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APPENDICES

A. Intrinsic viscosity and viscosity-averaged molecular weight (M_v) calculation

From the method described in section 4.3.2.1, efflux time of serially diluted chitosan solutions in acetic acid measured using Ubbelohde viscometer was presented in Table A-1, A-2, A-3, and A-4. The reduced viscosity, η_{red}, was calculated by:

$$\eta_{red} = \frac{(\eta/\eta_0 - 1)}{c} \approx \frac{(t/t_0 - 1)}{c} = \frac{(\eta_r - 1)}{c}$$

where η is the viscosity of the chitosan solution at the polymer concentration c, and η_0 is the solvent viscosity while η_r is denoted as relative viscosity. t and t_0 are efflux times of polymer solution and solvent, respectively. Secondly, the inherent viscosity (η_i) was calculated as:

$$\eta_i = \frac{\ln \eta_r}{c}$$

Table A-1 Efflux time of XLMW chitosan.

Samples (mg/ml)	0.00	0.25	0.50	0.75	1.00
	199.28	201.93	204.13	212.87	217.08
Efflux time (sec)	199.68	202.04	206.22	211.79	217.25
	201.25	201.82	206.08	211.93	217.12
Average efflux time (sec)	200.07	201.93	205.48	212.20	217.15

Table A-2 Efflux time of LMW chitosan.

Samples (mg/ml)	0.00	0.25	0.50	0.75	1.00
	199.28	208.38	218.53	227.00	241.56
Efflux time (sec)	199.68	209.38	219.78	226.82	238.28
	201.25	209.34	218.63	227.84	237.78
Average efflux time (sec)	200.07	209.03	218.98	227.22	239.21

Table A-3 Efflux time of MMW chitosan.

Samples (mg/ml)	0.00	0.25	0.50	0.75	1.00
Efflux time (sec)	199.28	227.80	257.66	292.09	326.40
	199.68	228.16	258.53	292.09	324.94
	201.25	227.62	257.32	291.94	324.56
Average efflux time (sec)	200.07	227.86	257.84	292.04	325.30

Table A-4 Efflux time of HMW chitosan.

Samples (mg/ml)	0.00	0.25	0.50	0.75	1.00
Efflux time (sec)	199.28	267.28	347.78	455.94	565.35
	199.68	266.31	346.82	461.06	567.36
	201.25	267.04	347.91	458.75	566.24
Average efflux time (sec)	200.07	266.88	347.50	458.58	566.32

An example of the calculation of η_r, η_{red}, and η_i for the case of XLMW chitosan were shown as follows:

$$\eta_r \approx \frac{201.93}{200.07} = 1.0093$$

$$\eta_{red} = \frac{(1.0093 - 1)}{0.25} = 0.0372 \text{ ml/mg}$$

$$\eta_i = \frac{\ln 1.0093}{0.25} = 0.0370 \text{ ml/mg}$$

Table A-5 η_r, η_{red}, and η_i of XLMW chitosan.

Samples (mg/ml)	0.25	0.50	0.75	1.00
η_r	1.0093	1.0270	1.0606	1.0854
η_{red} (ml/mg)	0.0372	0.0540	0.0808	0.0854
η_i (ml/mg)	0.0370	0.0533	0.0785	0.0819

Table A-6 η_r, η_{red}, and η_i of LMW chitosan.

Samples (mg/ml)	0.25	0.50	0.75	1.00
η_r	1.0448	1.0945	1.1357	1.1956
η_{red} (ml/mg)	0.1792	0.1890	0.1809	0.1956
η_i (ml/mg)	0.1753	0.1806	0.1697	0.1787

Table A-7 η_r, η_{red}, and η_i of MMW chitosan.

Samples (mg/ml)	0.25	0.50	0.75	1.00
η_r	1.1389	1.2887	1.4597	1.6259
η_{red} (ml/mg)	0.5556	0.5775	0.6129	0.6259
η_i (ml/mg)	0.5203	0.5073	0.5043	0.4861

Table A-8 η_r, η_{red}, and η_i of HMW chitosan.

Samples (mg/ml)	0.25	0.50	0.75	1.00
η_r	1.3339	1.7369	2.2921	2.8306
η_{red} (ml/mg)	1.3357	1.4738	1.7228	1.8306
η_i (ml/mg)	1.1525	1.1042	1.1060	1.0405

