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APPENDICES

APPENDIX A Effects of Microwave Exposure Time and Temperature on the %Weight Loss and Degree of Swelling of CM-chitin and CM-chtiosan

Table A1 Effect of microwave exposure time on the %Weight loss of (A) CM-chitin and (B) CM-chitosan films at 100°C

(A)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	100	100	100	100	0
10	100	100	100	100	0
20	100	100	100	100	0
30	61.82	61.81	60.65	61.43	0.672
50	27.25	28.93	31.32	29.17	2.04
70	26.92	30.68	28.37	28.65	1.89

(B)

Time (min)	%WL			Average	Standrad deviation
0	100	100	100	100	0
5	100	100	100	100	0
10	100	100	100	100	0
20	52.07	46.37	52.79	50.41	3.51
30	32.10	35.05	34.34	33.83	1.53
50	30.34	29.53	33.33	31.06	2.00
70	25.06	27.62	26.26	26.31	1.28

Table A2 Effect of microwave exposure time on the %Weight loss of (A) CM-chitin and (B) CM-chitosan films at 110°C

(A)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	78.58	82.11	84.06	81.58	2.77
10	37.24	43.84	41.19	40.75	3.32
20	31.39	31.97	29.23	30.86	1.44
30	23.52	21.97	23.53	23.00	0.89
50	21.04	15.72	18.85	18.53	2.67
70	18.59	20.27	20.51	19.79	1.04

(B)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	55.25	53.05	53.94	54.08	1.10
10	37.12	34.06	32.86	34.68	2.19
20	29.62	31.20	31.10	30.64	0.88
30	24.72	25.48	24.34	24.84	0.58
50	21.73	21.69	20.86	21.42	0.49
70	21.02	18.41	21.24	20.22	1.57

Table A3 Effect of microwave exposure time on the %Weight loss of (A) CM-chitin and (B) CM-chitosan films at 120°C.

(A)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	69.66	67.43	72.57	69.88	2.57
10	37.44	37.75	36.59	37.26	0.60
20	21.84	20.42	19.71	20.65	1.08
30	16.19	17.67	18.66	17.50	1.24
50	17.24	15.41	18.02	16.89	1.33
70	16.72	18.76	16.01	17.16	1.42

(B)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	43.52	42.19	51.81	45.84	5.21
10	28.82	29.76	24.93	27.83	2.56
20	18.30	18.67	18.09	18.35	0.29
30	15.22	15.39	14.73	15.11	0.34
50	13.14	18.75	13.26	15.05	3.20
70	17.42	16.46	18.30	17.39	0.92

Table A4 Effect of microwave exposure time on the Degree of swelling of (A) CM-chitin and (B) CM-chitosan films at 100°C

(A)

Time (min)	DS			Average	Standard deviation
5	-	-	-	-	-
10	-	-	-	-	-
20	-	-	-	-	-
30	58.67	63.07	61.50	61.08	2.22
50	10.75	11.50	12.68	11.64	0.97
70	6.32	8.45	7.89	7.55	1.10

(B)

Time (min)	DS			Average	Standard deviation
5	-	-	-	-	-
10	-	-	-	-	-
20	29.86	24.53	28.52	27.63	2.77
30	7.06	7.99	7.71	7.58	0.47
50	5.30	5.35	5.93	5.52	0.35
70	3.62	3.97	3.71	3.76	0.18

Table A5 Effect of microwave exposure time on the Degree of swelling of (A) CM-chitin and (B) CM-chitosan films at 110°C

(A)

Time (min)	DS			Average	Standard deviation
5	109.66	130.77	112.10	117.51	11.54
10	29.67	32.16	31.75	31.19	1.33
20	14.49	15.53	11.55	13.85	2.06
30	4.49	5.19	5.40	5.02	0.47
50	3.81	3.93	3.50	3.74	0.22
70	2.88	3.30	3.54	3.24	0.33

(B)

Time (min)	DS			Average	Standard deviation
5	37.74	33.20	32.56	34.50	2.82
10	11.45	9.96	8.54	9.98	1.45
20	4.70	7.08	6.04	5.94	1.19
30	3.53	3.80	4.00	3.77	0.23
50	3.44	2.60	2.96	3.00	0.42
70	2.67	2.76	2.72	2.71	0.04

Table A6 Effect of microwave exposure time on the Degree of swelling of (A) CM-chitin and (B) CM-chitosan films at 120°C

(A)

