

## REFERENCES

- Cho Y-W, Jang J, Park CR, Ko S-W. (2000) Preparation and solubility in acid and water of partially deacetylated chitins. Biomacromolecules,1:609–14.
- Goosen, M.F.A. (1997) Applications of chitin, chitosan, Pennsylvania: Technomic Publishing.
- Helbert, W., Cavaille, J. Y., and Dufresne, A. (1996) Polymer Composition, 17, 4, 604.
- Jayakumar, R., Prabakaran, M., Nair, S.V., Tokura, S., Tamura, H., and Selvamurugan, N. (2010) Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. Progress in Materials Science, 55, 675–709.
- Jayakumar, R., Prabakaran, M., Nair, S.V., Tokura, S., Tamura, H., and Selvamurugan, N.(2010) Biomedical applications of chitin and chitosan based nanomaterial-A short review. Carbohydrate Polymer, 82, 227-232.
- Jayakumar , R., Prabakaran, M., Nair, S.V., Tokura, S., Tamura, H., and Selvamurugan, N. (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnology Advances, 29, 322-337.
- Khor, E., and Lim, L.Y. (2003) Implantable applications of chitin and chitosan. Biomaterials, 24, 2339–2349.
- Kurita K. Chemistry and application of chitin and chitosan.(1995) Polymer Degradation and Stability,59,117–20.
- Kurita K (1997) Chitin and chitosan derivatives. In: Arshady R (ed) Desk reference of functional polymers: syntheses and applications. Washington,DC American Chemical Society, pp 239–259
- Kurita K (2006) Introduction of biologically active branches through controlled modification reactions of chitin and chitosan. In: Uragami T, Tokura S (eds) Material science of chitin and chitosan. Kodansha Scientific, Tokyo
- Kumar, M.N.V.R. (2000) A review of chitin and chitosan applications. Reactive & Functional Polymers, 46, 1–27.

- Li, J., Revol, J.F., and Marchessault, R. H. J. (1996) Applications of chitin and chitosan-derivatives for the detoxification of water and wastewater. Colloid Interface Science, 183, 365.
- Lu, Y., Weng, L., and Zhang, L. (2004) Morphology and Properties of Soy Protein Isolate Thermoplastics Reinforced with Chitin Whiskers. Biomacromolecules, 5, 1046-1051.
- Marchessault, R. H., Morehead, F. F., and Walter, N. M. (1959) Liquid Crystal Systems from Fibrillar Polysaccharides. Nature, 184, 632.
- Marumatsu, K., Masuda, S., yoshihara, Y., and Fujisawa, A. (2003) In vitro degradation behavior of freeze-dried carboxymethyl-chitin sponges processed by vacuum-heating and gamma irradiation. Polymer Degradation and Stability, 81, 327–332
- Morin, A. and Dufresne, A. (2002) Nanocomposites of Chitin Whiskers from Riftia Tubes and Poly(caprolactone). Macromolecules, 35, 2190–2199.
- Nair, K.G., and Dufresne, A. (2003) Crab shell chitin whisker reinforced natural rubber nanocomposites 1. Processing and swelling behavior. Biomacromolecules, 4, 657-665.
- Paillet, M. and Dufresne, A. (2001) Chitin Whisker Reinforced Thermoplastic Nanocomposites. Macromolecules, 34, 6527–6530.
- Pillai, C.K.S., Paul, W., Sharma, C.P. (2009) Chitin and chitosan polymers: Chemistry, solubility and fiber formation. Progress in Polymer Science, 34, 641–678.
- Raabe, D., Sachs, C., and Romano, P. (2005) The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material. Acta Materialia, 53, 4281–92.
- Revol, J.F., and Marchessault, R.H. (1993) In vitro chiral nematic ordering of chitin crystallites. International Journal Biological Macromolecules, 15, 329–35.
- Rinaudo, M. (2006) Chitin and chitosan: Properties and applications. Progress in Polymer Science, 31, 603–632.
- Sandford, PA., Sjak-Braek, G., and Anthonsen, T.(1989) Chitin and chitosan. London: Applied Science, 51–69.