Intrinsic viscosity ($[\eta]$) of deacetylated chitosan in aqueous acetic acid solutions was determined from the intercept of inherent viscosity (η_i) and reduced viscosity (η_{red}) plots where c was near zero (see Figure A-1). Intrinsic viscosity of four chitosan samples was summarized in Table A-9.
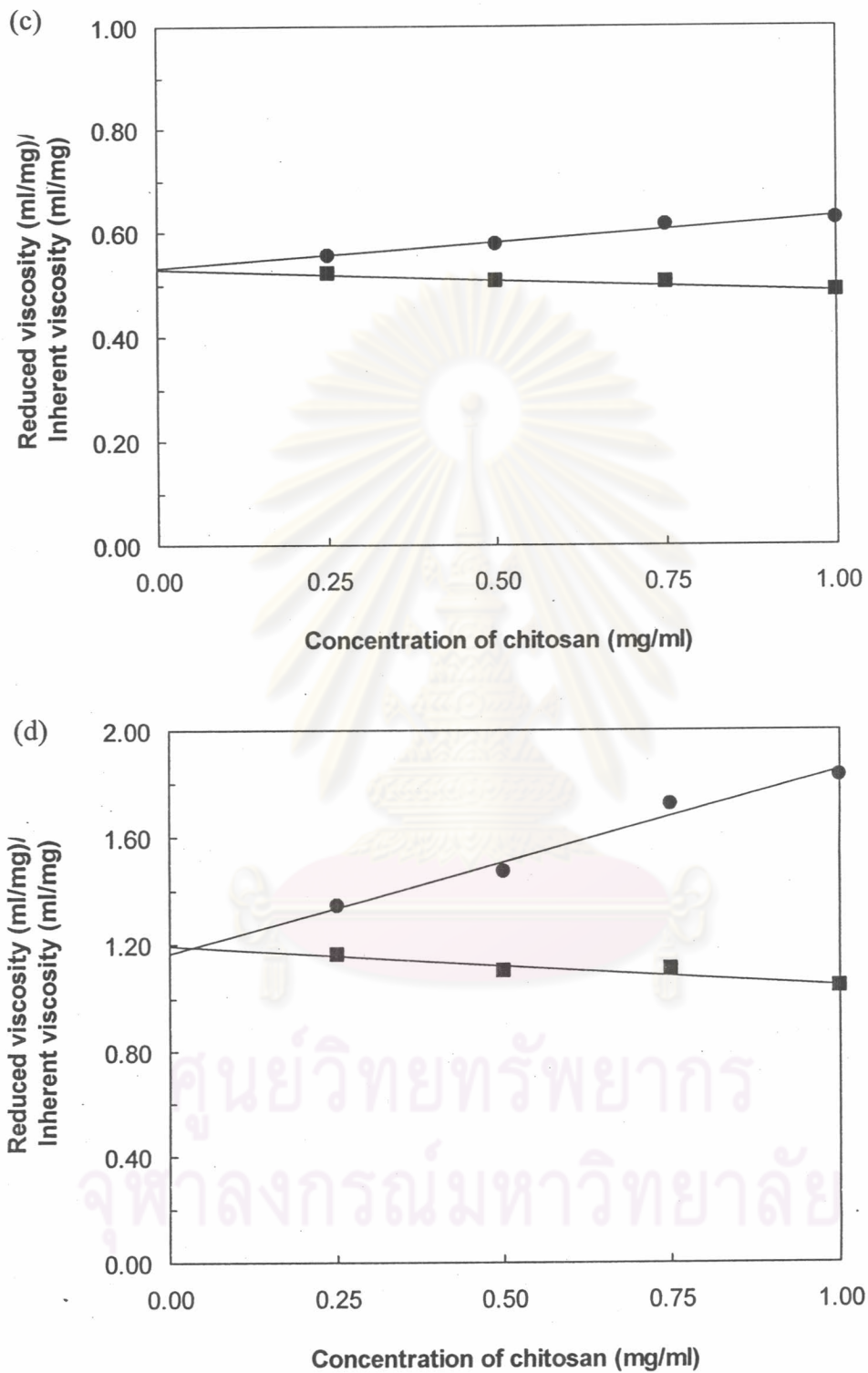
(a)

