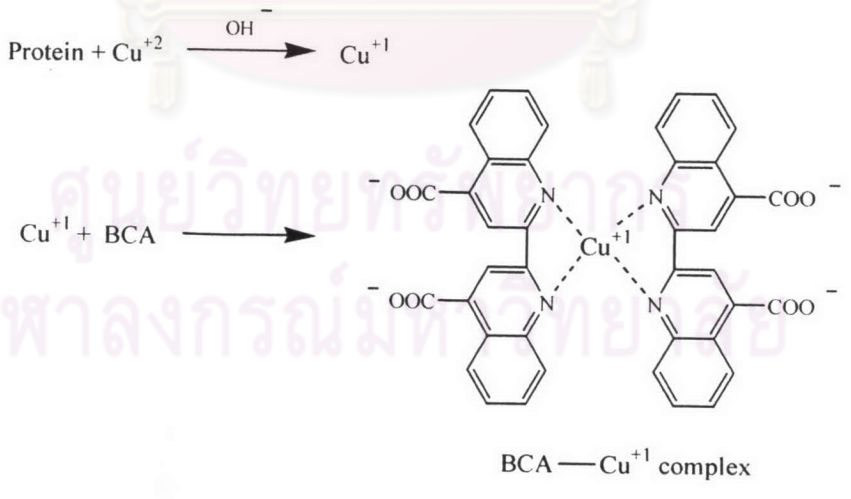

Figure B-1. Formation of purple complex between BCA and cuprous ion generated from the biuret reaction.

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).

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

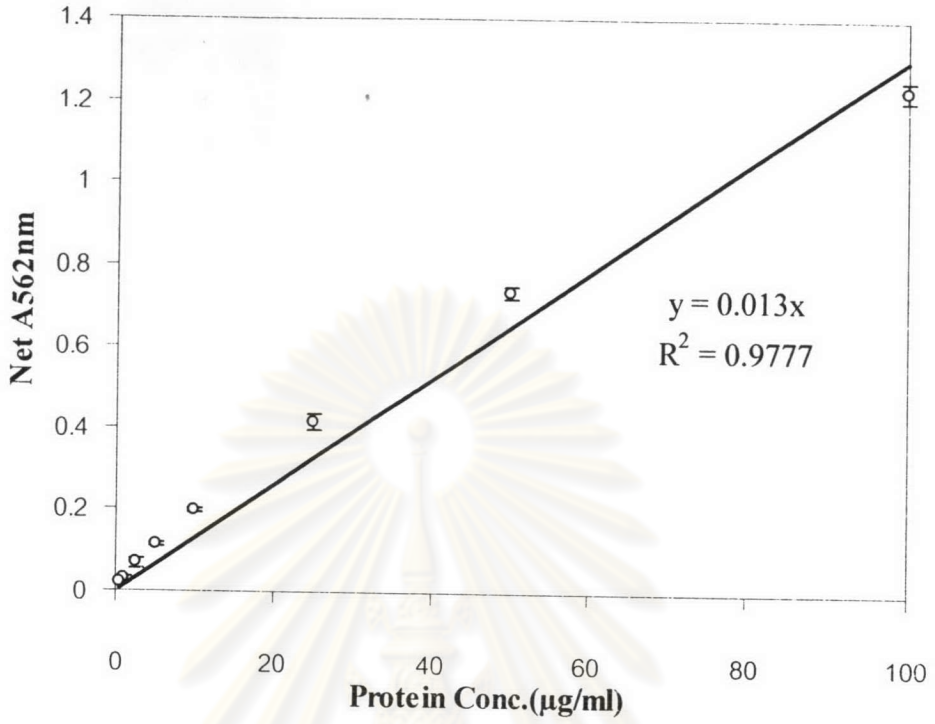

Figure B-2 A calibration curve of the amount of albumin adsorbed and the absorbance obtained from BCA microassay.

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)

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

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

Table B-2 The amount of protein adsorption per surface area ($\mu g/cm^2$) of modified chitosan film, as initial concentration1mg/ml.

Surface	The amount of protein adsorption ($\mu g/cm^2$)			
	BSA	FIB	LYZ	RNase
SFC-film 1	1.54 ± 0.17	1.84±0.45	4.57±0.18	0.50±0.15
SFC-film 2	1.07 ±0.28	1.20±0.40	2.86±0.44	1.19±0.19
SFC-film 3	0.46±0.26	0.88±0.33	3.36±0.38	1.23±0.3
Chitosan film	0.88±0.26	1.41±0.19	2.54±0.41	1.07±0.15
QAC-film 1	1.38±0.45	1.77±0.29	2.37±0.42	1.58±0.26
QAC-film 2	1.75±0.23	2.09±0.23	3.44±0.43	2.10±0.23
QAC-film 3	1.91±0.43	2.71±0.59	9.86±0.85	2.77±0.61

Table B-3 Air-Water contact angle of modified and unmodified chitosan film

Surface	Condition		Water contact angle (°)
	Rt (h)	Ratio	
SFC-film 1	24	1:0.5	71.2±2.6
SFC-film 2	24	1:1	67.8±3.8
SFC-film 3	24	1:5	62.0±1.9
Chitosan film	-		79.6±1.1
QAC-film1	2	1:3	74.2±2.2
QAC-film2	8	1:3	63.2±3.6
QAC-film3	8	1:12	61.0±1.7

VITAE

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