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APPENDICES

APPENDIX A

SIZE DISTRIBUTION OF FIBER DIAMETER OF CHITOSAN/PVA COMPOSITE

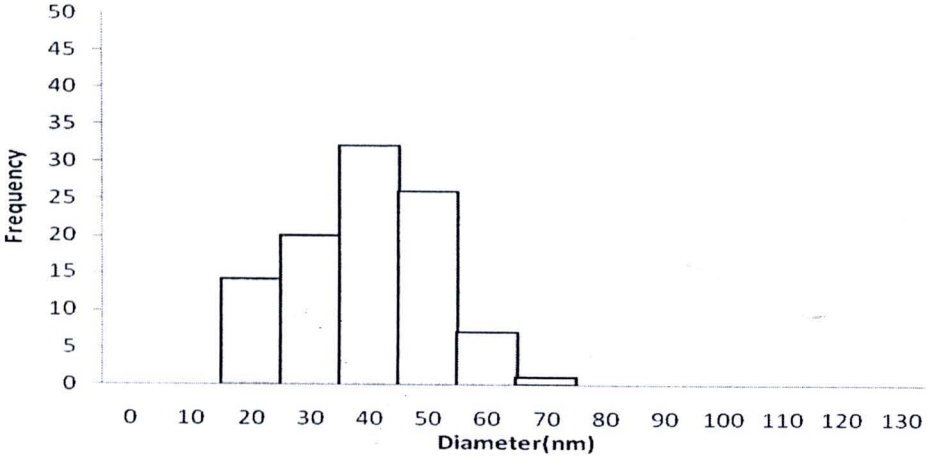

Figure A-1 Size distribution of fiber diameter of chitosan/PVA composite at 0.004 %w/v chitosan content, MW of chitosan of 100 kDa.

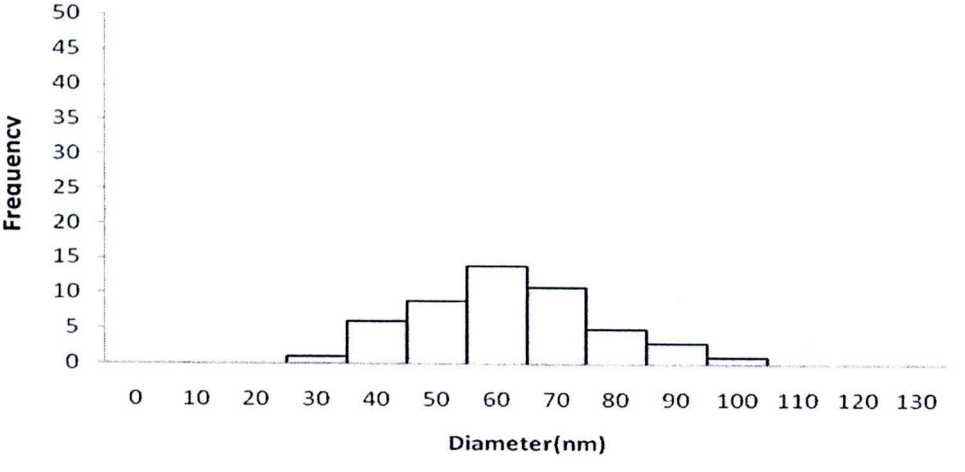

Figure A-2 Size distribution of fiber diameter of chitosan/PVA composite at 0.008 %w/v chitosan content, MW of chitosan of 100 kDa.