(b)
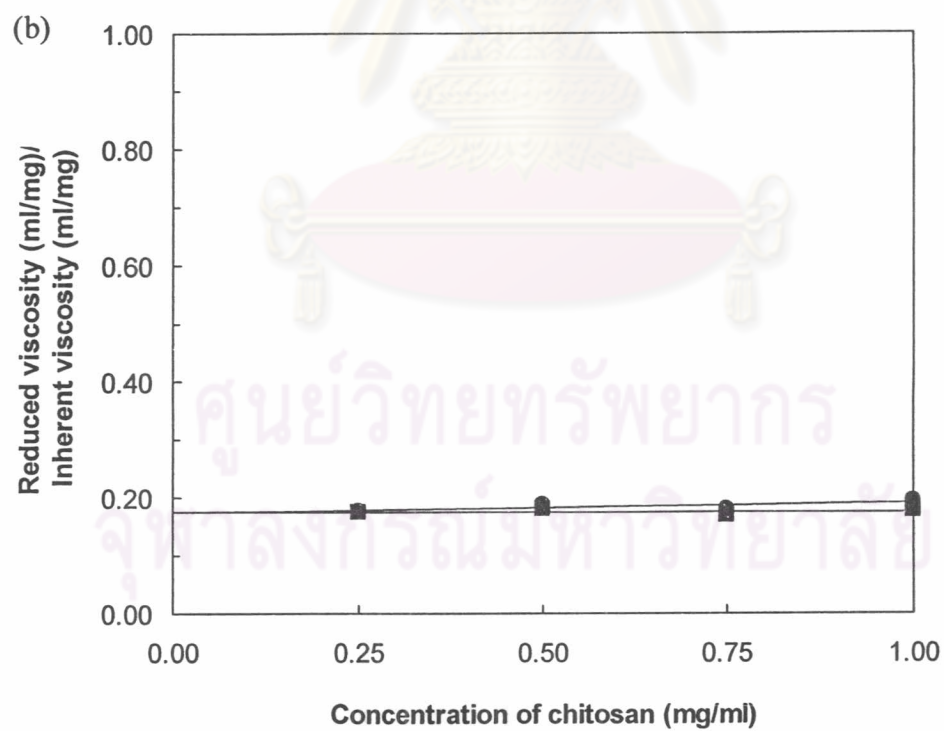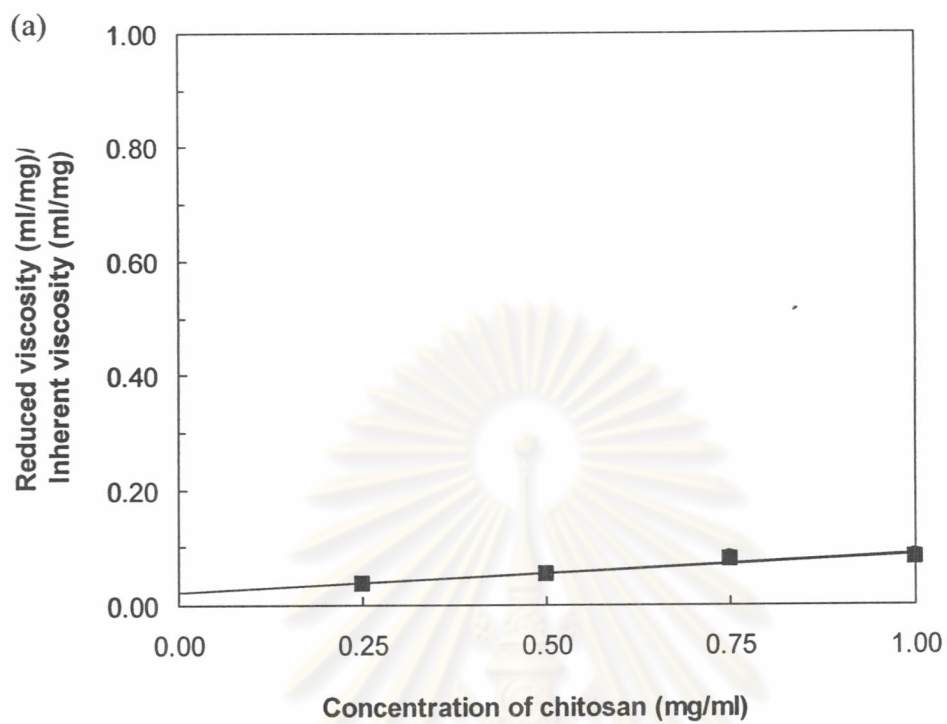
Figure A-1 Plot to determine intrinsic viscosity of (a) XLMW, (b) LMW, (c) MMW, and (d) HMW chtiosans: (●) reduced viscosity and (■) inherent viscosity.

Table A-9 [η] of chitosan samples.

Chitosan	$[\eta]$ (ml/g)
XLMW	22.1
LMW	176.1
MMW	531.2
HMW	1,182.5

The viscosity-averaged molecular weight was calculated based on the Mark-Houwink equation as follows:

$$[\eta] = KM^a$$

where K = 3.5×10^{-2} (ml/g) and a = 0.76.

An example of the calculation for XLMW chitosan was shown as follows:

$$22.1 = 3.5 \times 10^{-2} \times M_v^{0.76}$$
$$M_v^{0.76} = 631.4$$
$$M_v = (631.4)^{1/0.76}$$
$$M_v = 4,837.68 \approx 4.8k$$

The viscosity-averaged molecular weight of four chitosan samples was shown in Table A-10.

Table A-10 M_v of chitosan samples.

