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APPENDICES

Appendix A Structural and Physicochemical Characterization of Crude Biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from Petroleum-contaminated Soil

Table A1 Diameter of the clear zones on the oil surface obtained from oil displacement testing with the crude biosurfactant produced by *P. aeruginosa* SP4 and its fractions compared with Pluronic F-68 and SDS at a surfactant concentration of 20 mg/ml

Sample	Diameter of Clear Zone (cm)		
	I	II	III
PLURONIC F-68	3.0	2.6	2.4
SDS	11.2	12.0	11.6
BIOSURFACTANT	10.3	10.5	10.5
FRACTION A	10.5	10.6	10.5
FRACTION B	11.1	11.5	12.0
FRACTION C	13.0	13.6	13.0
FRACTION D	13.0	12.4	12.6
FRACTION E	10.6	11.0	10.0
FRACTION F	12.8	12.7	12.4

Table A2 Surface tension versus concentrations of the crude biosurfactant produced by *P. aeruginosa* SP4 compared with Pluronic F-68 and SDS

Concentration (mg/l)	Surface Tension (mN/m)								
	Pluronic F-68			SDS			Biosurfactant		
	I	II	III	I	II	III	I	II	III
0.3	56.0	55.8	55.6	69.6	69.7	69.8	56.0	56.6	56.6
0.6	54.1	54.2	54.3	67.6	67.5	67.6	55.6	55.3	55.4
1.3	53.2	53.0	53.4	65.1	65.2	65.1	53.3	53.6	53.1
2.5	52.1	52.2	52.1	63.9	63.0	63.2	50.4	50.6	50.3
5	51.4	51.6	51.4	61.3	61.4	61.1	49.4	49.5	49.3
10	51.2	51.3	51.4	57.9	57.9	57.9	47.8	48.0	47.7
20	51.3	51.4	51.1	53.5	53.7	53.4	44.8	45.0	44.7
40	50.9	50.9	51.0	48.2	48.1	48.0	41.5	41.4	41.3
80	51.0	50.9	51.0	41.9	41.7	41.5	36.4	36.8	36.8
160	50.8	50.7	50.8	37.0	36.9	37.0	35.0	35.0	35.0
320	46.7	46.7	46.8	33.0	32.9	33.0	32.5	32.5	32.3
640	45.0	45.0	45.0	30.5	30.7	30.6	30.6	30.7	30.2
1,280	44.0	44.0	44.1	28.5	28.6	28.8	30.1	30.3	30.2
2,560	42.8	42.8	42.9	28.8	28.7	28.6	29.4	29.1	28.8

Table A3 Emulsification activity (E_{24}) of the crude biosurfactant produced by *P. aeruginosa* SP4 compared with Pluronic F-68 and SDS

Sample	Emulsification Activity (%)								
	Pluronic F-68			SDS			Biosurfactant		
	I	II	III	I	II	III	I	II	III
PENTANE	50.8	52.3	50.8	80.9	81.6	79.6	16.2	14.7	14.7
HEXANE	47.6	49.2	47.6	63.6	65.2	63.6	18.5	18.5	18.2
HEPTANE	51.6	53.1	50.0	87.8	86.7	86.7	13.4	13.4	12.2
TOLUENE	51.5	52.9	52.2	84.9	84.3	80.8	27.9	25.0	25.0
1-CHLORO BUTANE	75.8	73.0	75.8	76.1	77.6	74.6	14.7	11.8	13.2
PALM OIL	76.2	77.7	76.2	75.0	73.5	70.6	90.3	91.4	93.6
CRUDE OIL	54.6	52.7	50.9	56.5	54.8	53.2	57.9	59.7	56.1
SOYBEAN OIL	40.0	38.5	40.0	65.2	63.8	67.7	58.1	60.3	60.3
COCONUT OIL	49.2	47.6	49.2	74.6	73.1	74.6	54.6	54.2	54.7
OLIVE OIL	73.0	71.4	73.0	75.8	74.2	75.8	43.3	46.9	46.9

Table A4 Surface tension of the crude biosurfactant as compared with Pluronic F-68 and SDS after heat treatment at 30°C with different heating times

Time (min)	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
Pluronic F-68						
0	45.0	45.0	45.0	45.1	45.2	45.3
20	45.1	45.2	45.3	45.0	45.2	45.2
40	44.9	44.8	45.0	45.1	45.2	45.0
60	45.7	45.6	45.7	45.4	45.5	45.7
80	45.0	44.9	45.0	44.9	45.0	45.1
100	45.9	45.8	45.7	45.7	45.7	45.8
120	44.9	44.8	44.6	45.0	44.9	45.0
SDS						
0	28.6	28.6	28.7	28.9	28.7	28.8
20	28.4	28.3	28.4	28.6	28.5	28.6
40	28.3	28.4	28.2	28.6	28.4	28.6
60	28.5	28.4	28.5	28.4	28.5	28.6
80	28.1	28.2	28.1	28.3	28.3	28.3
100	28.5	28.6	28.7	28.5	28.5	28.7
120	28.2	28.3	28.1	28.3	28.2	28.1
Biosurfactant						
0	30.4	30.5	30.5	30.3	30.4	30.3
20	30.7	30.6	30.6	30.6	30.5	30.5
40	30.6	30.8	30.7	30.8	30.6	30.8
60	30.7	30.7	30.7	30.8	30.8	30.7
80	30.5	30.6	30.6	30.4	30.6	30.4
100	30.5	30.6	30.5	30.7	30.5	30.6
120	30.5	30.6	30.4	30.6	30.5	30.6

Table A5 Surface tension of the crude biosurfactant as compared with Pluronic F-68 and SDS after heat treatment at 50°C with different heating times

Time (min)	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
Pluronic F-68						
0	45.0	45.0	45.0	45.1	45.2	45.3
20	45.9	45.8	45.9	45.7	45.7	45.8
40	45.4	45.4	45.4	45.5	45.3	45.3
60	45.3	45.3	45.3	45.4	45.2	45.2
80	45.9	45.9	45.9	45.7	45.8	45.7
100	45.3	45.4	45.3	45.4	45.5	45.5
120	45.9	45.9	45.9	45.7	45.8	45.7
SDS						
0	28.6	28.6	28.7	28.9	28.7	28.8
20	28.6	28.5	28.7	28.5	28.5	28.7
40	28.6	28.9	28.7	28.6	28.6	28.9
60	28.4	28.7	28.6	28.5	28.9	28.8
80	28.5	28.4	28.4	28.3	28.2	28.1
100	28.3	28.2	28.1	28.5	28.4	28.2
120	28.6	28.1	28.2	28.3	28.5	28.6
Biosurfactant						
0	30.4	30.5	30.4	30.4	30.4	30.5
20	30.7	30.7	30.8	30.7	30.7	30.7
40	30.5	30.6	30.5	30.5	30.5	30.5
60	30.3	30.4	30.3	30.4	30.4	30.3
80	30.2	30.3	30.2	30.2	30.3	30.3
100	30.2	30.2	30.3	30.3	30.2	30.3
120	30.7	30.9	30.6	30.4	30.4	30.9

Table A6 Surface tension of the crude biosurfactant as compared with Pluronic F-68 and SDS after heat treatment at 70°C with different heating times

Time (min)	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
Pluronic F-68						
0	45.0	45.0	45.0	45.1	45.2	45.3
20	45.4	45.5	45.5	45.6	45.6	45.5
40	45.8	45.7	45.8	45.9	45.8	45.8
60	45.4	45.3	45.3	45.4	45.3	45.4
80	45.4	45.3	45.5	45.4	45.5	45.3
100	45.5	45.5	45.4	45.3	45.4	45.5
120	45.7	45.8	45.7	45.7	45.6	45.7
SDS						
0	28.6	28.6	28.7	28.9	28.7	28.8
20	28.5	28.6	28.8	28.5	28.6	28.7
40	28.5	28.6	28.5	28.5	28.6	28.8
60	28.4	28.5	28.3	28.6	28.8	28.6
80	28.5	28.6	28.5	28.6	28.5	28.6
100	28.2	28.3	28.3	28.4	28.6	28.6
120	28.2	28.1	28.2	27.9	28.1	28.3
Biosurfactant						
0	30.4	30.5	30.4	30.3	30.4	30.4
20	30.4	30.4	30.4	30.4	30.5	30.3
40	30.4	30.6	30.5	30.5	30.5	30.4
60	30.6	30.6	30.7	30.7	30.5	30.5
80	30.9	30.8	30.8	30.8	30.8	30.9
100	30.8	30.9	30.8	30.6	30.8	30.6
120	30.4	30.6	30.3	30.3	30.5	30.3

Table A7 Surface tension of the crude biosurfactant as compared with Pluronis F-68 and SDS after heat treatment at 90°C with different heating times