- Sriupayo, J., Supaphol, P., Blackwell, J., and Rujiravanit, R. (2005) Preparation and characterization of  $\alpha$ -chitin whisker-reinforced chitosan nanocomposite films with or without heat treatment. Carbohydrate Polymers, 62:130-136.
- Takai, O. (2008). Solution plasma processing (SPP). Pure and Applied Chemistry, 80, 2003–2011.
- Tamura H, Nagahama H, Tokura S.(2006) Preparation of chitin hydrogel under mild conditions. Cellulose,13:357–64.
- Tamura H, Tsurutaa Y, Itoyamab K, Worakitkanchanakul W, Rujiravanit R, Tokura S. (2004) Preparation of chitosan filament applying new coagulation system. Carbohydrate Polymers,56.205–11.
- Tamura H, Nagahama H, Tokura S. (2007) Preparation of chitin hydrogel under mild conditions. Japan Apply Polymer Science,104,3909–16.
- Tamura H, Sawada M, Nagahama H, Higuchi T, Tokura S. (2006) Influence of amide content on the crystal structure of chitin. Holzforschung;60,480–4.
- Tokura S, Miura Y, Johmen M, Nishi N, and Nishimura S.I. (1994) Journal Control Release, 28, 235–41.
- Tokura, S., Baba, S., Uraki, Y., Miura, Y., Nishi, N., and Hasegawa, O. (1990) Controlled functionalization of the polysaccharide chitin. Carbohydrate Polymers, 13, 273–81.
- Uragami T, Kurita K, Fukamizo T (eds) (2001) Chitin and chitosan in life science. :Tokyo, Kodansha Scientific, pp 178–185
- Vasnev, V.A., Tarasov, A.I., Markova, G.D., Vinogradova, S.V., and Garkusha, O.G. (2006) Synthesis and properties of acylated chitin and chitosan derivatives. Carbohydrate Polymers, 64, 184–189
- Watanabe, K., Saiki, I., Uraki, Y., Tokura, S., and Azuma, I. (1990) Regioselective Conjugation of Chitosan with a Laminin-related Peptide. Chemical Pharmaceutical Bull. 38, 506–9.
- Wongpanit, P., Sanchavanakit, N., Pavasant, P., Supaphol, P., Tokura, S., and Rujiravanit, R. (2005). Preparation and Characterization of Microwave-treated Carboxymethyl Chitin and Carboxymethyl Chitosan Films for Potential Use in Wound Care Application. Macromolecular Bioscience, 5, 1001–1012

- Yi H, Wu L-Q, Bentley WE, Ghodssi R, Rubloff GW, Culver JN, et al. Biofabrication with chitosan. *Biomacromolecules* 2005;6:2881–94.
- Zaini, M. J., Fuad, M. Y. A., Ismail, H., Mansor, M. S., and Mustafah, J. (1996) Preparation and properties of polypropylene composites reinforced with wheat and flax straw fibres: Part II Analysis of composite microstructure and mechanical properties. *Polymer International*, 40, 51-55.

## APPENDICES

## Appendix A Characterization of Raw Materials

**Table A1** Percent yield of chitin

Samples	Dry weight (%)
Dry shrimp shell	100
Decalcification product	54.17
Deproteination product	20.12
Chitin	25.71

**Table A2** Percent yield of chitin hydrogel

Samples	Dry weight (%)
Chitin powder	100
Chitin hydrogel	65.8

**Table A3** Degree of deacetylation of chitin and chitin hydrogel

Type of chitin	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
Chitin powder	32.51	34.74	36.62	34.62	2.05
Chitin hydrogel	5.42	3.87	36.64	35.31	1.38

## Appendix B Preparation of Chitin Hydrogel by Using Solution Plasma

**Table B1** Solid content of chitin hydrogel and the ratio of alcohol solution without NaOH system for deacetylation reaction