Chitosan	M_v
XLMW	4.8k
LMW	74k
MMW	320k
HMW	880k

B. The results from gel permeation chromatography (GPC)

Polymer Laboratories
PL LogiCal GPC Software

12:23 Wed Nov 16 2005

Unknown   E1116.006
XLMW

Acquired : 11:10 Wed Nov 16 2005
Operator   Temsiri Wangtaveesab
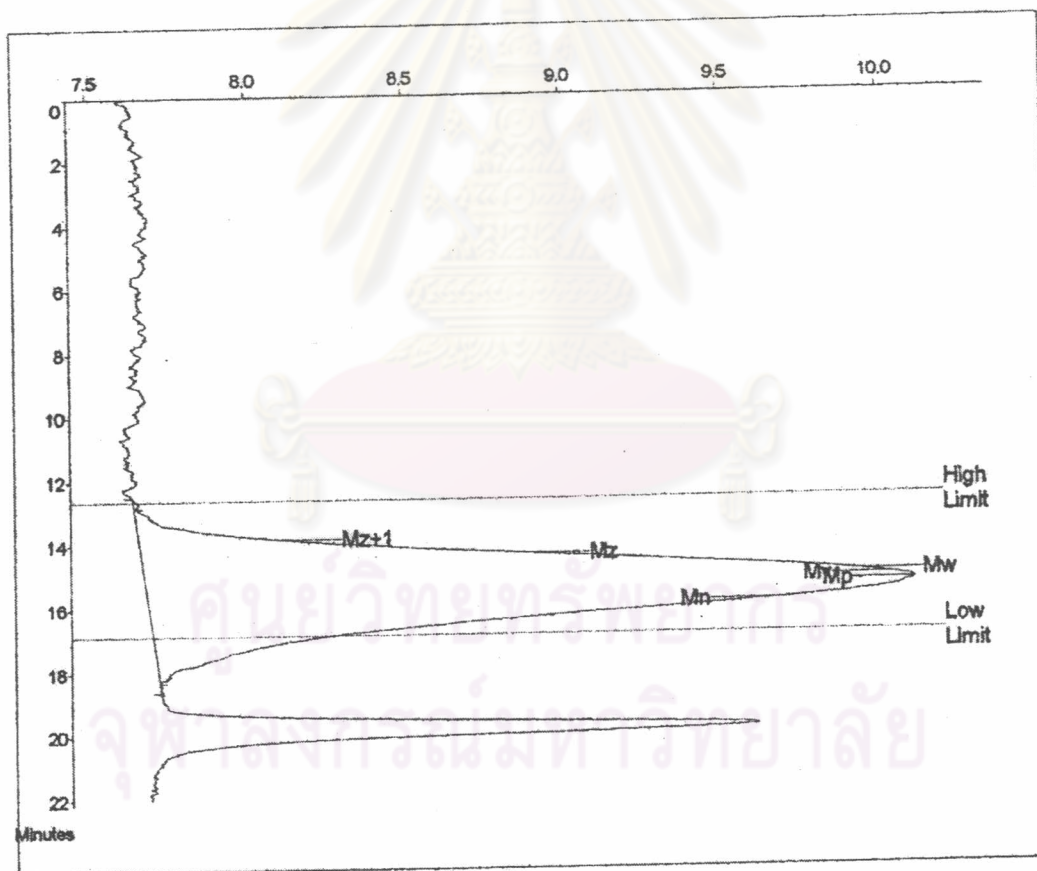
Concentration   :
Injection Volume :
Solvent   :   0.5M acetate buffer
Column Set   :   Ultrahydrogel linear

Detector   :   RI
Temperature :   30 degree celcius
Flow Rate   :   0.600
Standards   :   Pullulans

Method   74
Comments :

Calibration Using :   Narrow Standards
Calibration Limits :   12.63   to   16.87   Mins
Flow Rate Marker :   found at :   Not Found

Curve Used :   1st Order Polynomial
Last Calibrated :   Tue Nov 15 16:05:20 2005
in Standards at :   0.00   Mins

Broad Peak Start :   12.83   End : 18.32   Mins

Molecular Weight Averages

Mp =	35836	Mz =	106565	
Mn =	18422	Mz+1 =	189592	
Mw =	49222	Mv =	43249	
Polydispersity =	2.672	Peak Area =	63965	