Time (min)	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
Pluronic F-68						
0	45.0	45.0	45.0	45.1	45.2	45.3
20	45.0	45.0	45.1	45.0	45.1	45.1
40	44.9	44.9	45.0	44.9	45.0	45.0
60	45.2	45.3	45.2	45.3	45.4	45.3
80	45.4	45.4	45.4	45.5	45.6	45.5
100	45.6	45.6	45.7	45.8	45.7	45.5
120	45.4	45.4	45.5	45.4	45.6	45.3
SDS						
0	28.6	28.6	28.7	28.9	28.7	28.8
20	28.2	28.3	28.6	28.2	28.6	28.3
40	28.6	28.7	28.8	28.8	28.8	28.9
60	28.9	28.8	28.6	28.9	28.7	28.8
80	28.6	28.8	28.5	28.6	28.8	28.8
100	28.6	28.7	28.6	28.6	28.7	28.8
120	28.8	28.8	29.0	28.9	29.0	28.9
Biosurfactant						
0	30.8	30.9	30.9	30.9	30.9	31.0
20	31.6	31.6	31.4	31.4	31.6	31.5
40	31.5	31.3	31.5	31.4	31.4	31.5
60	31.0	30.8	30.9	30.9	30.8	30.9
80	31.0	31.1	31.1	31.0	31.0	31.1
100	31.2	31.2	31.0	31.1	31.1	31.2
120	31.3	31.4	31.4	31.3	31.3	31.4

Table A8 Effect of autoclave treatment (15 minutes at 121°C) on surface tension of the crude biosurfactant as compared with Pluronic F-68 and SDS

Sample	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
Before Autoclaving						
PLURONIC F-68	45.0	45.0	45.0	45.1	45.2	45.3
SDS	28.6	28.6	28.7	28.9	28.7	28.8
BIOSURFACTANT	30.4	30.5	30.5	30.3	30.4	30.3
After Autoclaving						
PLURONIC F-68	46.5	46.5	46.4	46.3	46.3	46.5
SDS	35.1	34.8	34.8	34.9	35.0	35.1
BIOSURFACTANT	31.7	31.5	31.8	31.5	31.7	31.5

Table A9 Effect of pH on surface activity of Pluronis F-68

pH	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
3	46.2	46.4	46.5	46.5	46.6	46.2
4	47.0	47.0	47.1	46.8	46.9	47.0
5	46.0	46.1	46.1	46.1	46.1	46.0
6	46.0	45.9	45.9	46.1	46.1	46.0
7	45.0	45.0	45.0	45.1	45.2	45.0
8	44.4	44.5	44.5	44.4	44.4	44.4
9	43.2	43.2	43.3	43.5	43.5	43.2
10	43.8	43.8	43.9	43.9	43.8	43.8
11	43.9	43.9	44.0	43.8	43.9	43.9

Table A10 Effect of pH on surface activity of SDS

pH	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
3	29.2	29.2	29.3	29.4	29.3	29.2
4	29.4	29.6	29.6	29.5	29.6	29.4
5	29.7	29.6	29.6	29.7	29.8	29.7
6	29.5	29.5	29.5	29.6	29.6	29.5
7	29.6	29.7	29.7	29.7	29.8	29.6
8	29.4	29.6	29.6	29.5	29.6	29.4
9	29.5	29.6	29.7	29.6	29.7	29.5
10	29.7	29.7	29.8	29.7	29.8	29.7
11	29.8	29.8	29.9	29.8	29.9	29.8

Table A11 Effect of pH on surface activity of the crude biosurfactant produced by *P. aeruginosa* SP4

pH	Surface Tension (mN/m)					
	I	II	III	IV	V	VI
3	29.0	29.1	29.1	29.1	29.0	29.0
4	29.2	29.3	29.2	29.2	29.3	29.2
5	30.3	30.2	30.2	30.2	30.0	30.3
6	31.3	31.3	31.0	31.3	31.0	31.3
7	31.9	32.2	32.1	31.9	31.8	31.9
8	31.9	31.9	32.2	32.3	32.3	31.9
9	32.0	31.9	32.0	32.0	32.1	32.0
10	32.0	31.9	32.1	32.0	32.2	32.0
11	31.6	31.9	31.5	31.5	31.8	31.6

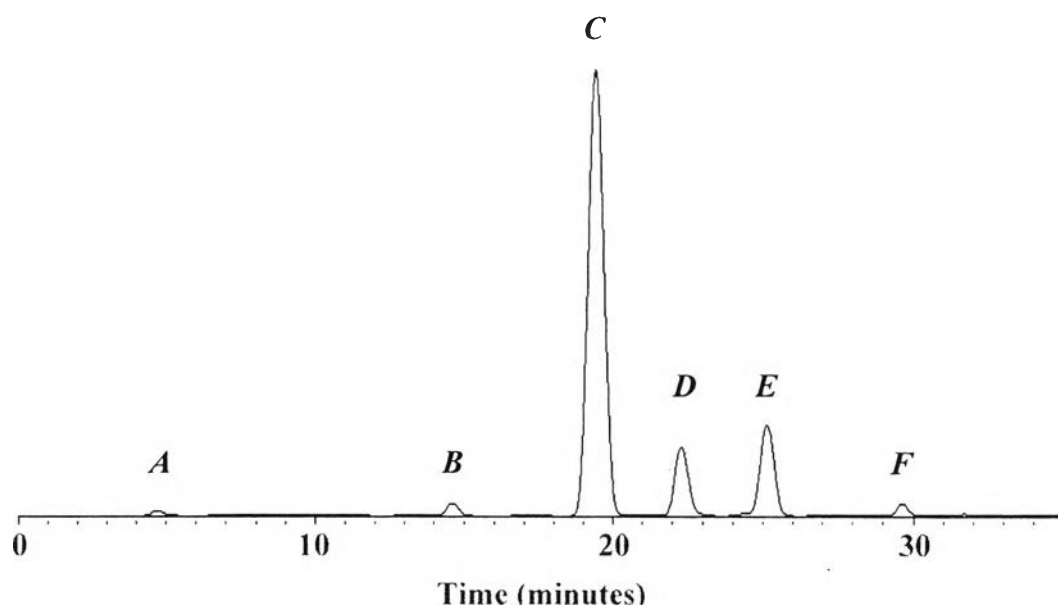


Figure A1 HPLC-ELSD chromatogram of the crude biosurfactant produced by *P. aeruginosa* SP4.

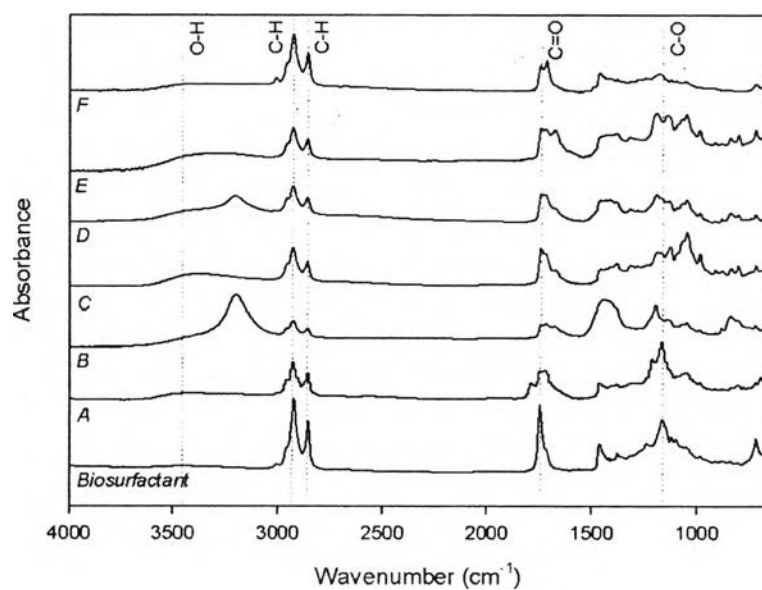


Figure A2 ATR-FTIR spectra of the crude biosurfactant produced by *P. aeruginosa* SP4 and its fractions.

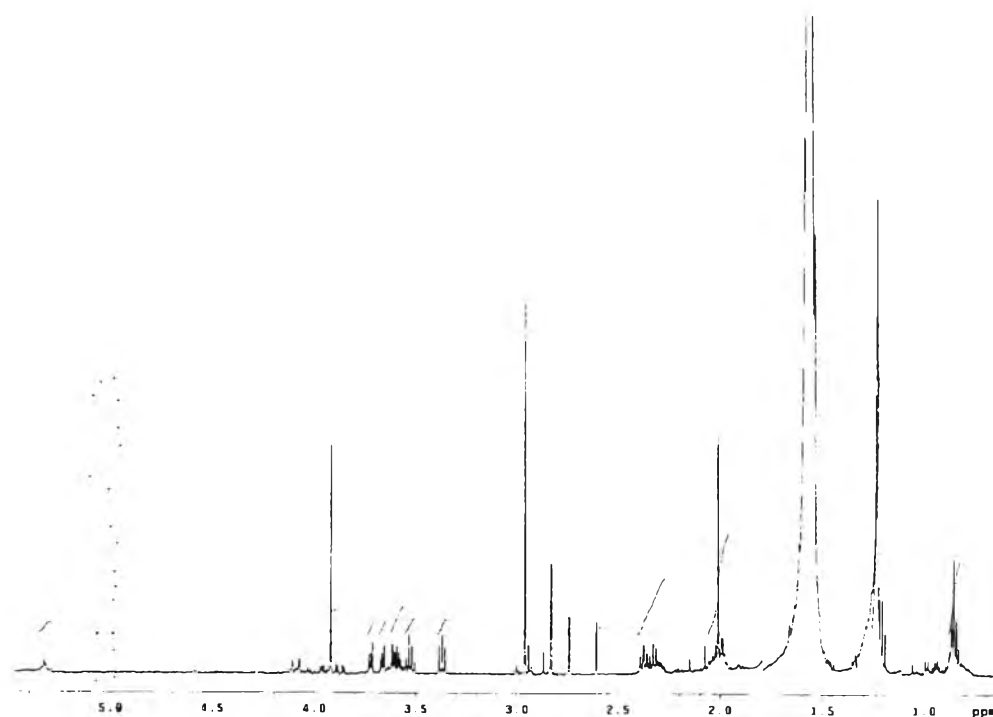


Figure A3 ¹H NMR spectrum of fraction A fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