Ratio of Alcohol :H <sub>2</sub> O	Chitin hydrogel (ml)	Alcohol (ml)	Water (ml)	Total volume (ml)	Amount of chitin hydrogel (g)
90 : 10	10	90	0	100	1.82
50 : 50	10	50	40	100	1.82
10 : 90	10	10	80	100	1.82

**Table B2** Solid content of chitin hydrogel and the ratio of alcohol solution with NaOH system for deacetylation reaction

NaOH concentration (%)	Chitin hydrogel (ml)	50% NaOH w/v (ml)	Alcohol (ml)	Total volume (ml)	Amount of chitin hydrogel (g)
1%	10	1.8	88.2	100	1.82
5%	10	9	81	100	1.82
10%	10	18	72	100	1.82
12%	10	21.6	68.4	100	1.82



### Appendix C Deacetylation of Chitin Hydrogel without NaOH Concentration

**Table C1** Effect of type of alcohol and alcohol concentration on degree of deacetylation by using solution plasma technique

Ratio of methanol :H <sub>2</sub> O	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
90:10	43.54	44.32	43.43	43.76	0.48521473
50:50	45.44	43.43	46.54	45.14	1.57703308
10:90	54.64	54.43	53.43	54.17	0.64655497
Ratio of ethanol :H <sub>2</sub> O	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
90:10	43.5	46.44	44.58	44.84	1.48714492
50:50	45.65	47.98	48.98	47.53	1.70869346
10:90	55.5	53.44	54.58	54.50	1.03195607
Ratio of propanol :H <sub>2</sub> O	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
90:10	44.5	47.44	48.58	46.84	2.10513658
50:50	47.54	48.67	49.91	48.70	1.18542538
10:90	59.98	56.98	58.98	58.64	1.52752523

**Table C2** Effect of type of alcohol and alcohol concentration on degree of deacetylation by using conventional heat treatment

Ratio of methanol :H <sub>2</sub> O	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
90:10	35.64	37.87	35.86	36.46	1.2289155
50:50	36.56	38.45	39.98	38.33	1.713155
10:90	39.43	38.45	40.32	39.40	0.9353609
Ratio of ethanol :H <sub>2</sub> O	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
90:10	35.67	37.89	36.64	36.73	1.1129391
50:50	37.55	39.87	39.9	39.11	1.3481963
10:90	40.87	41.54	40.34	40.92	0.6013596
Ratio of propanol :H <sub>2</sub> O	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
90:10	36.47	35.04	38.79	36.77	1.8925204
50:50	39.67	39.43	38.22	39.11	0.7771958
10:90	40.32	40.53	39.32	40.06	0.646555

**Table C3** Effect of cycles of plasma treatment time on degree of deacetylation

1 cycle of treatment time					
Type of Alcohol	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
Methanol	42.78	43.07	46.11	43.99	1.844568604
Ethanol	43.5	46.44	44.58	44.84	1.487144916
Propanol	44.5	47.44	48.58	46.84	2.105136575
2 cycles of treatment time					
Type of Alcohol	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
Methanol	48.64	47.86	45.88	47.46	1.422814113
Ethanol	54.76	56.97	54.87	55.53	1.245404887
Propanol	60.54	58.76	57.78	59.03	1.399190242
3 cycles of treatment time					
Type of Alcohol	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
Methanol	49.87	53.78	52.17	51.94	1.965120861
Ethanol	56.67	56.89	57.77	57.11	0.582065288
Propanol	62.87	62.91	58.44	61.41	2.569286542

### Appendix D Deacetylation of Chitin Hydrogel with NaOH concentration

**Table D1** Effect of no NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using solution plasma technique

No NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	47.28	43.49	44.77	45.18	1.9279782
2	53.096	51.36	54.71	53.06	1.6753702
3	54.87	55.77	54.87	55.17	0.5196152
4	56.71	54.52	59.23	56.82	2.356926
5	57.52	59.54	54.98	57.35	2.2849362

