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APPENDIX

## APPENDIX A

## Particle Size and Zeta Potential

Table 1A Mean particle size of GH-loaded  $\text{Ca}^{2+}$  alginate-chitosan particles and GH-loaded  $\text{Ca}^{2+}$  alginate-*N*-butylchitosan particles with different %DS of chitosan

Sample	Particle size (nm)			Mean (nm)	SD
	Set no. 1	Set no. 2	Set no. 3		
GH-ALG-CTS	292.2	306.6	285.6	294.8	10.7
GH-ALG-10%NBC	345.8	333.8	320.5	333.4	12.7
GH-ALG-37%NBC	358.5	349.5	385.1	364.4	18.5
GH-ALG-46%NBC	392.2	422.1	380.6	398.3	21.4

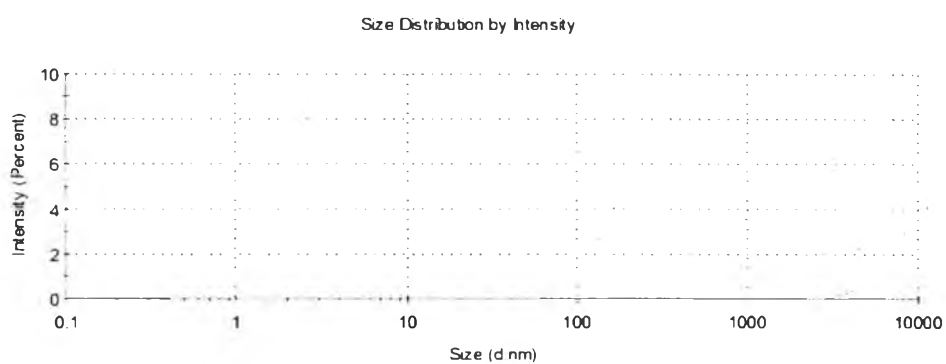


Figure 1A Size distribution of GH-ALG-CTS particles

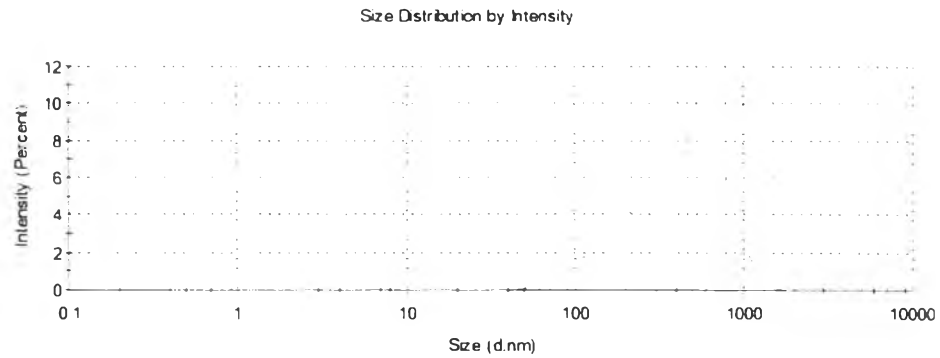


Figure 2A Size distribution of GH-ALG-10%NBC particles

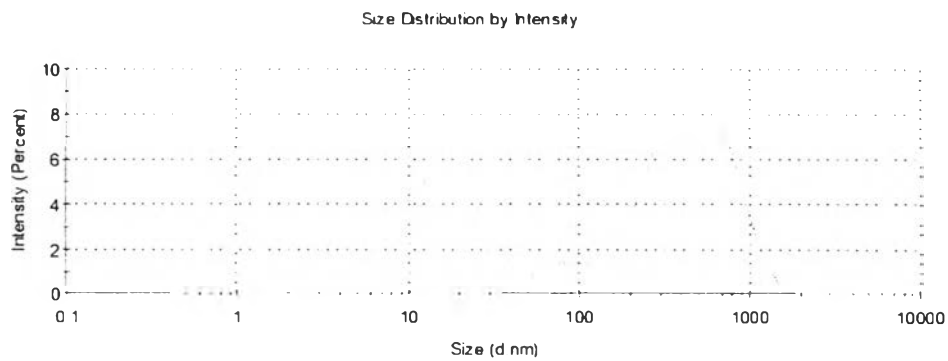


Figure 3A Size distribution of GH-ALG-37%NBC particles

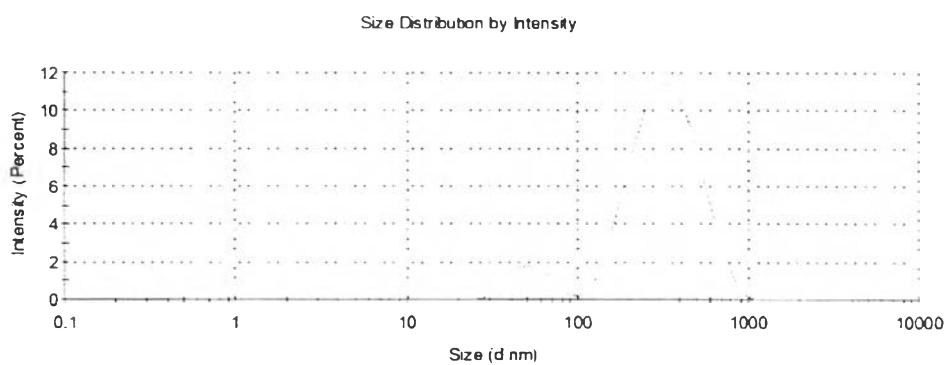


Figure 4A Size distribution of GH-ALG-46%NBC particles