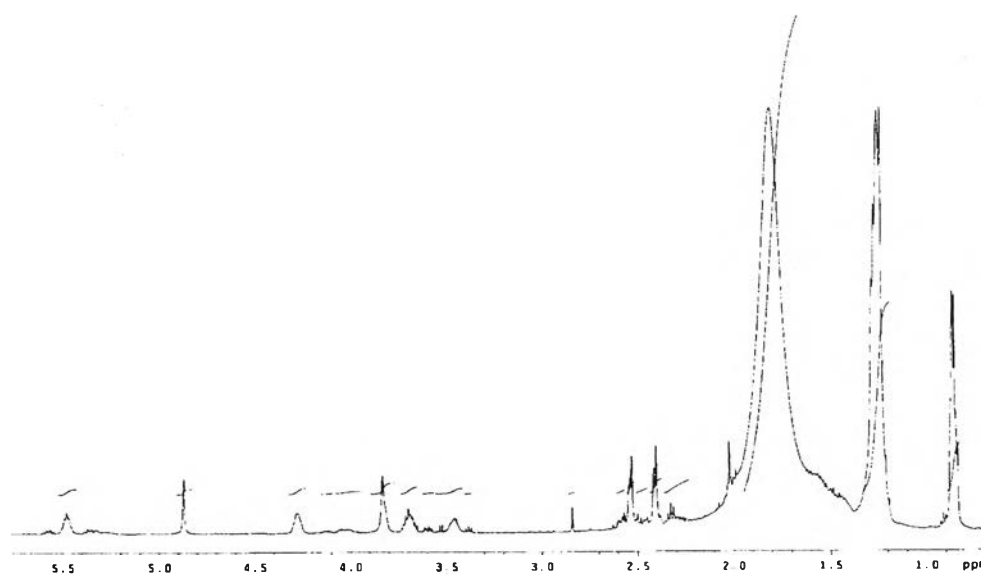


Figure A4 ¹H NMR spectrum of fraction B fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

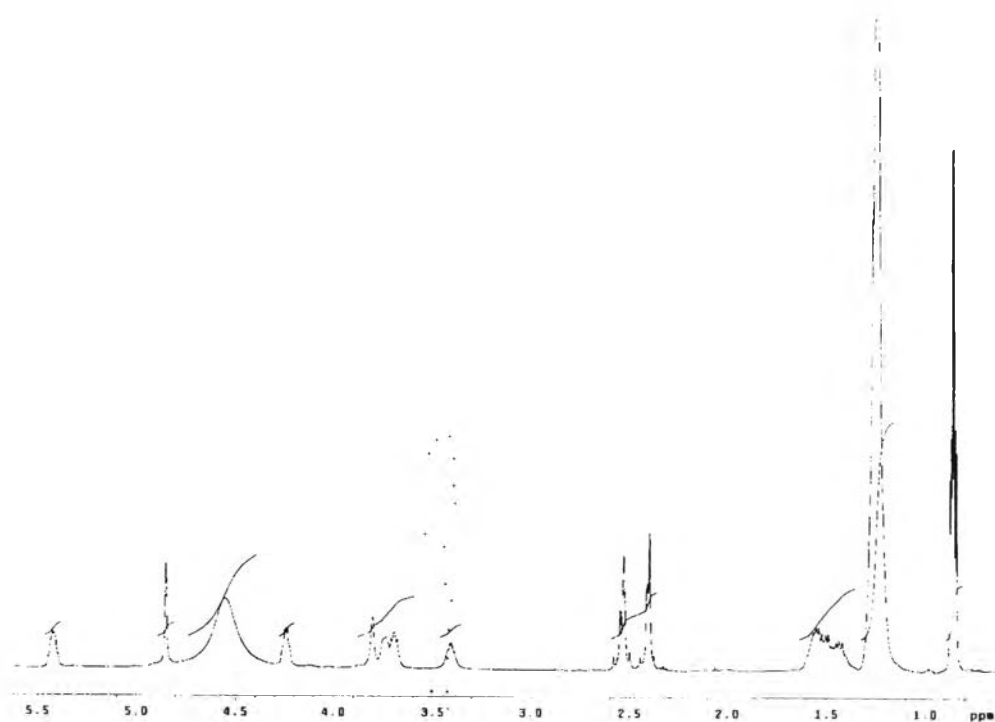


Figure A5 ¹H NMR spectrum of fraction C fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

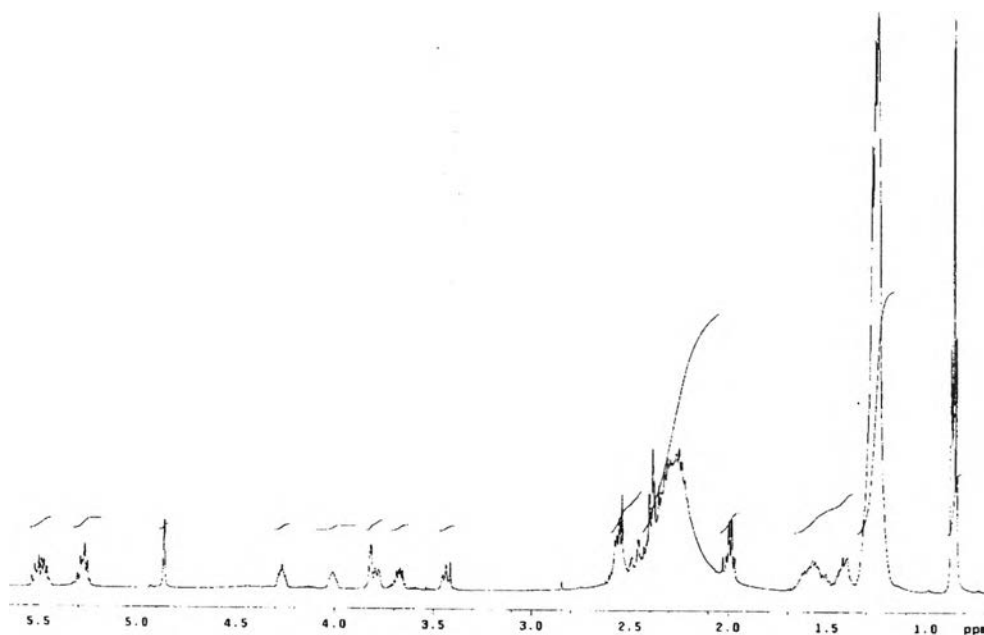


Figure A6 ¹H NMR spectrum of fraction D fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

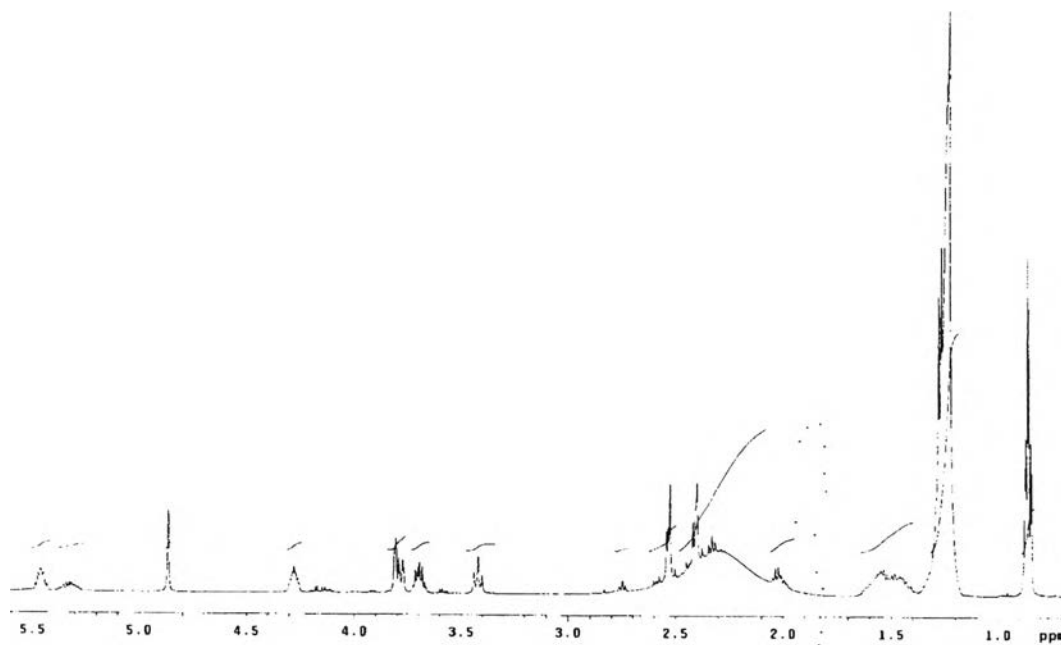


Figure A7 ¹H NMR spectrum of fraction E fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