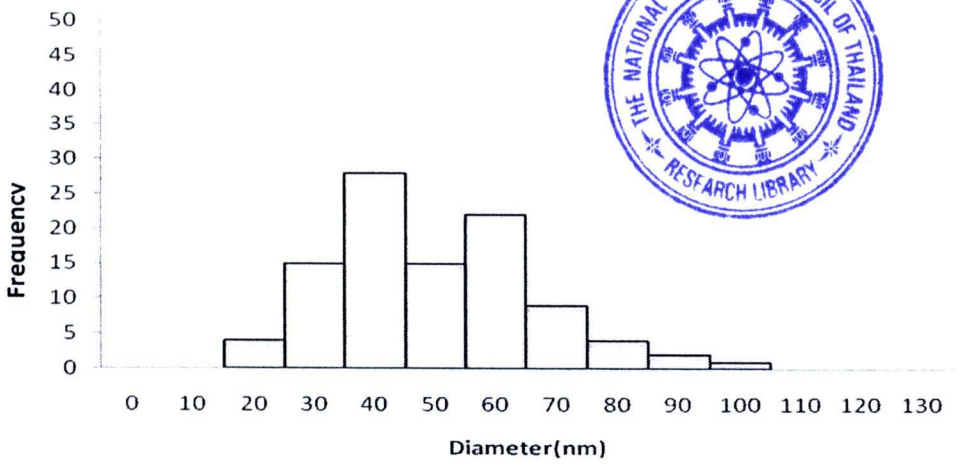

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Figure A-3 Size distribution of fiber diameter of chitosan/PVA composite at 0.012 %w/v chitosan content, MW of chitosan of 100 kDa.

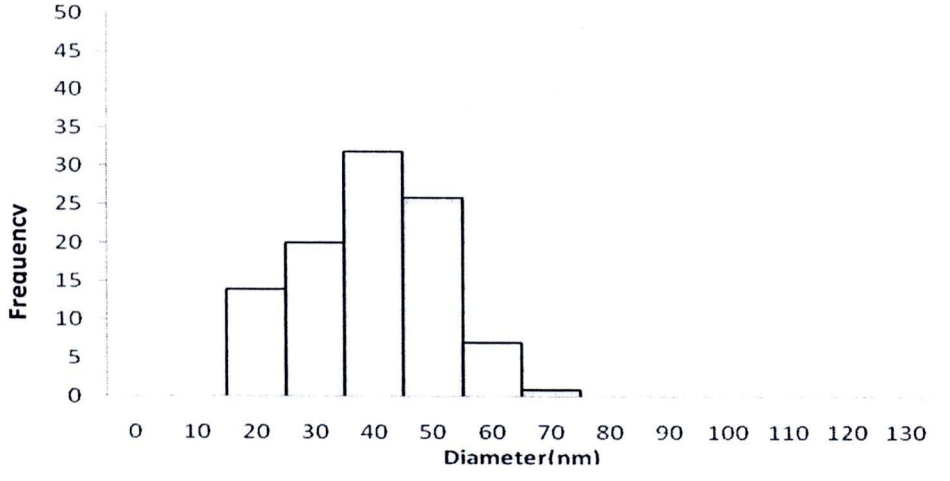


Figure A-4 Size distribution of fiber diameter of chitosan/PVA composite at 0.016 %w/v chitosan content, MW of chitosan of 100 kDa.

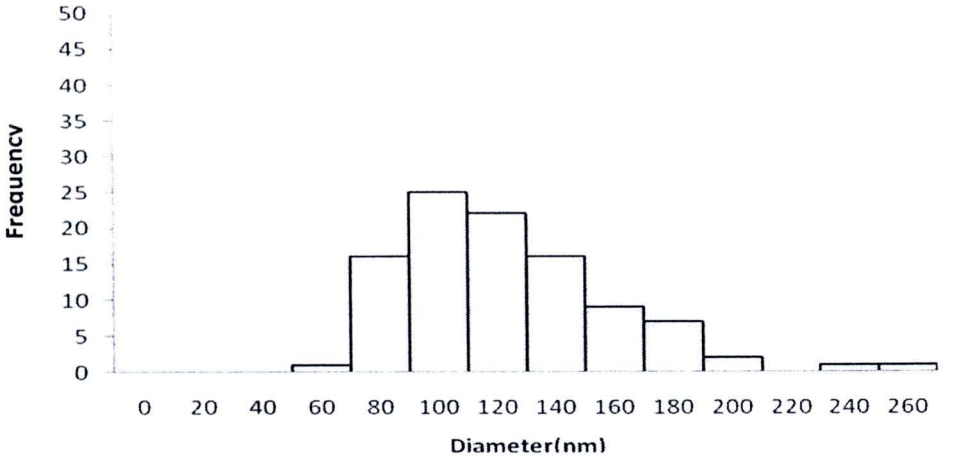


Figure A-5 Size distribution of fiber diameter of chitosan/PVA composite at 0.003 %w/v chitosan content, MW of chitosan of 400 kDa.