Table 2A Zeta potential of GH-loaded  $\text{Ca}^{2+}$  alginate-chitosan particles and GH-loaded  $\text{Ca}^{2+}$  alginate-*N*-butylchitosan particles with different %DS of chitosan

Sample	Zeta potential (mV)			Mean (mV)	SD
	Set no. 1	Set no. 2	Set no. 3		
GH-ALG-CTS	-28.1	-28.3	-29.1	-28.5	0.53
GH-ALG-10%NBC	-30.4	-28.7	-27.9	-29.0	1.28
GH-ALG-37%NBC	-27.2	-27.7	-28.1	-27.6	0.47
GH-ALG-46%NBC	-26.6	-26.2	-25.7	-26.2	0.45

Table 3A Mean particle size of GH-loaded  $\text{Ca}^{2+}$  alginate-chitosan particles and GH-loaded  $\text{Ca}^{2+}$  alginate-*N*-butylchitosan particles in filtrate

Sample	Particle size (nm)			Mean (nm)	SD
	Set no. 1	Set no. 2	Set no. 3		
GH-ALG-CTS	184.5	179.5	185.1	183.0	3.1
GH-ALG-46%NBC	101.8	113.6	90.5	102	11.6



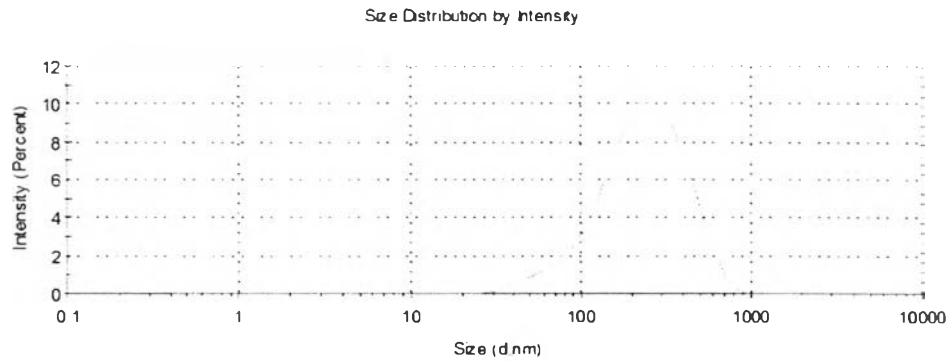


Figure 5A Size distribution of GH-ALG-CTS particles in filtrate

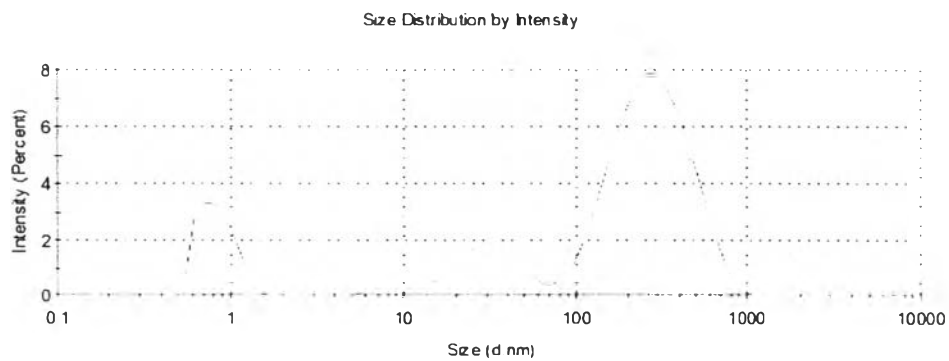


Figure 6A Size distribution of GH-ALG-46%NBC particles in filtrate

Table 4A Mean particle size of GH-ALG-CTS particles in suspension for stability tests for 60 days