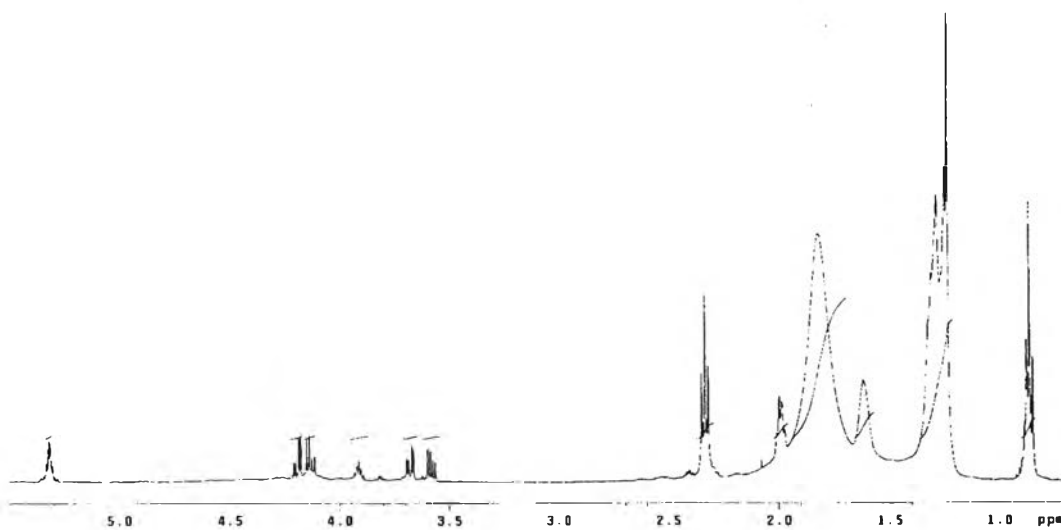


Figure A8 ¹H NMR spectrum of fraction F fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

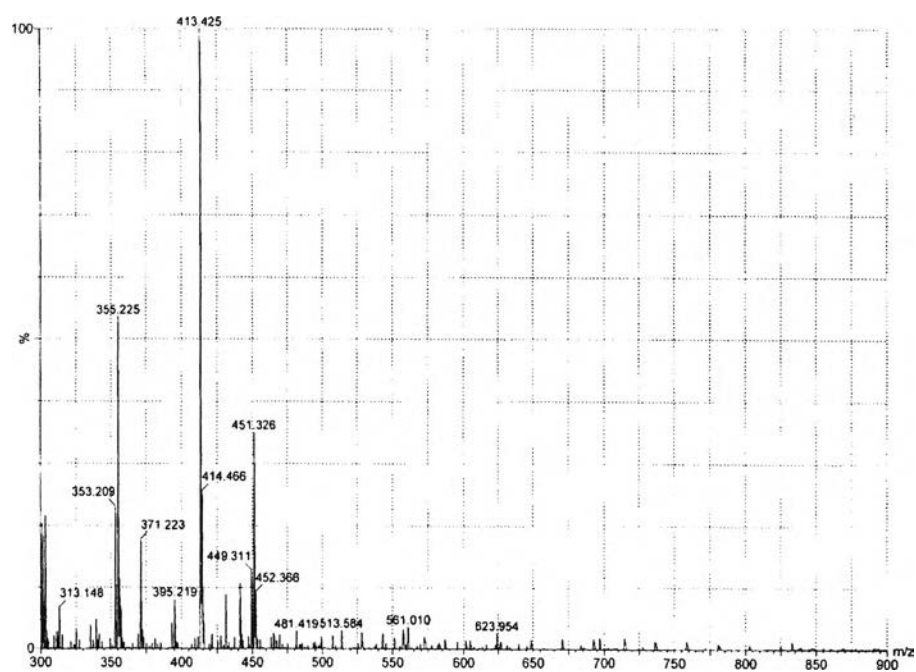


Figure A9 Mass spectrum of fraction A fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

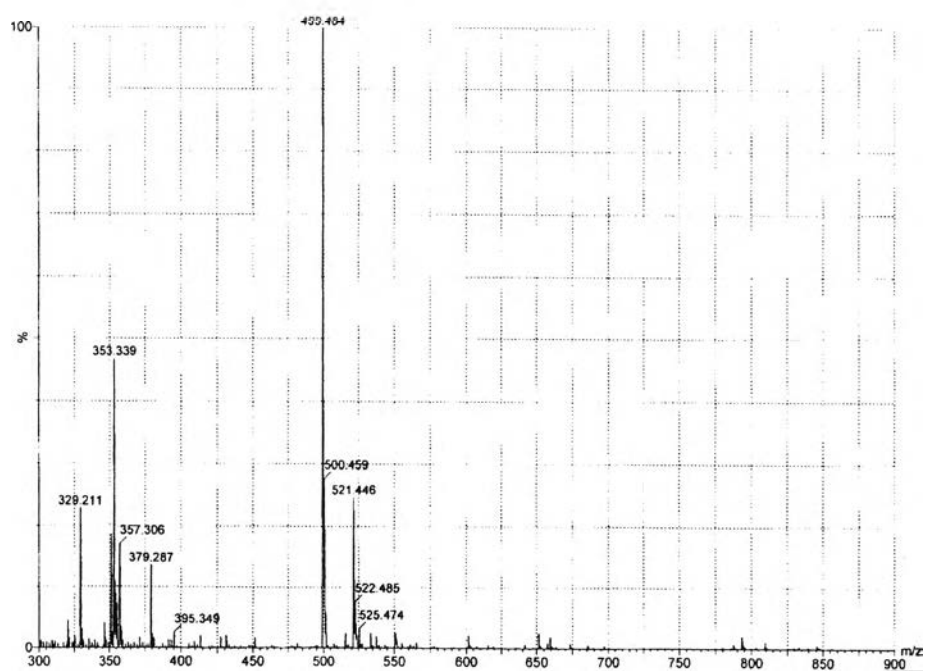


Figure A10 Mass spectrum of fraction B fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

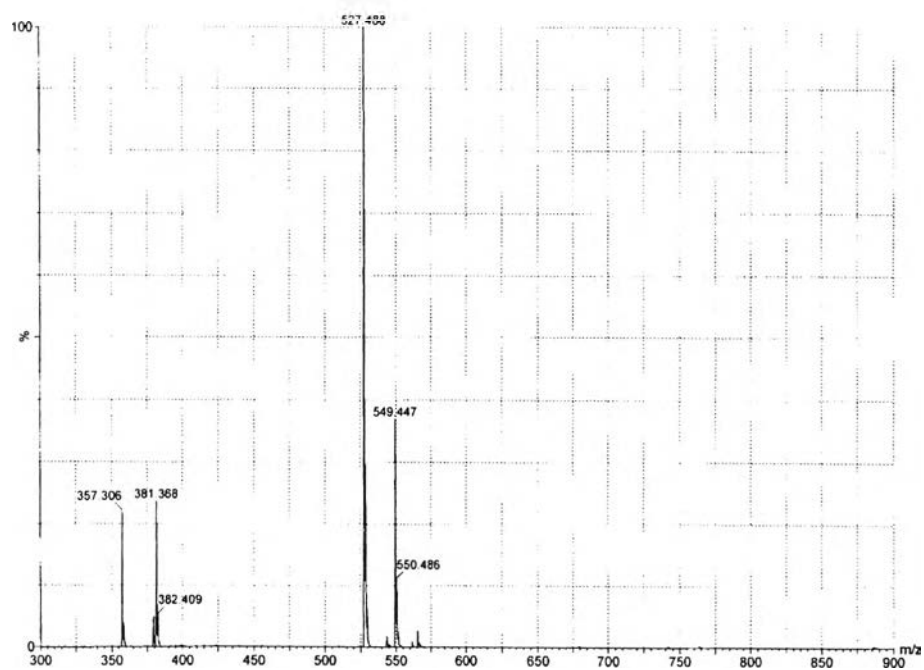


Figure A11 Mass spectrum of fraction C fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