Figure B-1 GPC curve of XLMW chitosan.
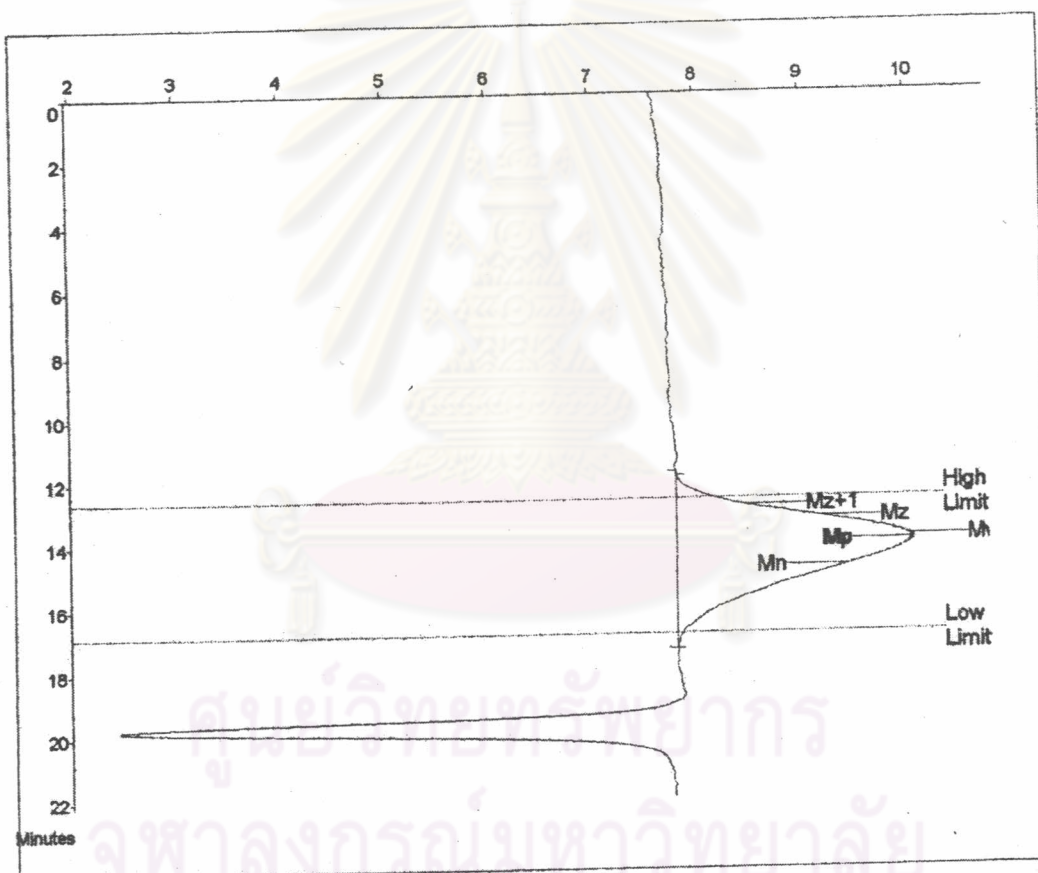
Polymer Laboratories
PL LogiCal GPC Software

Unknown   E1116.007
LMW

Acquired   :   11:36 Wed Nov 16 2005
Operator   :   Temsiri Wangtaveesab

Concentration   :
Injection Volume :
Solvent   :   0.5M acetate buffer
Column Set   :   Ultrahydrogel linear

Detector   :   RI
Temperature   :   30 degree celcius
Flow Rate   :   0.600
Standards   :   Pullulans

Method   74
Comments :

Calibration Using :   Narrow Standards
Calibration Limits :   12.63   to 16.87   Mins
Flow Rate Marker :   found at :   Not Found

Curve Used :   1st Order Polynomial
Last Calibrated :   Tue Nov 15 16:05:20 2005
in Standards at :   0.00   Mins

Broad Peak Start :   11.92   End : 17.35   Mins

Molecular Weight Averages

Mp =	159872	Mz =	372363
Mn =	64330	Mz+1 =	589677
Mw =	182806	Mv =	160867
Polydispersity =	2.842	Peak Area =	61265

Figure B-2 GPC curve of LMW chitosan.