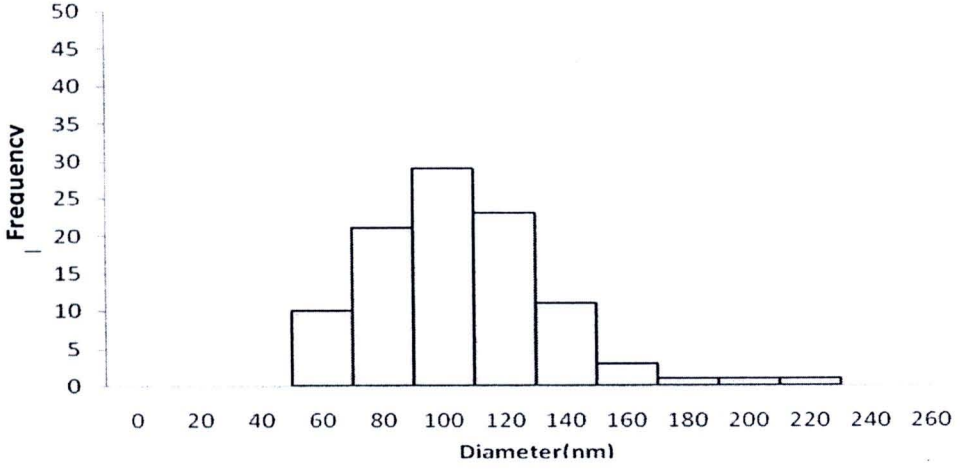


Figure A-6 Size distribution of fiber diameter of chitosan/PVA composite at 0.006%w/v chitosan content, MW of chitosan of 400 kDa.

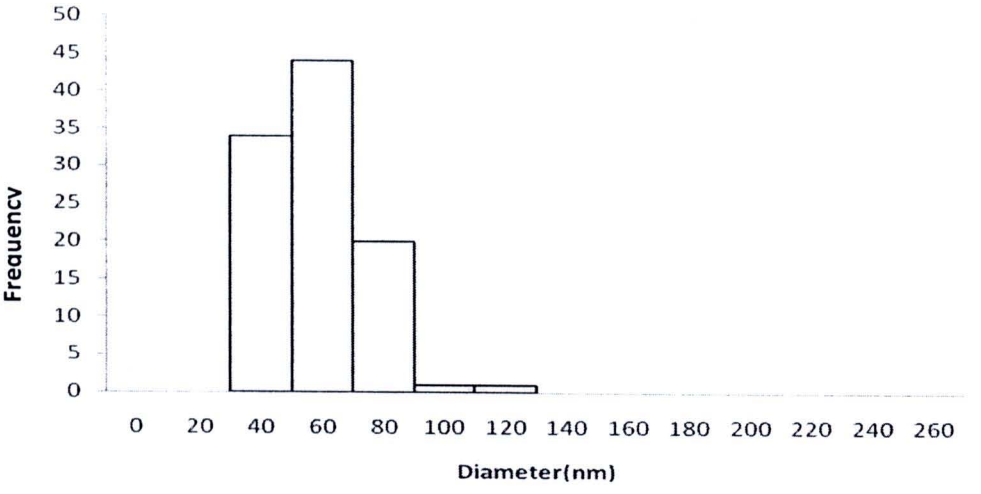


Figure A-7 Size distribution of fiber diameter of chitosan/PVA composite at 0.009%w/v chitosan content, MW of chitosan of 400 kDa.