Time (min)	DS			Average	Standard deviation
5	114.45	94.90	91.02	100.12	12.55
10	23.72	25.37	24.05	24.38	0.87
20	5.49	4.63	5.32	5.14	0.45
30	4.44	3.64	4.82	4.30	0.60
50	2.71	2.29	2.52	2.50	0.21
70	2.10	2.32	2.23	2.21	0.11

(B)

Time (min)	DS			Average	Standard deviation
5	16.20	14.78	13.70	14.89	1.25
10	5.54	5.82	5.13	5.49	0.34
20	3.30	2.90	3.24	3.14	0.21
30	2.10	2.17	2.05	2.10	0.06
50	1.83	2.14	2.48	2.15	0.32
70	2.01	2.13	2.33	2.15	0.16

Table A7 Effect of microwave exposure temperature on the %Weight loss of (A) CM-chitin and (B) CM-chitosan films at experimental time was 50 min

(A)

Temperature (°C)	%WL			Average	Standard deviation
80	100	100	100	100	0
90	89.09	91.32	84.35	88.25	3.55
100	27.25	28.93	31.32	29.16	2.04
110	21.04	15.72	18.85	18.53	2.67
120	17.24	15.41	18.02	16.89	1.33
130	20.04	17.62	19.58	19.08	1.28
140	19.56	20.07	15.00	18.21	2.79

(B)

Temperature (°C)	%WL			Average	Standard deviation
80	100	100	100	100	0
90	55.60	52.10	57.21	54.97	2.61
100	30.34	29.53	33.33	31.06	2.00
110	21.73	21.69	20.86	21.42	0.49
120	13.14	18.75	13.26	15.05	3.20
130	19.97	18.34	17.47	18.59	1.26
140	18.13	17.39	17.89	17.80	0.37

Table A8 Effect of microwave exposure temperature on the Degree of swelling of (A) CM-chitin and (B) CM-chitosan films at experimental time was 50 min

(A)

Temperature (°C)	DS			Average	Standard deviation
90	123.11	140.90	142.88	135.63	10.88
100	10.75	11.50	12.6861	11.64	0.97
110	3.81	3.93	3.5045	3.74	0.21
120	2.71	2.29	2.5261	2.50	0.21
130	2.09	2.0832	2.0744	2.08	0.01
140	1.70	1.87	1.6490	1.73	0.11

(B)

Temperature (°C)	DS			Average	Standard deviation
90	36.56	31.07	39.33	35.65	4.20
100	5.30	5.35	5.93	5.52	0.35
110	3.44	2.60	2.96	3.00	0.42
120	1.83	2.14	2.48	2.15	0.32
130	2.21	2.19	2.27	2.22	0.04
140	1.85	1.93	1.52	1.76	0.21

APPENDIX B Effects of Autoclave Exposure Time on the %Weight Loss and Degree of Swelling of CM-chitin and CM-chtiosan at 120°C

Table B1 Effect of autoclave exposure time on the %Weight loss of (A) CM-chitin and (B) CM-chitosan films at 120°C

(A)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	100	100	100	100	0
10	100	100	100	100	0
20	100	100	100	100	0
30	100	100	100	100	0
50	36.23	31.07	33.42	33.57	2.58
70	22.36	25.15	20.70	22.73	2.24

(B)

Time (min)	%WL			Average	Standard deviation
0	100	100	100	100	0
5	100	100	100	100	0
10	100	100	100	100	0
20	100	100	100	100	0
30	41.96	59.91	50.85	50.90	8.97
50	23.90	30.62	21.73	25.41	4.63
70	21.57	19.96	20.26	20.59	0.85

Table B2 Effect of autoclave exposure time on the Degree of swelling of (A) CM-chitin and (B) CM-chitosan films at 120°C

(A)

Time (min)	DS			Average	Standard deviation
5	-	-	-	-	-
10	-	-	-	-	-
20	-	-	-	-	-
30	-	-	-	-	-
50	22.17	27.98	22.54	24.23	3.25
70	5.07	4.45	4.02	4.51	0.52

(B)

Time (min)	DS			Average	Standard deviation
5	-	-	-	-	-
10	-	-	-	-	-
20	-	-	-	-	-
30	43.97	49.84	39.00	44.27	5.42
50	3.66	5.90	2.96	4.17	1.53
70	2.47	2.85	2.95	2.75	0.25