Polymer Laboratories
PL LogiCal GPC Software

12:25 Wed Nov 16 2005

Unknown   E1116.008
NMW

Acquired : 12:02 Wed Nov 16 2005
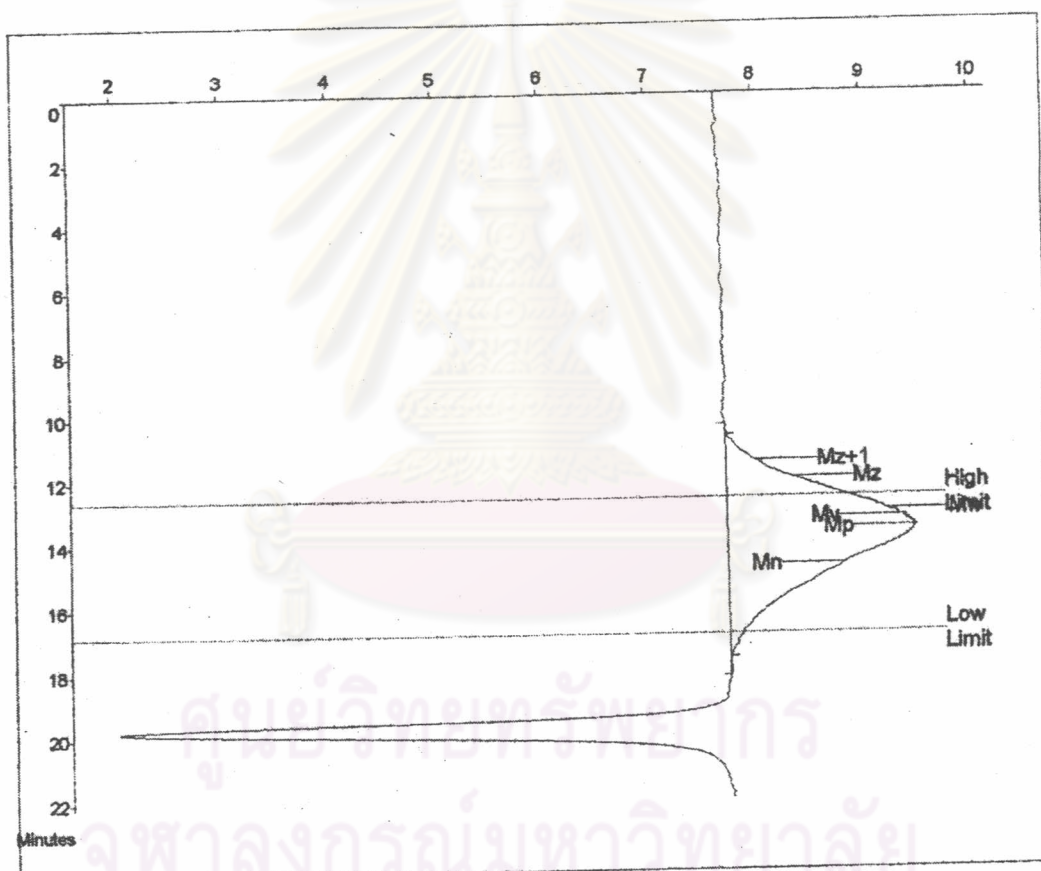Operator   Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent   :   0.5M acetate buffer
Column Set   :   Ultrahydrogel linear

Detector   : RI
Temperature : 30 degree celcius
Flow Rate   : 0.600
Standards   : Pullulans

Method   74
Comments :

Calibration Using :   Narrow Standards
Calibration Limits :   12.63   to 16.87   Mins
Flow Rate Marker :   found at :   Not Found

Curve Used :   1st Order Polynomial
Last Calibrated :   Tue Nov 15 16:05:20 2005
in Standards at :   0.00   Mins

Broad Peak Start :   10.67   End : 17.60   Mins

Molecular Weight Averages			
Mp =	248882	Mz =	1556320
Mn =	68299	Mz+1 =	2902136
Mw =	459716	Mv =	364724
Polydispersity =	6.731	Peak Area =	62763

Figure B-3 GPC curve of MMW chitosan.

Polymer Laboratories
PL LogiCal GPC Software

13:44 Wed Nov 18 2005

Unknown   E1116.009
HMW

Acquired : 12:28 Wed Nov 16 2005
Operator   Temsiri Wangtaveesab

Concentration :
Injection Volume :
Solvent   :   0.5M acetate buffer
Column Set   :   Ultrahydrogel linear

Detector   :   RI
Temperature :   30 degree celcius
Flow Rate   :   0.600
Standards   :   Pullulans

Method   74
Comments :

Calibration Using :   Narrow Standards
Calibration Limits :   12.63   to 16.87   Mins
Flow Rate Marker :   found at :   Not Found

Curve Used :   1st Order Polynomial
Last Calibrated :   Tue Nov 15 16:05:20 2005
in Standards at :   0.00   Mins