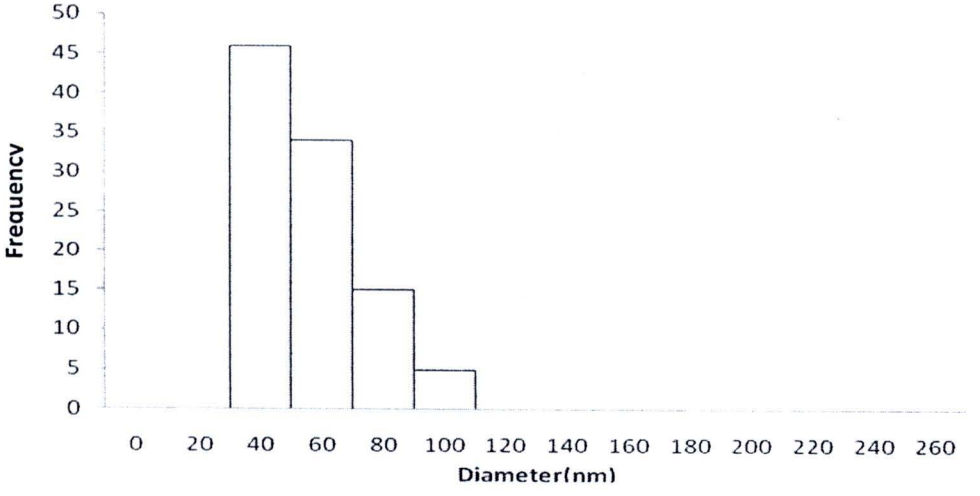


Figure A-8 Size distribution of fiber diameter of chitosan/PVA composite at 0.012%w/v chitosan content, MW of chitosan of 400 kDa.

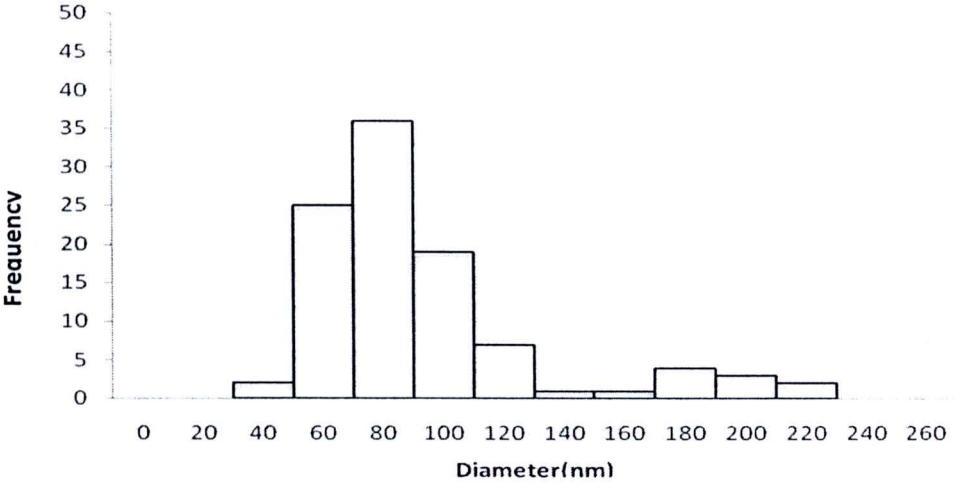


Figure A-9 Size distribution of fiber diameter of chitosan/PVA composite at 0.002 %w/v chitosan content, MW of chitosan of 760 kDa.

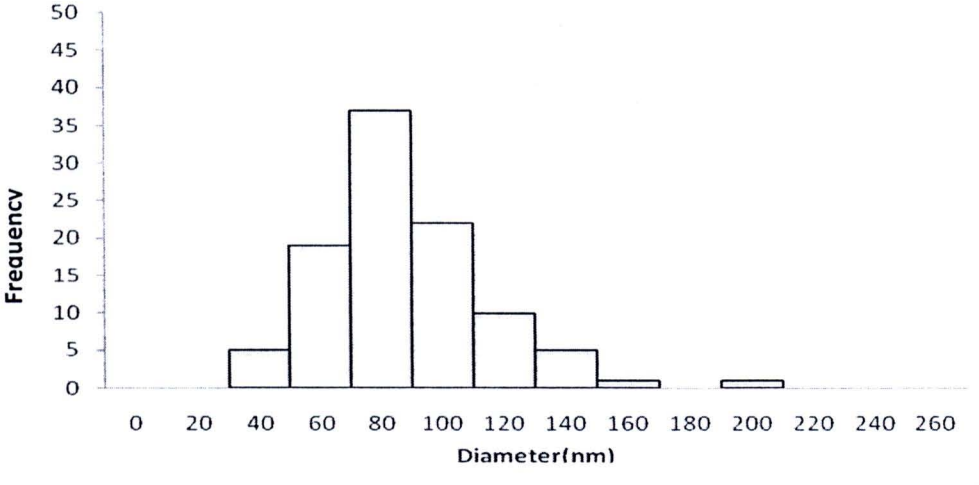


Figure A-10 Size distribution of fiber diameter of chitosan/PVA composite at 0.004 %w/v chitosan content, MW of chitosan of 760 kDa.

