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APPENDICES

APPENDIX A

Protein adsorption assay

Table a. Protein used in this study

Protein	Source	M_w (kD)[1]	pI[2]	Shape
Lysozyme	Chicken egg	14	11	Ellipsoid
Albumin	Bovine serum	69	4.8	Ellipsoid

[1] Molecular weight of protein

[2] Isoelectric point of protein

Bicinchoninic acid assay

Bicinchoninic acid assay is a method for measuring the amount of proteins. The standard reagents were used in this method e.g. 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 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. Figure a shows complexation between bicinchoninic acid and Cu^{1+}.

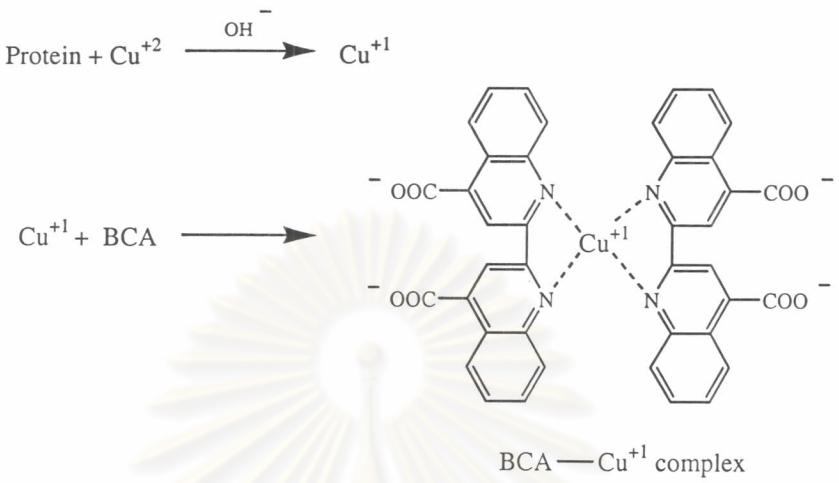

Figure a. Formation of purple complex with BCA and cuprous ion generated from the biuret reaction.

APPENDIX B

^{1}H NMR spectrum of chitosan films grafting with MTEG-ald (1:30:30)

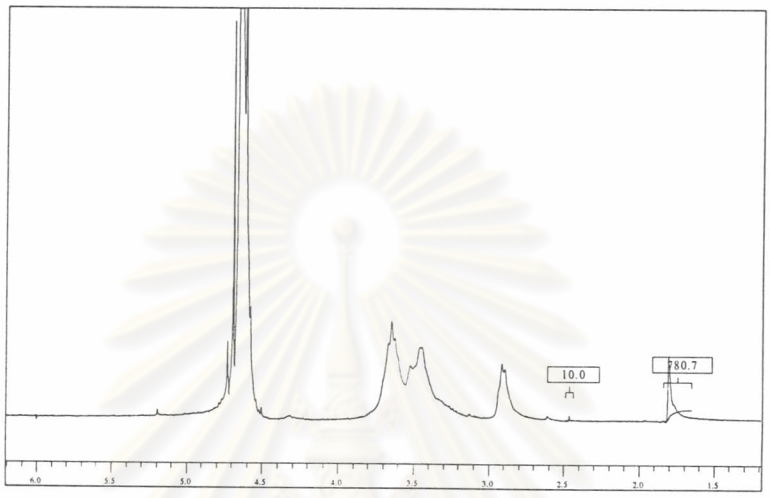

Figure b-1. ^{1}H NMR spectrum of modified chitosan films by grafting with MTEG-ald (1:30:30) (solvent: 1 % CD_3COOD in D_2O, 25 °C)

Table b-1 Information obtained from ^{1}H NMR spectrum of modified chitosan films by grafting with MTEG-ald (1:30:30)

	δ ppm	**Integration**
-C<u>H</u>$_2$NH- of GlcN	2.46	10/2
-C<u>H</u>$_3$ of GlcNAc	1.81	780.7/3

From the data in Table 3.5, at least 0.23 % of MTEG was grafted on the chitosan films (1:30:30).

ATR-IR spectrum of chitosan films by grafting with MTEG-ald (1:30:30)

Modified chitosan showed new peaks at 1462 cm^{-1} for the C-H deformation ($CH_{2\,(s)}$, $CH_{3\,(as)}$) and at 1348 cm^{-1} for the C-H deformation ($CH_{3\,(s)}$).