1 Cycle of plasma treatment = 1 Hour

**Table D2** Effect of 1% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using solution plasma technique

1% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	54.01	54.54	56.94	55.16	1.5612922
2	57.01	55.54	59.72	57.42	2.1204323
3	57.82	61.57	60.58	59.99	1.9433734
4	62.82	60.73	61.94	61.83	1.0493331
5	62.59	64.34	64.98	63.97	1.2372146

1 Cycle of plasma treatment = 1 Hour

**Table D3** Effect of 5% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using solution plasma technique

5% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	55.43	55.97	59.27	56.89	2.0787496
2	65.24	60.05	65.76	63.68	3.1572826
3	67.24	67.05	65.03	66.44	1.2247857
4	69.01	71.22	70.59	70.27	1.1385224
5	72.01	71.25	70.35	71.20	0.8309834

1 Cycle of plasma treatment = 1 Hour

**Table D4** Effect of 10% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using solution plasma technique

10% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	55.87	57.87	58.93	57.56	1.5538769
2	69.65	68.87	69.43	69.32	0.4021608
3	70.59	75.75	68.65	71.66	3.6696776
4	70.59	74.86	76.65	74.03	3.1134279
5	74.76	76.89	75.87	75.84	1.0653169

1 Cycle of plasma treatment = 1 Hour

**Table D5** Effect of 12% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using solution plasma technique

12% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	56.42	58.82	62.23	59.16	2.9195947
2	72.87	70.56	69.54	70.99	1.706136
3	70.43	75.87	78.76	75.02	4.2295508
4	76.76	78.54	79.98	78.43	1.6129889
5	76.08	78.97	80.32	78.46	2.1661102

1 Cycle of plasma treatment = 1 Hour



**Table D6** Effect of 5% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using conventional heat treatment

5% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	37.45	38.87	39.45	38.59	1.0289801
2	39.54	40.65	41.87	40.69	1.1654327
3	45.78	46.56	47.98	46.77	1.1154072
4	50.76	51.97	53.56	52.10	1.404291
5	56.43	49.71	55.34	53.83	3.6065542

1 Cycle of plasma treatment = 1 Hour

**Table D7** Effect of 10% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using conventional heat treatment

10% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	38.43	40.54	42.34	40.44	1.9570471
2	41.43	45.34	45.32	44.03	2.2516883
3	50.32	51.98	55.45	52.58	2.6176771
4	55.53	56.84	56.34	56.24	0.661085
5	56.32	59.34	59.43	58.36	1.7701507

1 Cycle of plasma treatment = 1 Hour

**Table D8** Effect of 12% w/v of NaOH concentration in methanol solvent on degree of deacetylation for deacetylation chitin hydrogel by using conventional heat treatment

12% (w/v) NaOH concentration					
Cycle of plasma treatment	Degree of deacetylation (%DD)				
	1	2	3	Average	SD
0	35.42	33.87	36.64	35.31	1.3882723
1	38.54	36.32	35.34	36.73	1.6395528
2	43.43	49.65	46.87	46.65	3.1158305
3	50.35	51.54	54.97	52.29	2.3987983
4	55.34	58.23	59.54	57.70	2.148961
5	56.65	58.87	59.97	58.50	1.6911929

1 Cycle of plasma treatment = 1 Hour

### Appendix E Solubility of Plasma Treated Chitin Hydrogel

**Table E1** Effect of 5% w/v of NaOH concentration in methanol solvent on solubility of plasma treated chitin hydrogel

5% (w/v) NaOH concentration					
Cycle of plasma treatment	% solubility of plasma treated chitin hydrogel				
	1	2	3	Average	SD
0	0	0	0	0	0
1	15.72	18.87	22.13	18.90667	3.205157
2	17.82	17.16	19.64	18.20667	1.284419
3	28.3	25.8	28.96	27.68667	1.666893
4	38.76	38.5	39.08	38.78	0.290517
5	49.75	48.75	48.92	49.14	0.53507