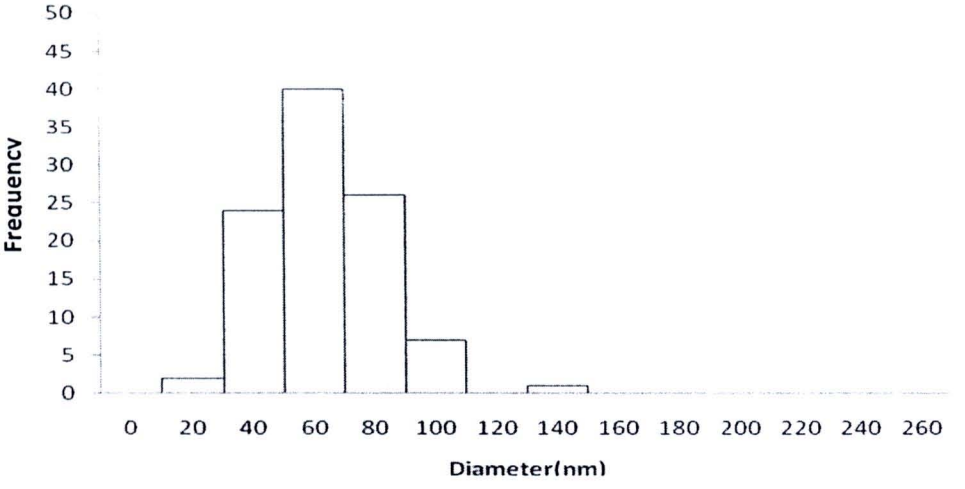


Figure A-11 Size distribution of fiber diameter of chitosan/PVA composite at 0.006 %w/v chitosan content, MW of chitosan of 760 kDa.

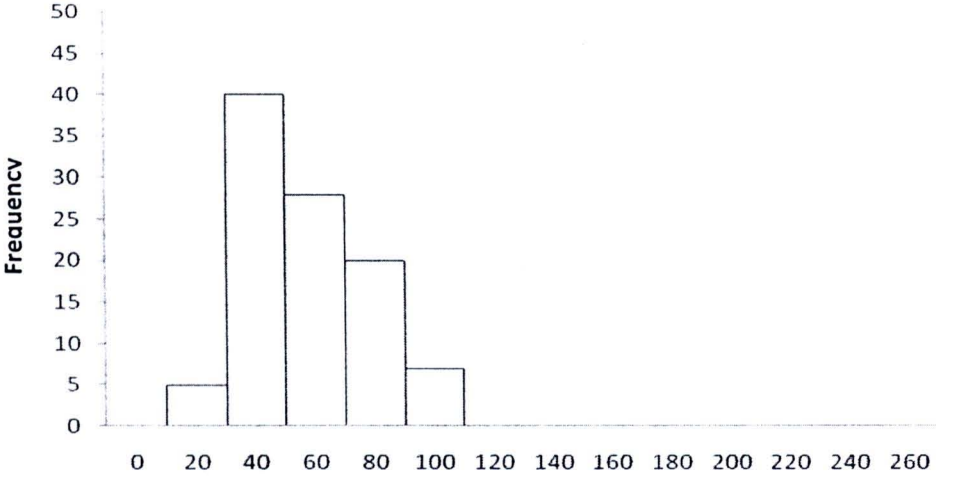


Figure A-12 Size distribution of fiber diameter of chitosan/PVA composite at 0.008 %w/v chitosan content, MW of chitosan of 760 kDa.

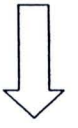
APPENDIX B

STANDARD CURVES FOR BACTERIAL CELLS CONCENTRATION

B.1 Preparation of Standard Curve for Bacterial Cells Concentration

In order to prepare the standard curve for bacterial cells concentration measurement, the following procedure is followed.