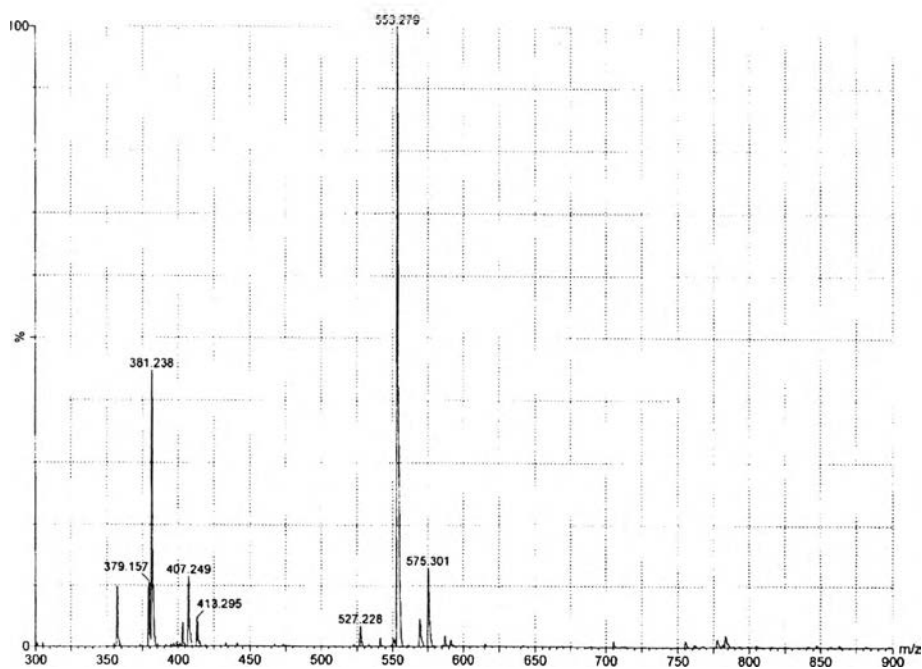


Figure A12 Mass spectrum of fraction D fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

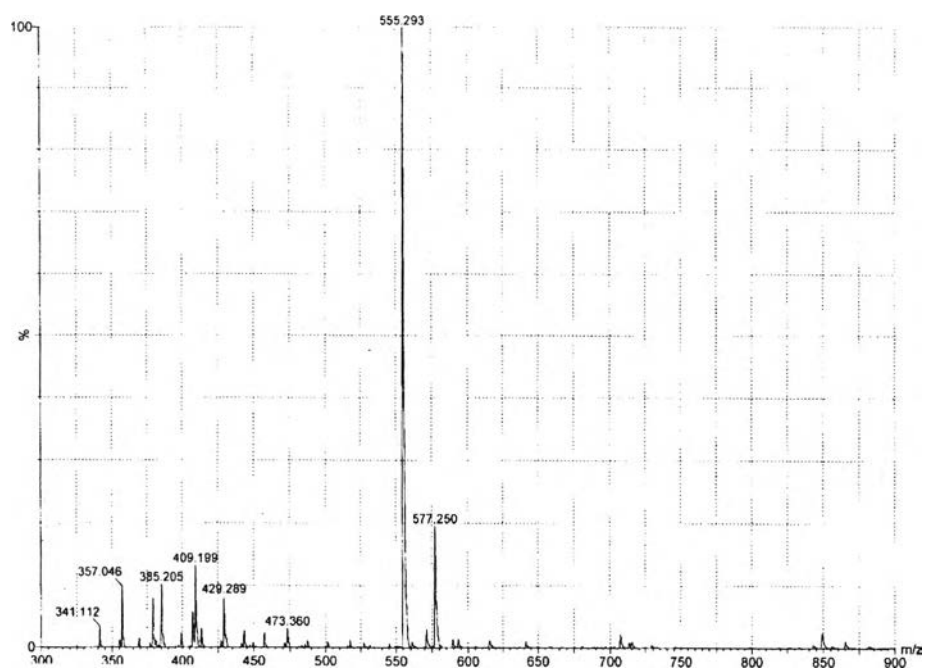


Figure A13 Mass spectrum of fraction E fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

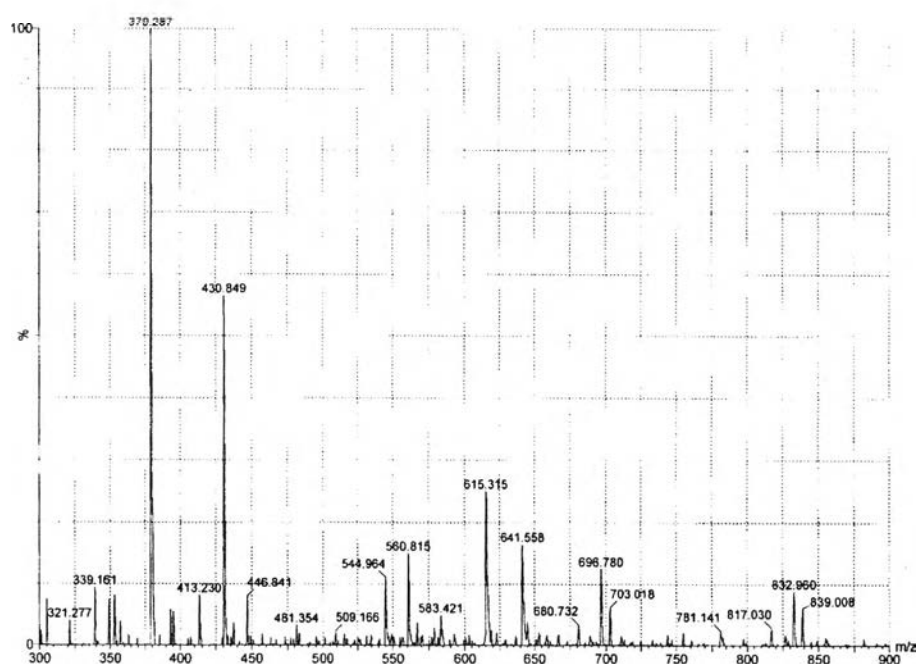


Figure A14 Mass spectrum of fraction F fractionated from the crude biosurfactant produced by *P. aeruginosa* SP4.

Appendix B Solution Properties and Vesicle Formation of Rhamnolipid Biosurfactants produced by *Pseudomonas aeruginosa* SP4

Table B1 Surface tension *versus* rhamnolipid biosurfactant concentration in a PBS solution

Concentration (mg/l)	Surface Tension (mN/m)		
	I	II	III
0.3	64.5	64.5	64.5
0.6	64.6	64.5	64.5
1.3	64.5	64.5	64.6
2.5	64.7	64.8	64.8
5	64.2	64.1	64.3
10	64.4	64.5	64.5
20	65.0	65.1	65.1
40	64.6	64.6	64.6
80	45.1	45.0	45.0
160	41.5	41.5	41.5
320	34.3	34.3	34.3
640	30.3	30.3	30.3
1,280	29.3	29.3	29.2
2,560	29.3	29.3	29.2

Table B2 Surface tension *versus* rhamnolipid biosurfactant concentration in a PBS solution containing NaCl

Concentration (mg/l)	Surface Tension (mN/m)								
	PBS + 0.1 M NaCl			PBS + 0.2 M NaCl			PBS + 0.4 M NaCl		
	I	II	III	I	II	III	I	II	III
0.3	64.8	64.8	64.8	65.7	65.7	65.9	62.3	62.3	62.3
0.6	64.1	64.1	64.2	66.5	66.5	66.5	62.3	62.3	62.3
1.3	65.5	65.5	65.6	66.5	66.5	66.4	62.7	62.8	62.8
2.5	65.7	65.6	65.6	65.5	65.5	65.5	62.8	62.8	62.9
5	64.6	64.6	64.5	65.5	65.6	65.7	62.4	62.4	62.4
10	64.2	64.3	64.2	64.3	64.3	64.4	62.7	62.8	62.7
20	64.5	64.6	64.5	64.2	64.1	64.1	62.9	62.9	62.9
40	64.0	63.9	63.9	65.0	65.0	65.0	62.4	62.4	62.4
80	59.3	59.4	59.2	42.3	42.3	42.3	43.6	43.7	43.6
160	39.8	39.8	39.8	38.8	38.7	38.7	35.6	35.6	35.6
320	34.7	34.8	34.8	33.7	33.8	33.7	32.2	32.2	32.2
640	29.9	29.8	29.8	30.3	30.3	30.3	30.8	30.8	30.8
1,280	29.9	29.8	29.8	28.6	28.6	28.6	29.5	29.5	29.4
2,560	29.9	29.4	29.5	28.8	28.7	28.4	28.9	28.9	28.9

Table B3 Surface tension *versus* rhamnolipid biosurfactant concentration in a PBS solution containing C₂H₅OH

Concentration (mg/l)	Surface Tension (mN/m)								
	PBS + 0.1 M C ₂ H ₅ OH			PBS + 0.2 M C ₂ H ₅ OH			PBS + 0.4 M C ₂ H ₅ OH		
	I	II	III	I	II	III	I	II	III
0.3	64.5	64.5	64.6	63.3	63.4	63.4	58.6	58.7	58.7
0.6	64.2	64.2	64.0	62.4	62.3	62.3	59.6	59.8	59.6
1.3	64.4	64.4	64.4	63.3	63.3	63.4	59.9	59.7	59.8
2.5	64.9	64.9	64.8	63.4	63.4	63.3	59.2	59.6	59.4
5	64.1	64.1	64.2	63.4	63.4	63.3	58.4	58.6	58.7
10	64.1	64.2	64.2	63.4	63.3	63.3	57.3	57.5	57.5
20	64.8	64.9	64.8	63.4	63.4	63.6	57.9	58.0	57.8
40	64.3	64.3	64.3	63.9	63.8	63.9	58.8	58.9	58.6
80	43.8	43.9	43.8	42.6	42.6	42.4	58.7	58.8	58.9
160	41.9	41.9	41.8	41.6	41.8	41.8	39.1	39.1	39.2
320	39.9	39.8	39.9	39.1	39.1	39.3	35.0	35.0	35.0
640	29.8	29.8	29.9	34.8	34.8	34.7	32.5	32.5	32.5
1,280	29.4	29.3	29.5	29.5	29.5	29.4	28.9	29.0	28.9
2,560	28.3	28.4	28.4	28.5	29.0	28.6	28.4	28.4	28.4