1 Cycle of plasma treatment = 1 Hour

**Table E2** Effect of 10% w/v of NaOH concentration in methanol solvent on solubility of plasma treated chitin hydrogel

10% (w/v) NaOH concentration					
Cycle of plasma treatment	% solubility of plasma treated chitin hydrogel				
	1	2	3	Average	SD
0	0	0	0	0	0
1	24.37	28.92	22.2	25.16333	3.429524
2	36.76	38.59	39.84	38.39667	1.549075
3	49.83	47.07	48.7	48.53333	1.387528
4	70.62	72.59	71.99	71.73333	1.009769
5	79.73	78.97	80.32	79.67333	0.676782

1 Cycle of plasma treatment = 1 Hour

**Table E3** Effect of 12% w/v of NaOH concentration in methanol solvent on solubility of plasma treated chitin hydrogel

12% (w/v) NaOH concentration					
Cycle of plasma treatment	% solubility of plasma treated chitin hydrogel				
	1	2	3	Average	SD
0	0	0	0	0	0
1	38.44	38.82	35.23	37.49667	1.972165
2	60.28	60.56	59.54	60.12667	0.527004
3	81.35	85.87	81.76	82.99333	2.499687
4	100	100	98	99.33333	1.154701
5	100	100	100	100	0

1 Cycle of plasma treatment = 1 Hour

## Appendix F Yield of Plasma Treated Chitin Hydrogel

**Table F1** Yield of plasma treated chitin hydrogel with 5% w/v NaOH concentration

5% (w/v) NaOH concentration					
Cycle of plasma treatment	% Yield of plasma treated chitin hydrogel				
	1	2	3	Average	SD
0	100	100	100	100	0
1	81.32	83.87	82.13	82.44	1.302958
2	73.82	71.16	69.64	71.54	2.11575
3	58.37	55.87	53.96	56.06667	2.211568
4	48.76	48.5	52.08	49.78	1.996096
5	39.75	38.75	34.92	37.80667	2.549438

1 Cycle of plasma treatment = 1 Hour

**Table F2** Yield of plasma treated chitin hydrogel with 10% w/v NaOH concentration

10% (w/v) NaOH concentration					
Cycle of plasma treatment	% Yield of plasma treated chitin hydrogel				
	1	2	3	Average	SD
0	100	100	100	100	0
1	84.37	88.92	87.24	86.84333	2.30079
2	70.76	68.55	67.89	69.06667	1.503141
3	60.13	55.67	58.71	58.17	2.278508
4	45.62	48.55	41.93	45.36667	3.317263
5	39.73	38.96	34.38	37.69	2.892283

1 Cycle of plasma treatment = 1 Hour



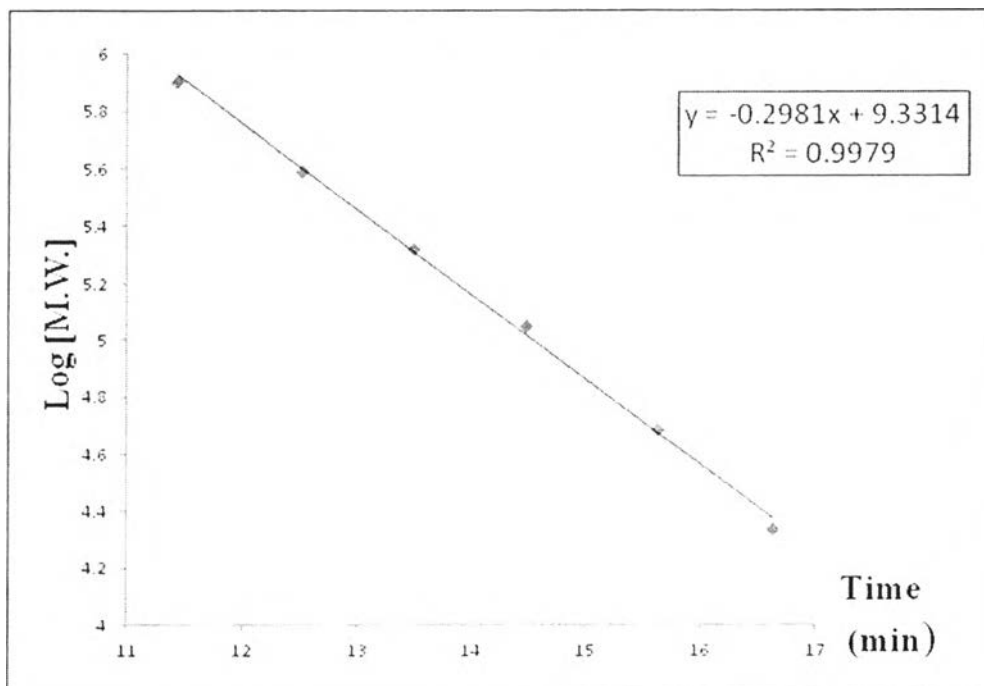
**Table F3** Yield of plasma treated chitin hydrogel with 12% w/v NaOH concentration