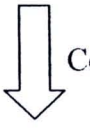
5 ml from broth medium



Incubated for 20h

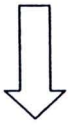
100 ml of medium (1% inoculum)

(Shaked at 250 rpm at 45°C for *Brevibacillus agri* strain 13 and at room temperature for *Acinetobacter baylyi* strain GFJ2)

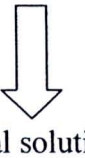


Centrifuge at 5000 rpm, 15 minutes

The high density cells were placed into 20 ml of bacterial solution

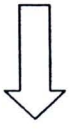


Measured optical density by spectrophotometer at 600 nm (OD_{600}).

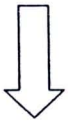


Diluted 10µl of the bacterial solution to 100µl of nutrient solution

(Serial dilutions $=10^{-8}$ -10^{-14} from the original medium solution)

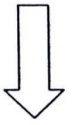


10µl of the serial solution was droped onto the agar plate and spread plate



Incubated for 20h

(45°C for *Brevibacillus agri* strain 13 and at room temperature for *Acinetobacter baylyi* strain (GFJ2)



Counted the colonies forming units

B.2 Determination of Colony Forming Units per ml (CFU/ml)

For example

From the spectrophotometer, OD_{600} = 1.0

From 10µl of the serial solution was counted = 6 colonies (CFU)

Before the dilution those 6 colonies were in a volume = 10^{-12} (1/µl)

Thus the original concentration = $\frac{6 \text{ colonies}}{10^{-2} \times 10^{-12}}$

Therefore, at OD_{600} is 1.0 = 6×10^{14} CFU/ml

The standard curve of bacterial cells were shown in Figure B-1 and Figure B-2 for Gram-positive *Brevibacillus agri* strain No13 and Gram-negative *Acinetobacter baylyi* strain GFJ2, respectively.

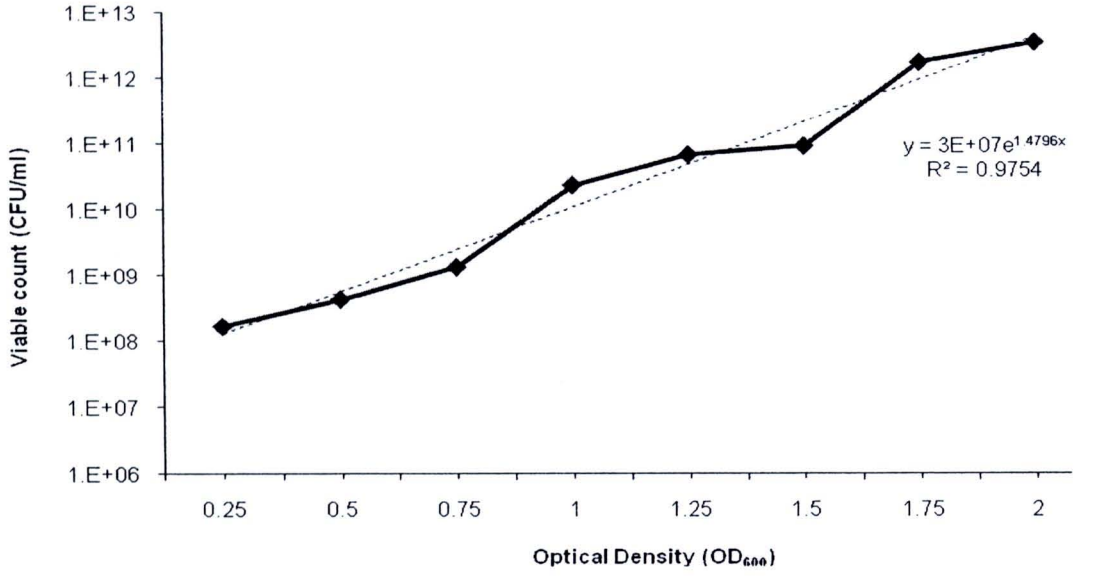


Figure B-1 The standard curve of Gram-positive *Brevibacillus agri* strain No13 between optical density (OD_{600}) and the colony forming units (CFU/mL) at 45°C, 24 hours incubated time by using the spread plate technique.