Broad Peak Start :   9.58   End : 16.57   Mins
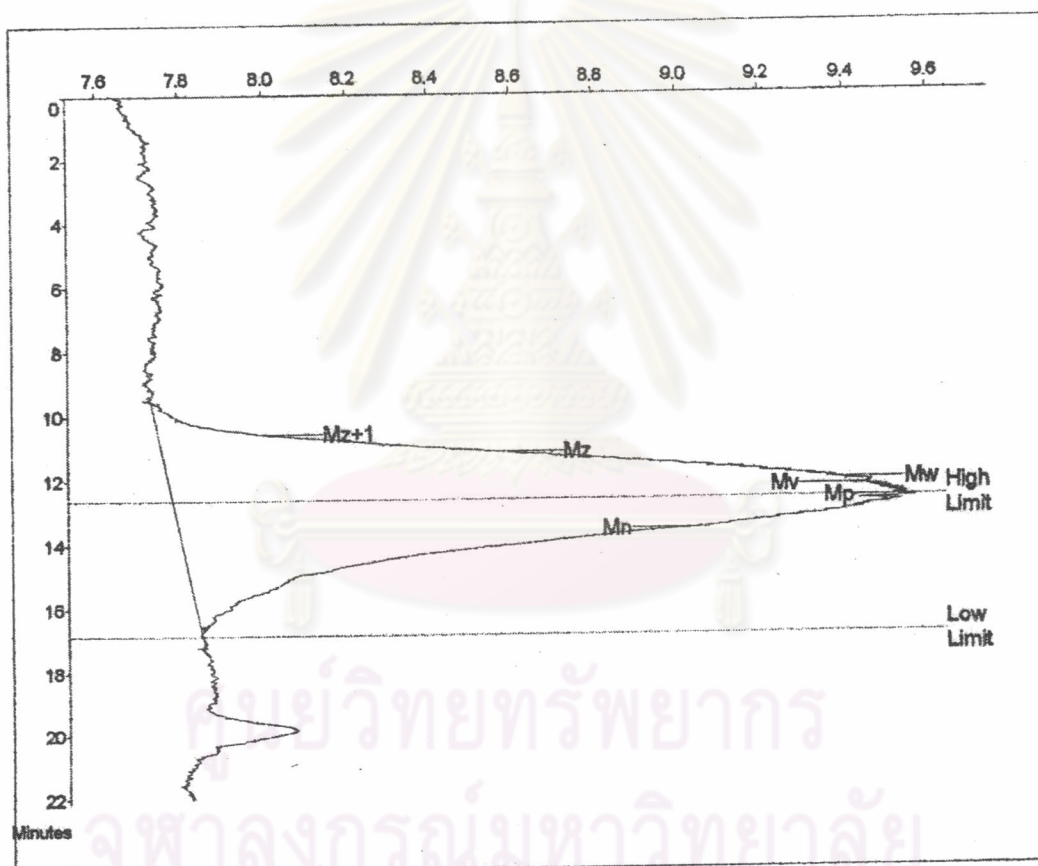
Molecular Weight Averages			
Mp =	759843	Mz =	4246336
Mn =	260510	Mz+1 =	8232196
Mw =	1450160	Mv =	1190035
Polydispersity =	5.567	Peak Area =	62261

Figure B-4 GPC curve of HMW chitosan.

C. Degree of deacetylation calculation

From the method described in section 4.3.2.3, the degrees of deacetylation of chitosans were calculated from the absorbance at the wavenumber of 1320 and 1420 cm^{-1}. The two absorbent peaks represented C-N stretching and C-H deformations, respectively. The degrees of acetylation (DA) could be calculated by using the following equation:

$$DA\ (\%) = 31.92(A_{1320}/A_{1420}) - 12.20$$

where A_{1320} and A_{1420} represented for absorbance at the wavenumber of 1320 and 1420 cm^{-1}, respectively. The calculation of degree of deacetylation of XLMW chitosan was shown as an example as follows:

$$DA = 31.92(0.3776/0.4623) - 12.20\ \%$$
$$DA = 13.87\ \%$$

The degree of deacetylation (DD) can be calculated from $100 - DA\ (\%)$:

$$DD\ (\%) = 100 - DA\ (\%)$$
$$DD = 100 - 13.87\ \%$$
$$DD = 86.13\ \%$$

Table C-1 Absorbance at the wavenumber of 1320 and 1420 cm^{-1} and the results of degree of deacetylation of four chitosan samples.

Chitosan	A_{1320}	A_{1420}	DD (%)
XLMW	0.3776	0.4623	86.13
LMW	0.3362	0.4418	87.91
MMW	0.3811	0.4380	84.43
HMW	0.3341	0.4044	85.83

D. Standard curve for cell viability determination

Standard curve for L929 mouse connective tissue fibroblasts:

Table D-1 Absorbance at 570 nm from mitochondrial activity (MTT) assay
for standard curve of L929 mouse connective tissue fibroblasts.

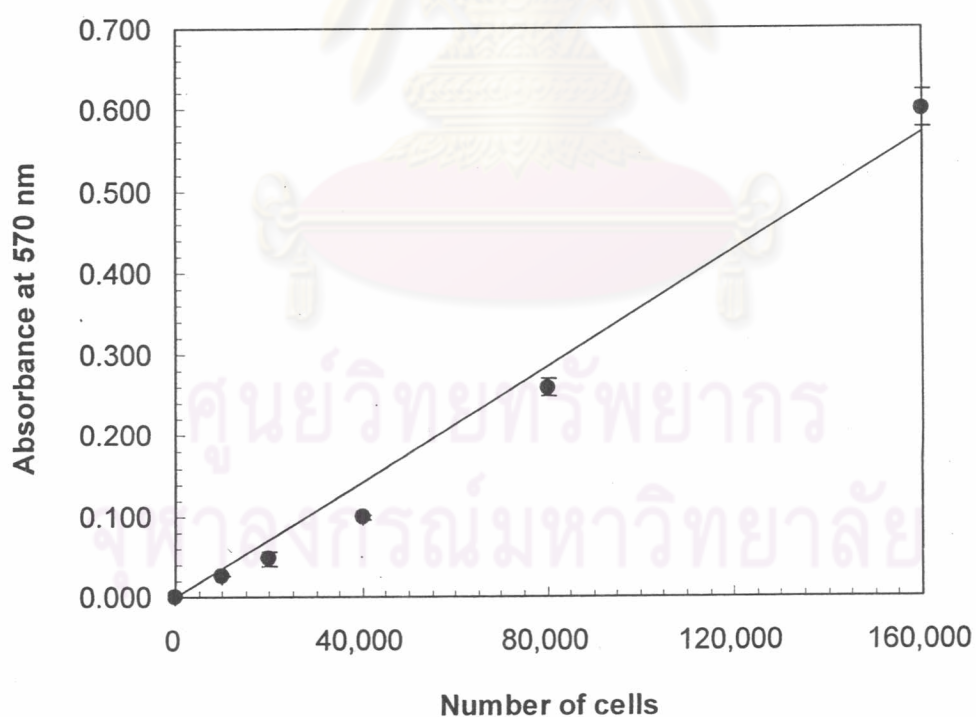| Replication no.	Number of cells					
	0	10,000	20,000	40,000	80,000	160,000
1	0	0.024	0.047	0.098	0.263	0.611
2	0	0.024	0.043	0.089	0.261	0.603
3	0	0.027	0.050	0.110	0.254	0.584
Average	0	0.025	0.047	0.099	0.259	0.599
SD	0	0.002	0.004	0.011	0.005	0.014