APPENDIX C Viscosity-Average Molecular Weight of CM-chitin

Table C1 Viscosity-average molecular weight of CM-chitin

Conc. (g/100ml)	Time (sec.)				η_{sp}/C	$\ln(\eta_{rel})/C$
	X_1	X_2	X_3	Average		
0.0000	105.38	105.28	105.12	105.26		
0.00625	111.03	110.50	110.72	110.75	8.0798	7.8824
0.0125	121.75	121.93	122.19	121.95	8.4232	8.0086
0.0250	147.84	147.75	147.28	147.62	8.6853	7.8599
0.0500	210.03	209.69	209.78	209.83	9.4657	7.7499
0.1000	105.38	105.28	105.12	105.26	10.9415	7.3915

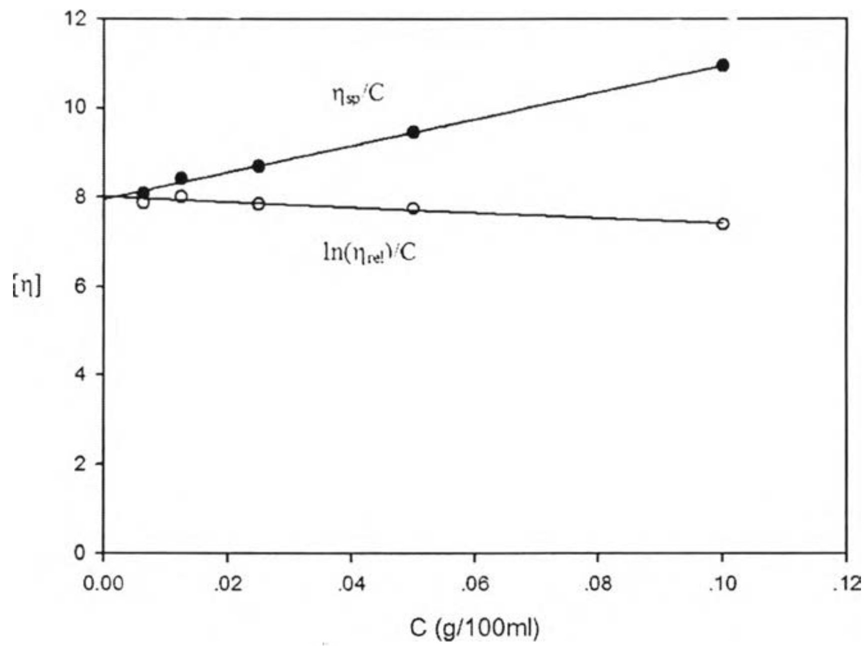

Figure C1 η_{sp}/C and $\ln(\eta_{rel})/C$ against concentration of CM-chitin solution.

The viscosity-average molecular weight of CM-chitin was determined based on Mark-Houwink equation. The K and a values were according to Kaneko *et al.* (1982).

$$[\eta] = 7.92 \times 10^{-5} M^{1}$$

Where [η] = intrinsic viscosity

M = Viscosity-average molecular weight

Interception: [η] of CM-chitin was 7.98

From calculation; M = 100,757 Da

The viscosity-average molecular weight of CM-chitin obtained from calculation was 100 kDa.

APPENDIX D Cytotoxicity of Microwave-Treated CM-chitin and CM-chitosan

Table D1 Direct cytotoxicity: a number of living cell after polymer films were deposited on confluent cell cultures for (A) 24 h and (B) 48 h

(A)

Types of polymers	Polystyrene	Chitosan	CM-chitosan	Chitin	CM-chitin
Number of cells (% of control)	97.81	88.76	44.85	83.30	97.03
	102.95	84.68	63.41	93.16	111.83
	99.31	86.90	67.81	91.15	91.86
	100	90.99	92.35	104.25	90.20
	99.45	97.77	86.69	100.63	98.64
	100.00	97.45	68.92	102.31	104.22
Average	100	91.09	70.67	95.79	98.96
Standard deviation	1.71	5.46	17.06	8.01	8.04

(B)

Types of polymers	Polystyrene	Chitosan	CM-chitosan	Chitin	CM-chitin
Number of cells (% of control)	98.39	65.33	18.80	67.54	71.89
	103.39	76.21	21.93	78.79	83.86
	98.20	81.68	39.01	83.01	95.03
	100.78	93.19	43.41	97.25	88.78
	97.83	100.90	48.94	101.32	87.80
	101.44	97.25	55.94	94.87	96.27
Average	100	85.76	38.00	87.13	87.27
Standard deviation	2.22	13.72	14.82	12.91	8.85

Table D2 Direct cytotoxicity: Total protein of cells after polymer films were deposited on confluent cell cultures for (A) 24 h and (B) 48 h