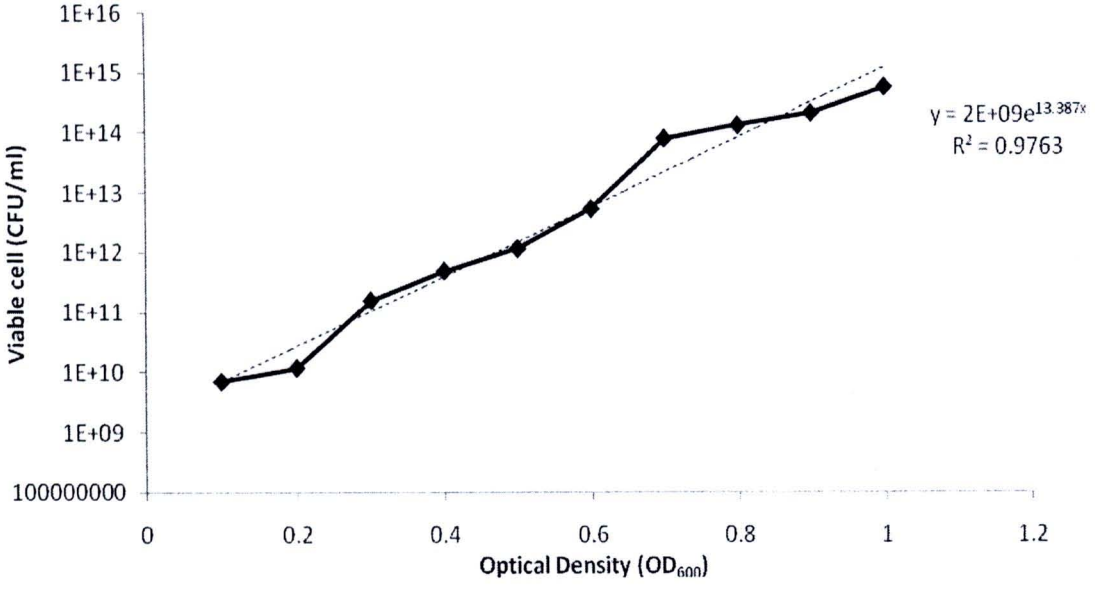


Figure B-2 The standard curve of Gram-negative *Acinetobacter baylyi* strain GFJ2 between optical density (OD_{600}) and the colony forming units (CFU/mL) at room temperature, 24 hours incubated time by using the spread plate technique.

APPENDIX C

DETERMINATION OF NUMBER OF CELLS ATTACHED ON CHITOSAN NANOFIBERS

In order to determine the bacterial cells attached on chitosan nanofibers, the optical density of the initial bacterial cell solution and that of the washed solution was measured by spectrophotometer at 600 nm (OD_{600}). The Colony Forming Unit (CFU) was calculated from a standard calibration curve for *Acinetobacter baylyi* strain GFJ2 and *Brevibacillus agri* strain 13 as followed.

For example, Gram-negative *Acinetobacter baylyi* strain GFJ2 of the hydrolyzed chitosan at 6h and 12h for incubation time.

OD_{600} of the initial bacterial cells solution is 0.88 $=2.6545\times10^{14}$ CFU/ml

100µl of the initial bacterial cells solution $=2.6545\times10^{14}\times(10^{-1})$

Thus, CFU of initial bacterial cells $=2.6545\times10^{13}$

OD_{600} of the first washed solution is 0.1507 $=1.5038\times10^{10}$ CFU/ml

500µl of the first of washed solution $=1.5038\times10^{10}\times(5\times10^{-1})$

CFU

Thus, CFU of the first washed solution $=7.519\times10^{9}$

OD_{600} of the second washed solution is 0.1993 $=2.8823\times10^{10}$ CFU/ml

500µl of the second of washed solution $=2.8823\times10^{10}\times(5\times10^{-1})$

CFU

Thus, CFU of the second washed solution $=1.4412\times10^{10}$

Since Total CFU attachment = $CFU_{initial} - CFU_{first\ washed} - CFU_{second\ washed}$

$= (2.6545\times10^{13}) - (7.519\times10^{9}) - (1.4412\times10^{10})$

$= 2.6503\times10^{13}$ cells

Therefore, the total bacterial cells were attached on the support = 2.6503×10^{13} cell