Day	Particle size (nm)			Mean (nm)	SD
	Set no. 1	Set no. 2	Set no. 3		
0	404.2	393.8	397.9	397.6	5.7
7	372.0	375.0	382.0	376.3	5.1
15	361.0	357.3	364.7	361.0	3.7
45	347.0	366.3	345.1	352.8	11.7
60	485.7	482.5	495.0	487.7	6.5

**Table 5A** Mean particle size of GH-ALG-46%NBC particles in suspension for stability tests for 60 days

Day	Particle size (nm)			Mean (nm)	SD
	Set no. 1	Set no. 2	Set no. 3		
0	532.5	532.1	551.7	538.8	11.02
7	597.0	587.0	572.0	585.3	12.6
15	578.1	575.4	558.6	570.7	10.6
45	540.8	541.4	547.1	543.1	3.5
60	692.7	695.0	699.4	697.7	3.4

**Table 6A** Zeta potential of GH-ALG-CTS particles in suspension for stability tests for 60 days

Day	Zeta potential (mV)			Mean (mV)	SD
	Set no. 1	Set no. 2	Set no. 3		
0	-27.8	-28.7	-28.8	-28.4	0.6
7	-28.9	-30.2	-30.0	-29.7	0.7
15	-12.8	-12.1	-12.9	-12.6	0.4
45	-9.8	-12.8	-11.9	-11.5	1.5
60	-13.7	-12.8	-12.3	-12.9	0.7





Table 7A Zeta potential of GH-ALG-46%NBC particles in suspension for stability tests for 60 days

Day	Zeta potential (mV)			Mean (mV)	SD
	Set no. 1	Set no. 2	Set no. 3		
0	-28.0	-28.8	-31.0	-29.3	1.6
7	-28.3	-29.9	-30.6	-29.6	1.2
15	-19.0	-19.1	-19.6	-19.2	0.3
45	-13.3	-12.4	-17.4	-14.4	2.7
60	-8.1	-9.4	-8.7	-8.7	0.6



## APPENDIX B

## Calibration Curve

The concentration versus peak area data of GH in ultra-pure water at 245 nm and are presented in Table 1. They show a linear relationship with the correlation coefficient equal to 0.9984

Table 1B Peak area of GH in ultra-pure water determined at 245 nm

Concentration of GH in ultra-pure water ( $\mu\text{g/mL}$ )	Peak area of GH derivative
1	81262
5	504662
25	2325336
50	2711104
100	6298170
125	9568707
250	20782873
500	40830664
1000	77538581

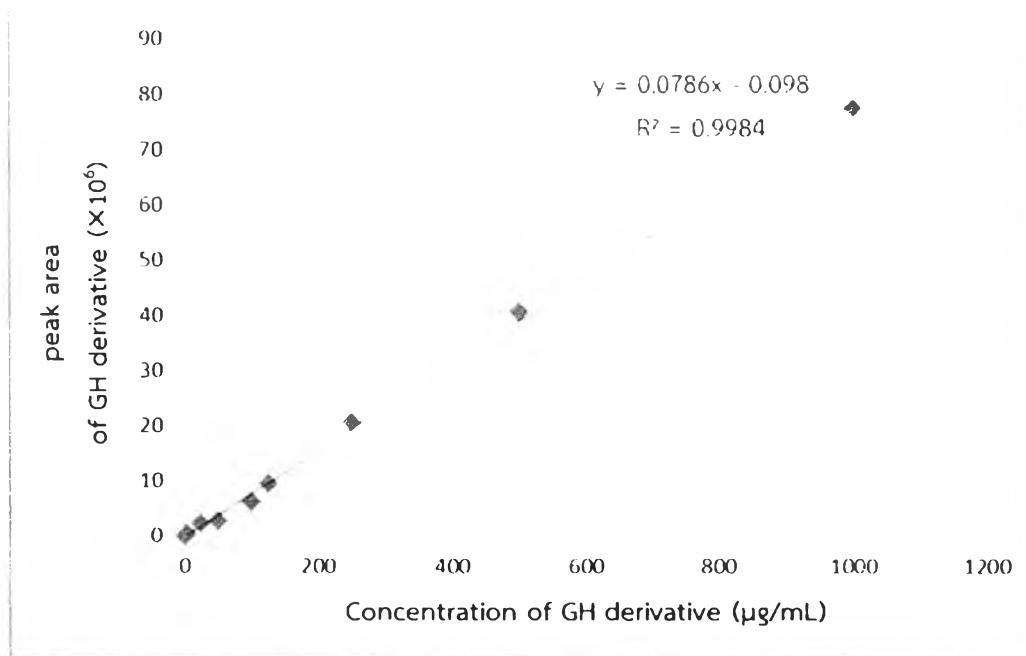


Figure 1B Standard calibration curve of GH derivative in ultra-pure water

$$\text{Amount of GH(mg)} = \frac{\text{peak area} + 0.098}{0.0786 \times 1000} \times \text{total vol. of sample (ml)}$$