Table B4 Turbidity (absorbance at 600 nm) of the biosurfactant solution at different concentrations prepared in a PBS solution

Concentration (mg/l)	Turbidity (Absorbance at 600 nm)		
	I	II	III
0.31	0.12	0.12	0.12
0.63	0.17	0.16	0.16
1.25	0.15	0.14	0.14
2.50	0.16	0.16	0.17
5	0.15	0.15	0.14
10	0.18	0.17	0.18
20	0.18	0.18	0.18
40	0.19	0.19	0.19
80	0.19	0.20	0.20
160	0.42	0.42	0.43
320	0.62	0.61	0.62
640	1.07	1.07	1.07
1,280	2.38	2.37	2.38
2,560	3.67	3.67	3.67

Table B5 Turbidity (absorbance at 600 nm) of the biosurfactant solution at different concentrations prepared in a PBS solution containing NaCl

Concentration (mg/l)	Turbidity (Absorbance at 600 nm)								
	PBS + 0.1 M NaCl			PBS + 0.2 M NaCl			PBS + 0.4 M NaCl		
	I	II	III	I	II	III	I	II	III
0.31	0.19	0.20	0.20	0.22	0.22	0.22	0.20	0.20	0.20
0.63	0.17	0.17	0.17	0.16	0.17	0.17	0.17	0.18	0.17
1.25	0.17	0.18	0.17	0.17	0.17	0.16	0.20	0.20	0.20
2.50	0.17	0.17	0.17	0.17	0.17	0.17	0.19	0.19	0.18
5	0.20	0.20	0.19	0.16	0.17	0.17	0.16	0.17	0.17
10	0.20	0.19	0.19	0.22	0.21	0.22	0.22	0.23	0.23
20	0.24	0.23	0.24	0.24	0.23	0.23	0.19	0.18	0.18
40	0.28	0.29	0.29	0.29	0.29	0.29	0.20	0.20	0.21
80	0.35	0.35	0.35	0.33	0.33	0.33	0.20	0.20	0.20
160	0.42	0.42	0.42	0.39	0.39	0.38	0.33	0.33	0.33
320	0.61	0.61	0.60	0.53	0.52	0.52	0.37	0.38	0.38
640	0.96	0.96	0.95	0.76	0.76	0.76	0.64	0.65	0.65
1,280	1.81	1.81	1.81	1.34	1.33	1.33	1.17	1.18	1.17
2,560	3.51	3.51	3.49	2.73	2.73	2.74	2.36	2.35	2.36

Table B6 Turbidity (absorbance at 600 nm) of the biosurfactant solution at different concentrations prepared in a PBS solution containing C₂H₅OH

Concentration (mg/l)	Turbidity (Absorbance at 600 nm)								
	PBS + 0.1 M C ₂ H ₅ OH			PBS + 0.2 M C ₂ H ₅ OH			PBS + 0.4 M C ₂ H ₅ OH		
	I	II	III	I	II	III	I	II	III
0.31	0.17	0.18	0.17	0.17	0.18	0.19	0.17	0.16	0.16
0.63	0.20	0.20	0.20	0.17	0.16	0.17	0.17	0.17	0.18
1.25	0.23	0.23	0.23	0.17	0.17	0.17	0.18	0.19	0.18
2.50	0.20	0.20	0.20	0.18	0.19	0.19	0.17	0.17	0.18
5	0.21	0.20	0.21	0.19	0.20	0.19	0.18	0.19	0.18
10	0.22	0.22	0.22	0.20	0.20	0.21	0.15	0.14	0.16
20	0.26	0.26	0.26	0.27	0.27	0.28	0.18	0.17	0.17
40	0.26	0.26	0.26	0.26	0.27	0.26	0.18	0.19	0.18
80	0.36	0.37	0.36	0.35	0.34	0.35	0.19	0.19	0.20
160	0.40	0.39	0.39	0.36	0.34	0.35	0.27	0.28	0.26
320	0.59	0.59	0.59	0.41	0.42	0.43	0.34	0.34	0.35
640	0.95	0.95	0.95	0.60	0.60	0.60	0.50	0.50	0.51
1,280	1.50	1.50	1.50	1.74	1.75	1.73	1.50	1.49	1.50
2,560	2.99	2.99	2.99	2.53	2.54	2.54	2.25	2.25	2.24

Table B7 Electrical conductivity of the biosurfactant solution at different concentrations prepared in a PBS solution

Concentration (mg/l)	Electrical Conductivity (mS)		
	I	II	III
0.31	5.79	5.74	5.88
0.63	6.30	6.36	6.31
1.25	6.32	6.35	6.38
2.50	6.25	6.52	6.71
5	6.74	6.59	6.96
10	6.85	6.97	6.74
20	7.09	7.09	7.09
40	7.45	7.44	7.41
80	7.87	7.73	7.96
160	6.87	6.81	6.82
320	6.24	6.37	6.37
640	6.02	6.04	6.07
1,280	5.98	5.89	5.83
2,560	5.85	5.96	6.05

Table B8 Electrical conductivity of the biosurfactant solution at different concentrations prepared in a PBS solution containing NaCl

Concentration (mg/l)	Electrical Conductivity (mS)								
	PBS + 0.1 M NaCl			PBS + 0.2 M NaCl			PBS + 0.4 M NaCl		
	I	II	III	I	II	III	I	II	III
0.31	9.87	9.88	9.81	9.87	9.86	9.82	14.70	14.64	14.64
0.63	10.04	10.05	10.06	10.90	10.86	11.02	16.64	16.65	16.61
1.25	10.21	10.22	10.17	13.32	13.43	13.23	17.54	17.65	17.45
2.50	10.50	10.44	10.63	15.15	15.04	15.30	18.90	18.80	19.05
5	10.06	10.18	10.26	17.01	17.15	16.91	20.08	20.01	20.10
10	10.08	10.01	10.01	17.98	18.02	17.88	21.04	21.56	21.46
20	10.14	10.02	9.98	17.99	17.78	18.18	21.22	20.96	22.92
40	10.13	10.10	10.22	18.97	19.12	17.78	21.41	20.93	22.33
80	10.61	10.66	10.68	19.33	20.46	20.15	21.78	21.77	21.76
160	9.61	9.65	9.70	18.00	18.01	18.06	21.44	21.59	21.31
320	8.43	8.47	8.40	14.19	14.14	14.11	18.58	18.68	18.43
640	6.98	6.82	6.77	12.05	12.10	12.01	14.08	14.02	14.04
1,280	6.82	6.72	6.96	10.52	10.52	10.57	12.24	12.22	12.26
2,560	6.71	6.73	6.74	10.26	10.17	10.34	12.30	12.26	12.23

Table B9 Electrical conductivity of the biosurfactant solution at different concentrations prepared in a PBS solution containing C₂H₅OH

Concentration (mg/l)	Electrical Conductivity (μS)								
	PBS + 0.1 M C ₂ H ₅ OH			PBS + 0.2 M C ₂ H ₅ OH			PBS + 0.4 M C ₂ H ₅ OH		
	I	II	III	I	II	III	I	II	III
0.31	4.67	4.62	4.67	4.84	4.98	4.75	6.71	6.78	6.71
0.63	4.86	4.93	4.72	4.91	5.01	4.88	6.91	7.20	6.76
1.25	4.99	4.82	5.00	5.07	5.08	5.09	7.26	7.29	7.20
2.50	5.51	5.55	5.52	5.65	5.52	5.45	7.54	7.59	7.51
5	5.95	6.01	6.88	6.01	5.91	6.14	7.61	7.64	7.80
10	6.17	6.34	5.97	6.14	6.25	5.97	7.68	7.98	7.37
20	6.25	6.14	6.38	6.38	6.14	6.58	7.81	8.08	7.68
40	6.41	6.43	6.41	6.53	6.56	6.60	7.94	8.04	8.06
80	6.65	6.60	6.69	6.60	6.63	6.67	8.06	8.22	7.80
160	5.95	5.90	5.99	6.80	6.80	6.89	8.22	8.45	8.00
320	5.27	5.23	5.26	5.74	5.77	5.71	6.64	6.68	6.60
640	4.91	4.78	5.17	5.17	5.18	5.16	5.87	5.96	5.75
1,280	4.71	4.73	4.71	4.80	4.80	4.79	5.37	5.10	5.63
2,560	4.53	4.55	4.59	4.62	4.66	4.64	4.94	4.82	4.90

Table B10 Scattered light intensity of the biosurfactant solution at different concentrations prepared in a PBS solution, a PBS solution containing NaCl, and a PBS solution containing C₂H₅OH