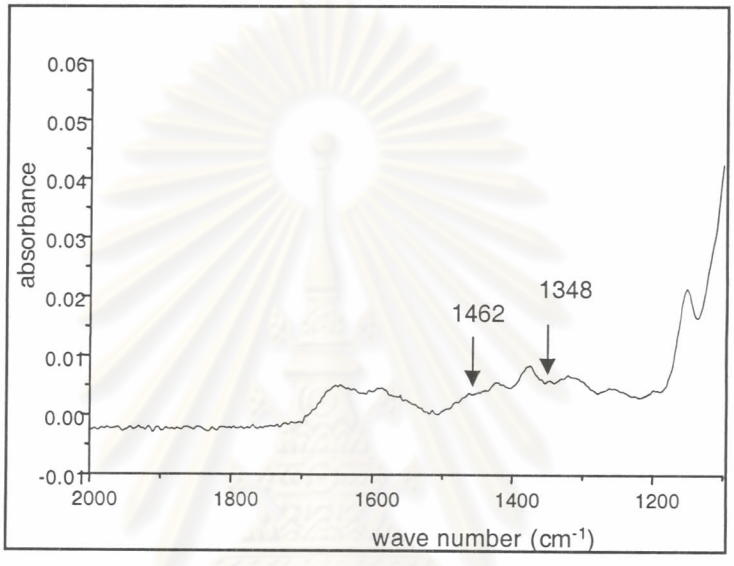

Figure b-2. ATR-IR spectrum of modified chitosan films by grafting with MTEG-ald (1:30:30)

APPENDIX C

^{1}H NMR spectrum of chitosan films by grafting with MPEG-ald (1:30:30)

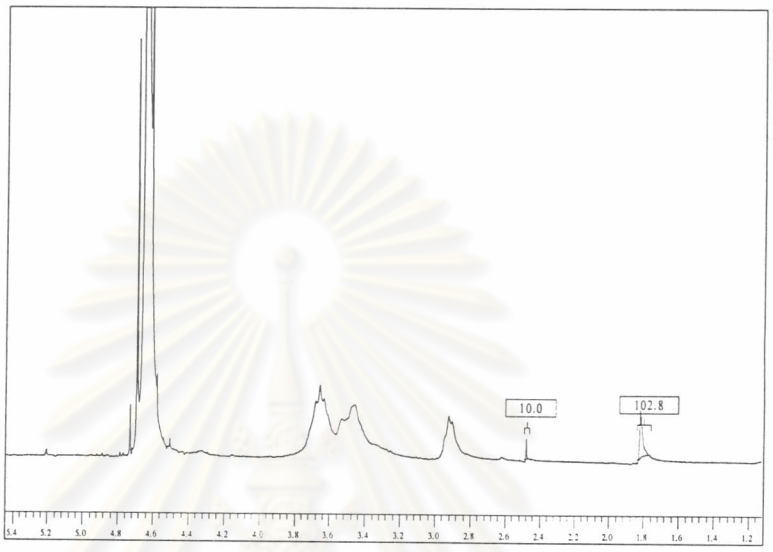

Figure c-1. ^{1}H NMR spectrum of chitosan films by grafting with MPEG-ald (1:30:30) (solvent: 1 % CD_3COOD in D_2O, 25 °C)

Table c-1 Information obtained from ^{1}H NMR spectrum of modified chitosan films by grafting with MPEG-ald (1:30:30)

	δ ppm	**Integration**
-C<u>H</u>$_2$NH- of GlcN	2.48	10/2
-C<u>H</u>$_3$ of GlcNAc	1.82	102.8/2

From the data in Table c-1, at least 1.76 % of MPEG was grafted on the chitosan films (1:30:30).

ATR-IR spectrum of chitosan films by grafting with MPEG-ald (1:30:30)

Modified chitosan showed new peaks at 1461 cm^{-1} for the C-H deformation ($CH_{2\,(s)}$, $CH_{3\,(as)}$) and at 1345 cm^{-1} for the C-H deformation ($CH_{3\,(s)}$).

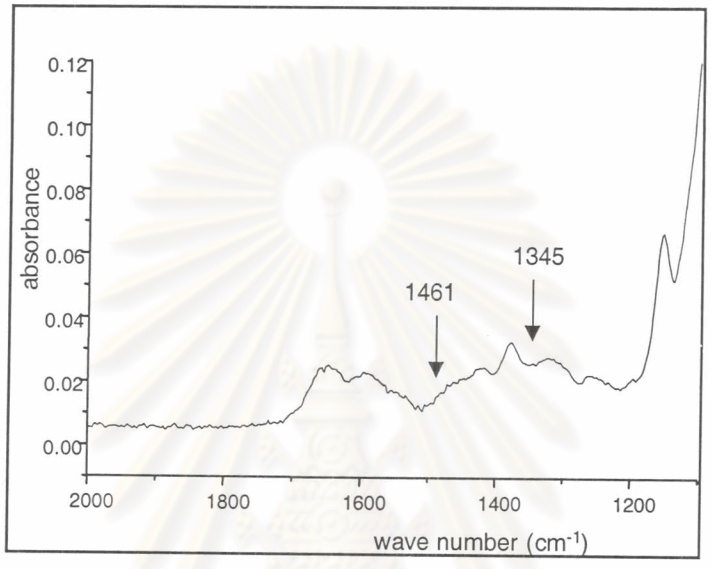

Figure c-2. ATR-IR spectrum of modified chitosan films by grafting with MPEG-ald (1:30:30)

VITAE

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