(A)

Types of polymers	Polystyrene	Chitosan	CM-chitosan	Chitin	CM-chitin
Total protein of cells (%)	95.86	105.43	102.23	80.14	121.88
	90.34	108.80	101.25	90.35	107.05
	113.79	110.49	105.15	95.07	101.29
Average	100	108.24	102.88	88.52	110.07
Standard deviation	12.25	2.57	2.028	7.63	10.62

(B)

Types of polymers	Polystyrene	Chitosan	CM-chitosan	Chitin	CM-chitin
Total protein of cells (%)	90.48	117.88	93.62	102.10	126.76
	116.24	118.72	137.97	114.55	136.58
	93.27	115.37	105.12	108.74	138.37
Average	100	117.32	112.24	108.46	133.90
Standard deviation	14.13	1.74	23.01	6.23	6.24

Table D3 Indirect cytotoxicity: a number of cells cultured in extraction medium at given concentration of (A) microwave-treated CM-chitin and (B) microwave-treated CM-chitosan

(A)

Concentration (mg/ml)	0.0156	0.03125	0.0625	0.125	0.25	0.5	1	2
Number of cells (% of control)	94.91	89.20	82.42	87.68	95.70	83.50	90.84	87.79
	97.68	85.31	97.34	86.83	90.62	88.24	88.41	85.81
	98.70	80.56	87.85	84.29	94.46	104.8	92.99	87.28
	107.34	82.59	78.81	79.83	98.81	94.63	92.09	76.38
	100	90.33	84.57	91.46	101.9	106.3	92.20	80.62
	90.67	83.05	81.75	90.33	95.19	99.94	86.15	92.76
	98.19	81.58	88.53	80.84	80.84	86.77	87.06	85.14
	83.78	88.41	83.72	74.74	86.32	93.89	92.25	85.31
Average	96.44	85.14	85.64	84.51	92.95	93.89	90.28	85.14
Standard deviation	6.68	3.58	5.48	5.48	6.57	8.53	2.55	5.54

(B)

Concentration (mg/ml)	0.0156	0.03125	0.0625	0.125	0.25	0.5	1	2
Number of cells (% of control)	92.57	90.09	93.63	102.0	90.62	89.50	80.83	75.23
	112.7	95.22	94.51	87.08	89.56	97.81	78.47	69.98
	98.11	99.29	98.05	103.2	106.5	101.0	87.32	69.92
	99.41	93.92	93.04	104.2	101.4	95.99	84.19	70.45
	93.04	113.7	93.57	107.9	96.81	94.81	84.90	75.23
	110.9	98.64	94.69	92.68	90.50	96.69	83.31	69.98
	84.02	92.51	85.96	88.32	89.62	90.97	84.31	69.92
	91.33	94.92	94.98	91.86	87.73	87.26	84.43	70.45
Average	97.81	97.28	93.57	97.16	94.10	94.28	83.49	71.40
Standard deviation	2.59	2.71	4.59	6.78	8.07	3.41	7.31	9.84

APPENDIX E Adhesicn of Fibroblasts on Microwave-Treated CM-chitin and CM-chitosan Films

Table E1 Adhesion of fibroblasts on the chitosan, chitin, microwave-treated CM-chitin, and microwave-treated CM-chitosan for (A) 1 h and (B) 2 h

(A)

Types of polymers	Chitosan	CM-chitosan	Chitin	CM-chitin
Number of cells (%)	87.88	17.88	89.85	7.18
	98.02	18.45	152.25	11.83
	114.08	15.49	114.22	7.60
	103.78	40.15	94.69	10.00
	98.48	43.93	117.42	12.00
	97.72	46.21	122.72	15.00
Average	100	30.35	115.19	10.60
Standard deviation	8.61	14.49	22.35	2.96

(B)

Types of polymers	Chitosan	CM-chitosan	Chitin	CM-chitin
Number of cells (%)	148.30	56.19	172.39	26.05
	173.80	28.30	166.61	32.81
	170.98	46.19	167.04	24.64
	181.81	76.51	227.27	24.24
	164.39	78.78	281.81	44.69
	181.81	73.48	286.36	53.78
Average	170.18	59.91	216.91	34.37
Standard deviation	12.62	20.08	56.81	12.26

CURRICULUM VITAE

Name: Mr. Panya Wongpanit

Date of Birth: January 15, 1981

Nationality: Thai

University Education:

1999-2003 Bachelor Degree of Engineering in Petrochemical and Polymeric Materials, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakhonpathom, Thailand.