Concentration (mg/l)	Average Count Rate (kcps)						
	PBS	0.1 M NaCl	0.2 M NaCl	0.4 M NaCl	0.1 M C ₂ H ₅ OH	0.2 M C ₂ H ₅ OH	0.4 M C ₂ H ₅ OH
0.3	24.4	73.4	11.6	33.4	11.9	38.1	17.5
0.6	26.6	82.2	11.5	32.2	22.7	38.8	15.3
1.3	28.6	89.2	13.5	32.3	18.0	38.6	28.1
2.5	32.8	91.2	21.8	32.5	20.6	58.0	29.3
5	37.2	97.9	21.5	35.7	33.7	90.3	32.5
10	42.4	100.2	20.0	42.7	46.8	153.1	61.6
20	65.5	131.9	46.9	35.8	11.9	38.1	17.5
40	183.4	434.2	123.9	151.6	22.7	38.8	15.3
80	468.6	462.5	670.4	549.9	703.1	532.8	469.4
160	465.6	423.4	499.7	499.4	458.8	1,100	1,000
320	471.6	425.0	482.1	497.4	440.9	468.7	489.0
640	478.0	428.8	480.4	488.8	439.0	445.5	420.3
1,280	475.6	422.5	477.0	488.0	443.2	440.0	425.5
2,560	470.3	421.2	475.5	488.4	445.2	441.4	428.4

Table B11 Contribution of the various-sized biosurfactant vesicles at different concentrations prepared in a PBS solution

Concentration (mg/l)	Contribution of Aggregate (%)	
	50-250 nm	>250 nm
160	59.1	40.9
320	37.5	62.5
640	20.1	79.9
1,280	0	100
2,560	0	100

Table B12 Contribution of the various-sized biosurfactant vesicles at different concentrations prepared in a PBS solution containing NaCl: (a) 50-250 nm and (b) >250 nm

Concentration (mg/l)	Contribution of Aggregate (%)					
	PBS + 0.1 M NaCl		PBS + 0.2 M NaCl		PBS + 0.4 M NaCl	
	(a)	(b)	(a)	(b)	(a)	(b)
160	42.5	57.5	24.6	75.4	10.0	90.0
320	50.6	49.4	25.2	74.8	50.6	49.5
640	57.0	43.0	30.5	69.5	46.8	53.2
1,280	0	100	0	100	54.0	46.0
2,560	0	100	0	100	42.6	57.4

Table B13 Contribution of the various-sized biosurfactant vesicles at different concentrations prepared in a PBS solution containing C₂H₅OH: (a) 50-250 nm and (b) >250 nm

Concentration (mg/l)	Contribution of Aggregate (%)					
	PBS + 0.1 M C ₂ H ₅ OH		PBS + 0.2 M C ₂ H ₅ OH		PBS + 0.4 M C ₂ H ₅ OH	
	(a)	(b)	(a)	(b)	(a)	(b)
160	20.9	79.1	66.4	33.6	37.1	62.9
320	4.2	95.8	39.0	61.0	100	0
640	0	100	21.9	78.1	100	0
1,280	0	100	0	100	100	0
2,560	0	100	0	100	100	0

Table B14 Encapsulation efficiency (*E*⁰%) of the biosurfactant vesicle formed in a PBS solution in the absence and presence of the additives at a biosurfactant concentration of 1,280 mg/l

Medium	Encapsulation Efficiency (%)		
	I	II	III
PBS	9.6	7.8	12.5
PBS + 0.1 M NaCl	6.4	8.2	7.7
PBS + 0.2 M NaCl	6.7	5.0	7.5
PBS + 0.4 M NaCl	5.8	6.1	6.2
PBS + 0.1 M C ₂ H ₅ OH	11.4	17.0	14.6
PBS + 0.2 M C ₂ H ₅ OH	13.2	18.3	17.1
PBS + 0.4 M C ₂ H ₅ OH	32.0	31.7	29.4

Appendix C Preparation and Characterization of Rhamnolipid Vesicles as Potential Nanocarrier Systems

Table C1 Solution turbidity (absorbance at 600 nm) of the rhamnolipid solution prepared in a PBS solution (pH 7.4) at a biosurfactant concentration of 0.13 wt.% at different cholesterol concentrations

Cholesterol Concentration (μM)	Turbidity (Absorbance at 600 nm)		
	I	II	III
0	0.99	0.99	0.99
25	0.99	0.99	0.99
50	0.99	0.99	0.99
100	0.71	0.71	0.71
200	0.65	0.65	0.65
400	0.60	0.60	0.60

Table C2 Zeta potential of the rhamnolipid solution prepared in a PBS solution (pH 7.4) at a biosurfactant concentration of 0.13 wt.% at different cholesterol concentrations

Cholesterol Concentration (μM)	Zeta Potential (mV)		
	I	II	III
0	-29.8	-29.9	-27.1
25	-28.6	-28.5	-28.5
50	-27.2	-25.4	-26.9
100	-17.2	-21.9	-20.4
200	-16.5	-16.1	-14.3
400	-13.2	-13.6	-13.2

Table C3 Size of the rhamnolipid vesicle prepared in a PBS solution (pH 7.4) at a biosurfactant concentration of 0.13 wt.% at different cholesterol concentrations obtained from the DLS measurement

Cholesterol Concentration (μM)	Hydrodynamic Diameter (nm)				
	I	II	III	IV	V
0	297.1	288.0	313.0	294.6	298.2
25	262.8	258.5	294.6	269.2	286.7
50	261.4	263.9	263.2	268.2	270.0
100	250.9	241.9	242.2	259.3	256.6
200	234.9	239.8	240.1	220.4	255.1
400	213.7	210.5	203.2	206.5	210.9

Table C4 Size of the rhamnolipid vesicle prepared in a PBS solution (pH 7.4) at a biosurfactant concentration of 0.13 wt.% at different cholesterol concentrations obtained from the TEM technique

Cholesterol Concentration (μM)	Vesicle Diameter (nm)							
	I	II	III	IV	V	VI	VII	VIII
0	813	829	811	808	782	723	923	780
25	634	663	638	656	608	699	702	712
50	554	547	501	508	515	498	494	488
100	443	449	462	494	395	361	354	514
200	399	372	411	414	420	409	404	415
400	339	300	289	346	337	353	365	340

Table C5 Encapsulation efficiency ($E\%$) of the rhamnolipid vesicles formed in a PBS solution (pH 7.4) at a biosurfactant concentration of 0.13 wt.% at various cholesterol concentrations and initial Sudan III concentrations

Cholesterol Concentration (μM)	Encapsulation Efficiency (%)								
	8.8 Mm Sudan III			17.5 μM Sudan III			35 μM Sudan III		
	I	II	III	I	II	III	I	II	III
0	46.8	45.9	45.1	20.5	19.5	19.6	6.6	6.8	7.5
25	68.9	67.0	70.5	33.2	31.8	33.2	16.0	17.7	19.9
50	70.0	78.2	80.8	35.0	35.2	32.8	21.3	22.5	23.3
100	91.1	82.5	90.8	54.6	59.8	57.5	23.6	22.3	21.7
200	65.0	65.7	66.5	39.0	36.6	37.2	10.1	10.6	11.0
400	60.1	50.0	46.9	26.2	27.8	24.8	11.3	12.8	11.1

Appendix D Surface-modified Polymeric Films by Rhamnolipid Biosurfactant from *Pseudomonas aeruginosa* SP4 for Biomedical Applications

Table D1 Adsorption isotherms of the rhamnolipid biosurfactant onto either silk fibroin or chitosan films from the SPR analysis

Concentration (mM)	Adsorbed Mass ($\mu\text{mol}/\text{m}^2$)							
	Silk Fibroin Film				Chitosan Film			
	I	II	III	IV	I	II	III	IV
0.03	0.16	0.26	0.07	0.08	0.13	0.15	0.12	0.16
0.06	0.25	0.13	0.18	0.08	0.24	0.19	0.16	0.24
0.12	0.79	0.21	0.43	0.43	0.25	0.19	0.14	0.44
0.24	1.25	0.65	0.98	0.96	0.60	0.56	0.51	0.35
0.49	3.16	2.47	2.47	2.37	0.74	0.75	0.74	0.72
0.97	3.66	4.52	4.03	4.11	2.25	2.17	1.80	1.70
1.95	4.16	4.69	4.04	4.66	2.31	2.33	2.69	1.98
3.90	4.58	4.43	4.59	4.17	2.39	2.33	2.50	2.47

(a) The adsorption of the biosurfactant onto the silk fibroin film:

From the SPR data, the adsorbed amount of the biosurfactant on the silk fibroin film was $4.5 \mu\text{mol}/\text{m}^2$.

$$\begin{aligned}
 4.5 \mu\text{mol}/\text{m}^2 &= 4.5 \times 10^{-6} \times 6.02 \times 10^{23} \text{ molecules}/\text{m}^2 \\
 &= 2.7 \times 10^{18} \text{ molecules}/\text{m}^2
 \end{aligned}$$

If the bilayer topography was assumed, one biosurfactant molecule should occupy $2 \times (2.7 \times 10^{18})^{-1} \text{ m}^2$.

$$\begin{aligned}
 2 \times (2.7 \times 10^{18})^{-1} \text{ m}^2 &= 2 \times (3.7 \times 10^{-19}) \text{ m}^2 \\
 &= 7.41 \times 10^{-19} \text{ m}^2 \\
 &= 74.1 \text{ \AA}^2
 \end{aligned}$$

Therefore, based on the assumption of bilayer topography, a surface area per molecule of 74.1 \AA^2 was obtained.