APPENDIX C

Amount of GH Release

Table 1C Amount of GH release from GH-ALG-CTS particles in PBS pH 7.4, 37°C

Time (h)	Amount of GH release (mg)			Mean	SD
	Set no.1	Set no.2	Set no.3		
0	0.5128	0.5024	0.4997	0.5050	0.0069
0.25	1.4056	1.6886	1.4307	1.5083	0.1567
0.5	1.7659	1.8190	1.6185	1.7345	0.1039
0.75	2.3084	2.1811	2.0633	2.1843	0.1226
1	2.5947	2.4404	2.4046	2.4799	0.1010
2	2.9403	3.1522	3.1415	3.0780	0.1194
3	3.2686	3.4326	3.3773	3.3595	0.0834
6	3.5099	3.5036	3.5260	3.5131	0.0116
12	3.5077	3.5279	3.5581	3.5312	0.0254
24	3.5711	3.5861	3.5647	3.5740	0.0110



Table 2C Amount of GH release from GH-ALG-10%NBC particles in PBS pH 7.4, 37°C

Time (h)	Amount of GH release (mg)			Mean	SD
	Set no.1	Set no.2	Set no.3		
0	0.4020	0.4026	0.3963	0.4003	0.0035
0.25	1.2422	1.1970	1.2313	1.2235	0.0236
0.5	1.5133	1.5306	1.5340	1.5259	0.0111
0.75	1.9669	1.9808	1.7829	1.9102	0.1104
1	2.1601	2.1617	2.1545	2.1588	0.0038
2	3.0748	2.6916	2.8041	2.8569	0.1970
3	3.5744	3.3438	3.5559	3.4914	0.1281
6	4.0691	4.0245	4.1014	4.0650	0.0386
12	4.0969	4.1018	4.0943	4.0977	0.0038
24	4.2248	4.2767	4.2595	4.2536	0.0264

Table 3C Amount of GH release from GH-ALG-37%NBC particles in PBS pH 7.4, 37°C

Time (h)	Amount of GH release (mg)			Mean	SD
	Set no.1	Set no.2	Set no.3		
0	0.3657	0.3633	0.3644	0.3644	0.0012
0.25	1.1917	1.1912	1.1973	1.1934	0.0034
0.5	1.4194	1.3994	1.3986	1.4058	0.0118
0.75	1.7409	1.8238	1.8588	1.8078	0.0606
1	2.2970	2.2342	2.3180	2.2831	0.0436
2	2.8354	2.7410	2.8289	2.8018	0.0528
3	3.1388	3.1527	3.3600	3.2172	0.1239
6	3.8026	3.8137	3.8139	3.8101	0.0065
12	3.8149	3.8164	3.8175	3.8163	0.0013
24	3.8704	3.8867	3.8646	3.8739	0.0115



Table 4C Amount of GH release from GH-ALG-46%NBC particles in PBS pH 7.4, 37°C

Time (h)	Amount of GH release (mg)			Mean	SD
	Set no.1	Set no.2	Set no.3		
0	0.4000	0.3653	0.4107	0.3920	0.0238
0.25	1.1913	1.1874	1.1977	1.1921	0.0052
0.5	1.2841	1.3025	1.2890	1.2919	0.0095
0.75	1.7124	1.7407	1.7786	1.7439	0.0333
1	2.1092	2.1405	2.1618	2.1372	0.0265
2	2.8203	2.8408	2.7707	2.8106	0.0360
3	3.5489	3.1249	3.5596	3.4111	0.2480
6	3.9416	4.1882	4.2370	4.1223	0.1584
12	4.2281	4.2962	4.2354	4.2532	0.0374
24	4.3229	4.3232	4.3260	4.3240	0.0017



## VITA

Mr. Wittaya Pamornwaranon was born on January 12, 1977 in Maehongsorn, Thailand. He received his Bachelor's Degree of Science in Industrial Chemistry from the Faculty of Science, Rajmangala Institute of Technology, Bangkok Technical Campus in 2000. After graduation, he worked at the Faculty of Science, Rajmangala Krungthep University, Bangkok, Thailand. He has been a graduate student in the Program of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University since 2010 and finished his study in July 2014.

Proceeding:

The poster presentation "Calcium Alginate- N-Butyl Chitosan Particles as Carriers for Controlled Release of Glucosamine Hydrochloride" in the 4th Polymer Conference of Thailand, Pathumwan Princess Hotel, Bangkok, Thailand March 20-21, 2014