Figure D-1   Standard curve for L929 mouse connective tissue fibroblasts.

Standard curve for Detroit 551 human dermal fibroblasts:

Table D-2 Absorbance at 570 nm from mitochondrial activity (MTT) assay
for standard curve of Detroit 551 human dermal fibroblasts.

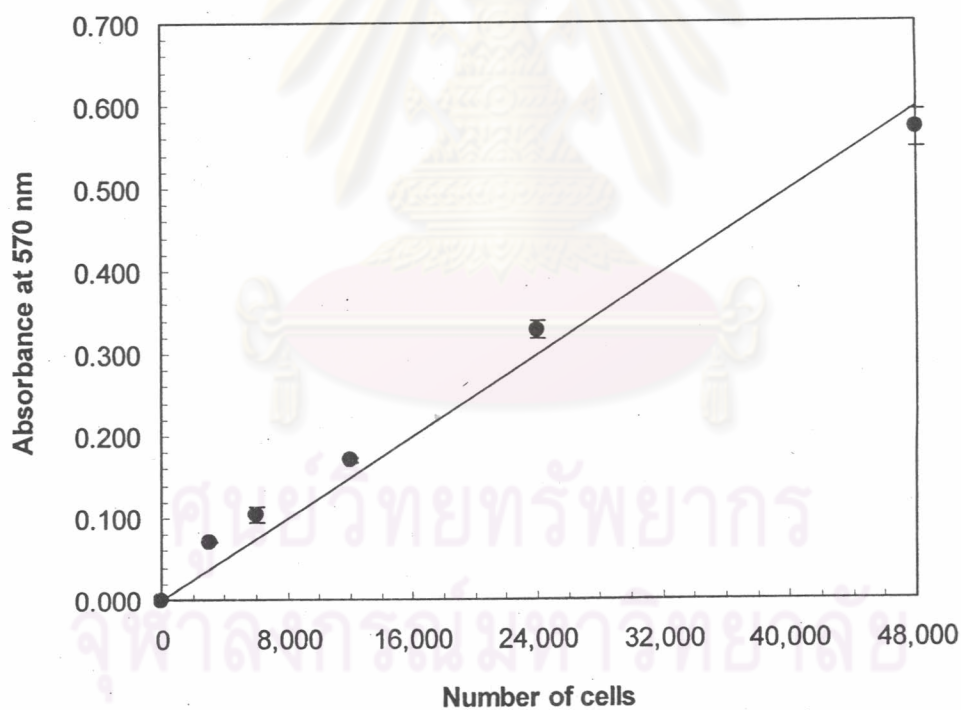| Replication no.	Number of cells					
	0	3,000	6,000	12,000	24,000	48,000
1	0	0.071	0.113	0.173	0.338	0.596
2	0	0.071	0.095	0.167	0.317	0.561
3	0	0.071	0.105	0.169	0.328	0.554
Average	0	0.071	0.104	0.170	0.328	0.570
SD	0	0.000	0.009	0.003	0.010	0.020

Figure D-2   Standard curve for Detroit 551 human dermal fibroblasts.

VITAE

Mr. Chalonglarp Tangsadthakun was born in Bangkok, Thailand on March 19, 1982. He finished the high school education in 1999 from Wat Suthivararam school. In 2003, he graduated from Faculty of Engineering, Chulalongkorn University with a Bachelor of Engineering (1st Class Honors) in chemical engineering. After the graduation, he pursued his graduate study to a Master of Engineering (chemical engineering), the Faculty of Engineering, Chulalongkorn University.