12% (w/v) NaOH concentration					
Cycle of plasma treatment	% Yield of plasma treated chitin hydrogel				
	1	2	3	Average	SD
0	100	100	100	100	0
1	80.44	82.82	75.27	79.51	3.859961
2	60.28	60.56	65.54	62.12667	2.959347
3	51.35	55.57	56.75	54.55667	2.839037
4	41.78	40.43	44.58	42.26333	2.116798
5	30.76	25.79	28.87	28.47333	2.508632

1 Cycle of plasma treatment = 1 Hour

## Appendix G Determination of Molecular Weight by GPC

**Figure G1** Calibration curve for GPC measurement using pullulan standard



**Table G1** The molecular weight of Treated chitin hydrogel in 12% NaOH concentration with different times by using solution Plasma

Condition	Treated chitin hydrogel 3 hours	Treated chitin hydrogel 4 hours	Treated chitin hydrogel 5 hours
$M_n$	91,141	88,942	87,689
$M_w$	245,880	230,840	220,149
$M_z$	627,817	540,912	466,782
PDI	2.69781	2.59541	2.51059

M is molecular weight and PDI is polydispersity index.

**Table G2** The molecular weight of plasma-treated chitin hydrogel obtained by varying NaOH concentrations at 5 hours of plasma treatment time

Condition	Plasma-treated chitin hydrogel 5% NaOH	Plasma-treated chitin hydrogel 10% NaOH	Plasma-treated chitin hydrogel 12% NaOH
$M_n$	103,134	105,596	87,689
$M_w$	228,674	222,543	220,149
$M_z$	349,047	324,807	466,782
PDI	2.21726	2.10749	2.51059

M is molecular weight and PDI is polydispersity index.

**Appendix H Antibacterial Activity of Plasma Treated Chitin Hydrogel****Table H1** Antibacterial activity of the obtained chitosan from plasma solution treatment against E.coli

Cycles of treatment time.	Bacterial Reduction Rate (%)
4	90.9 ± 6.4
5	96.96 ± 7.3

**Table H2** Antibacterial activity of the obtained chitosan from plasma solution treatment against S.aureus

Cycles of treatment time.	Bacterial Reduction Rate (%)
4	89.79 ± 5.4
5	95.91 ± 5.1

## CURRICULUM VITAE

**Name:** Ms. Maneekarn Kantakanun

**Date of Birth:** April 8, 1988

**Nationality:** Thai

**University Education:**

2007–2010 Bachelor Degree of Science in Materials Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

**Proceedings:**

1. Kantakanun, M.; Vanichvattanadecha, C.; Saito, N.; and Rujiravanit, R. (2013) Deacetylation of chitin hydrogel by using solution plasma . Proceedings of The 4<sup>th</sup> Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 19<sup>th</sup> PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.

**Presentations:**

1. Kantakanun, M.; Vanichvattanadecha, C.; Saito, N.; and Rujiravanit, R. (2013) Deacetylation of chitin hydrogel by using solution plasma. Poster presented at The 4<sup>th</sup> Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 19<sup>th</sup> PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.