(b) The adsorption of the biosurfactant onto the chitosan film:

From the SPR data, the adsorbed amount of the biosurfactant on the chitosan film was $2.3 \mu\text{mol}/\text{m}^2$.

$$\begin{aligned} 2.3 \mu\text{mol}/\text{m}^2 &= 2.3 \times 10^{-6} \times 6.02 \times 10^{23} \text{ molecules}/\text{m}^2 \\ &= 1.4 \times 10^{18} \text{ molecules}/\text{m}^2 \end{aligned}$$

If the monolayer topography was assumed, one biosurfactant molecule should occupy $(1.4 \times 10^{18})^{-1} \text{ m}^2$.

$$\begin{aligned} (1.4 \times 10^{18})^{-1} \text{ m}^2 &= 7.14 \times 10^{-19} \text{ m}^2 \\ &= 71.4 \text{ \AA}^2 \end{aligned}$$

Therefore, based on the assumption of monolayer topography, a surface area per molecule of 71.4 \AA^2 was obtained.

Table D2 Adsorption isotherms of the rhamnolipid biosurfactant onto either silk fibroin or chitosan films from the QCM-D experiment

Concentration (mM)	Adsorbed Mass ($\mu\text{mol}/\text{m}^2$)					
	I	II	III	IV	V	VI
Silk Fibroin Film						
0.03	2.9	2.9	2.9	2.9	2.9	2.9
0.06	13.8	13.8	13.8	13.8	13.8	13.8
0.12	29.0	29.0	29.0	29.1	29.0	29.0
0.24	34.3	34.3	34.3	34.3	34.3	34.3
0.49	37.0	37.0	37.0	37.0	37.0	37.0
0.97	38.8	38.8	38.8	38.8	38.8	38.8
1.95	39.8	39.8	39.8	39.8	39.8	39.8
3.90	39.9	39.9	39.9	39.9	39.9	39.9
Chitosan Film						
0.03	7.1	7.1	7.1	7.1	7.1	7.1
0.06	25.4	25.4	25.4	25.4	25.4	25.4
0.12	50.7	50.7	50.7	50.8	51.2	51.7
0.24	96.7	96.8	96.8	96.9	96.8	96.8
0.49	112.5	112.5	112.5	112.5	112.5	112.6
0.97	127.4	127.4	127.4	127.4	127.4	127.4
1.95	135.1	135.1	135.1	135.1	135.1	135.1
3.90	137.5	137.5	137.5	137.5	137.4	137.4

Table D3 The changes in the third overtone of the resonance frequency from the QCM-D experiment as a function of the biosurfactant concentration

Concentration (mM)	Frequency (Hz)					
	I	II	III	IV	V	VI
Silk Fibroin Film						
0.03	-8.2	-8.2	-8.6	-8.6	-8.1	-8.1
0.06	-39.5	-39.5	-39.5	-39.5	-39.5	-39.5
0.12	-83.3	-83.3	-83.3	-83.3	-83.3	-83.3
0.24	-98.3	-98.3	-98.3	-98.3	-98.3	-98.3
0.49	-106.1	-106.1	-106.0	-106.0	-106.0	-106.0
0.97	-111.3	-111.3	-111.3	-111.3	-111.3	-111.3
1.95	-114.0	-114.0	-114.0	-114.0	-114.0	-114.0
3.90	-114.3	-114.3	-114.3	-114.2	-114.2	-114.2
Chitosan Film						
0.03	-20.3	-20.3	-20.4	-20.4	-20.4	-20.4
0.06	-72.7	-72.7	-72.7	-72.7	-72.7	-72.8
0.12	-145.2	-145.2	-145.3	-145.3	-145.3	-145.3
0.24	-277.1	-277.1	-277.1	-277.2	-277.2	-277.3
0.49	-322.1	-322.1	-322.1	-322.1	-322.2	-322.2
0.97	-365.1	-365.1	-365.2	-365.2	-365.2	-365.2
1.95	-387.2	-387.2	-387.2	-387.2	-387.2	-387.2
3.90	-394.3	-394.2	-394.1	-394.0	-393.9	-393.8

Table D4 The changes in the third overtone of the dissipation from the QCM-D experiment as a function of the biosurfactant concentration

Concentration (mM)	Dissipation (10^{-6})					
	I	II	III	IV	V	VI
Silk Fibroin Film						
0.03	2.2	2.2	2.2	2.2	2.2	2.2
0.06	8.9	8.9	8.9	8.9	8.9	8.9
0.12	16.3	16.2	16.2	16.2	16.3	16.3
0.24	17.1	17.1	17.1	17.1	17.1	17.1
0.49	17.7	17.7	17.7	17.7	17.7	17.7
0.97	18.0	18.0	18.0	18.0	18.0	18.0
1.95	19.0	19.0	19.0	19.0	19.0	19.0
3.90	19.1	19.1	19.0	19.0	19.0	19.0
Chitosan Film						
0.03	4.2	4.2	4.2	4.2	4.2	4.2
0.06	13.6	13.6	13.6	13.6	13.6	13.6
0.12	25.0	25.0	25.0	25.0	25.0	25.0
0.24	28.1	27.3	26.5	25.7	24.9	24.2
0.49	27.2	27.3	27.3	27.3	27.3	27.3
0.97	30.2	30.2	30.2	30.2	30.2	30.2
1.95	35.6	35.6	35.6	35.6	35.6	35.6
3.90	37.9	37.9	37.8	37.8	37.8	37.7

Table D5 Water content percentages within the adsorbed layers of either silk fibroin or chitosan films as a function of the biosurfactant concentration

Concentration (mM)	Water Content (%)	
	Silk Fibroin Film	Chitosan Film
0.03	95.1	98.0
0.06	98.8	99.2
0.12	98.4	99.5
0.24	97.2	99.5
0.49	92.9	99.3
0.97	89.5	98.4
1.95	89.0	98.3
3.90	88.8	98.2

Table D6 Water contact angles of either silk fibroin or chitosan films as a function of the biosurfactant concentration

Concentration (mM)	Water Contact Angle (°)					
	Silk Fibroin Film			Chitosan Film		
	I	II	III	I	II	III
0	49.6	50.8	50.6	71.0	71.4	71.2
0.03	59.4	57.5	54.2	72.1	71.9	72.9
0.06	60.0	59.6	58.1	73.6	73.4	74.0
0.12	62.7	62.7	62.0	75.6	75.0	75.8
0.24	67.7	67.6	66.3	76.8	79.8	78.9
0.49	69.9	72.0	73.5	82.6	83.2	82.7
0.97	76.3	72.7	76.7	83.2	85.3	83.2
1.95	76.2	76.4	77.5	85.1	83.8	83.6
3.90	76.7	77.5	76.5	85.1	83.5	86.5

Table D7 Surface roughness (R_a) of unmodified silk fibroin, surface-modified silk fibroin films, unmodified chitosan, and surface-modified chitosan

Sample	Surface Roughness (nm)			
	I	II	III	IV
UNMODIFIED-SILK FIBROIN FILM	2.43	3.06	1.33	1.01
SURFACE-MODIFIED SILK FIBROIN FILM	4.34	3.51	1.66	1.26
UNMODIFIED-CHITOSAN FILM	1.57	1.25	1.50	1.18
SURFACE-MODIFIED CHITOSAN FILM	1.63	1.28	1.98	1.56

Table D8 Growth of human dermal fibroblasts on unmodified and surface-modified polymeric films

Sample	Fluorescent Intensity		
	I	II	III
Incubation Time of 1 day			
CONTROL	646	649	730
UNMODIFIED-SILK FIBROIN FILM	729	719	774
SURFACE-MODIFIED SILK FIBROIN FILM	728	760	708
UNMODIFIED-CHITOSAN FILM	689	744	717
SURFACE-MODIFIED CHITOSAN FILM	675	669	647
Incubation Time of 3 days			
CONTROL	1129	1131	1222
UNMODIFIED-SILK FIBROIN FILM	1470	1403	1585
SURFACE-MODIFIED SILK FIBROIN FILM	1357	1476	1240
UNMODIFIED-CHITOSAN FILM	1103	1190	1157
SURFACE-MODIFIED CHITOSAN FILM	1106	1236	1178
Incubation Time of 5 days			
CONTROL	2424	2541	2462
UNMODIFIED-SILK FIBROIN FILM	2800	2794	2947
SURFACE-MODIFIED SILK FIBROIN FILM	2420	2476	2669
UNMODIFIED-CHITOSAN FILM	2199	2359	2137
SURFACE-MODIFIED CHITOSAN FILM	2397	2485	2411

Table D9 Growth of human dermal keratinocytes on unmodified and surface-modified polymeric films

Sample	Optical Density (Absorbance at 570 nm)			
	1 day		7 days	
	I	II	I	II
CONTROL	0.29	0.31	0.69	0.74
UNMODIFIED-SILK FIBROIN FILM	0.27	0.28	0.62	0.63
SURFACE-MODIFIED SILK FIBROIN FILM	0.25	0.27	0.62	0.67
UNMODIFIED-CHITOSAN FILM	0.20	0.22	0.55	0.52
SURFACE-MODIFIED CHITOSAN FILM	0.25	0.24	0.60	0.60

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