APPENDIX D

FT-IR SPECTRA OF CHITOSAN

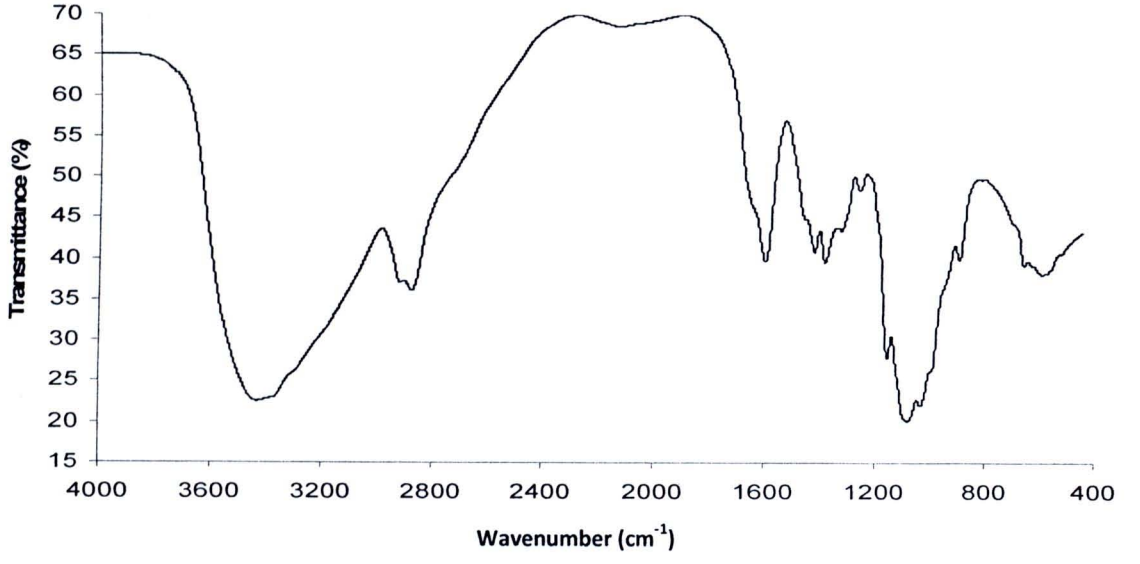


Figure D-1 FT-IR spectra of the chitosan hydrolyzed for 6 h.

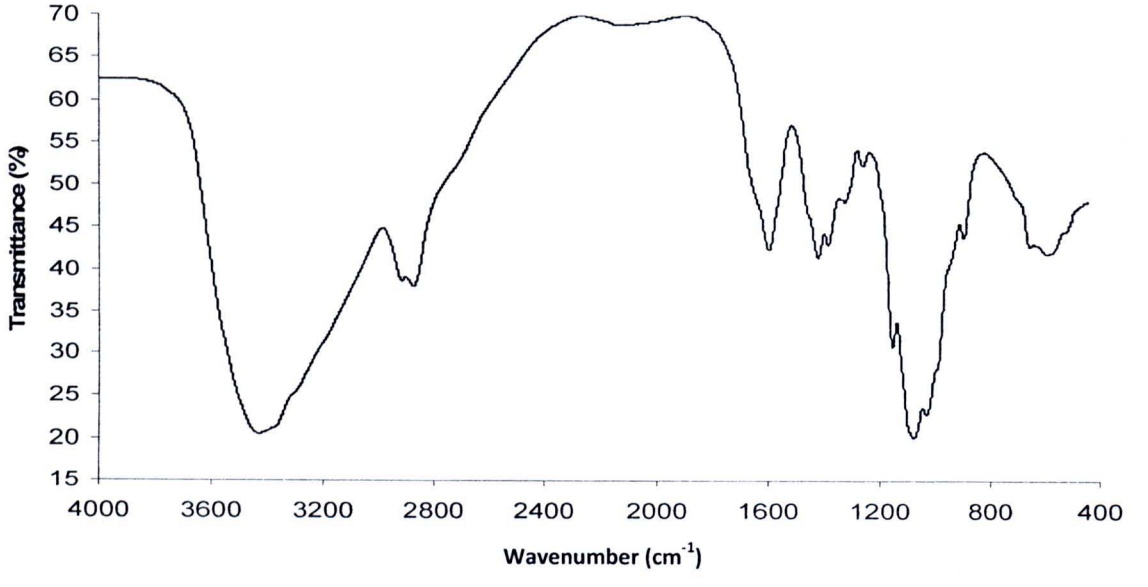


Figure D-2 FT-IR spectra of the chitosan hydrolyzed for 12 h.

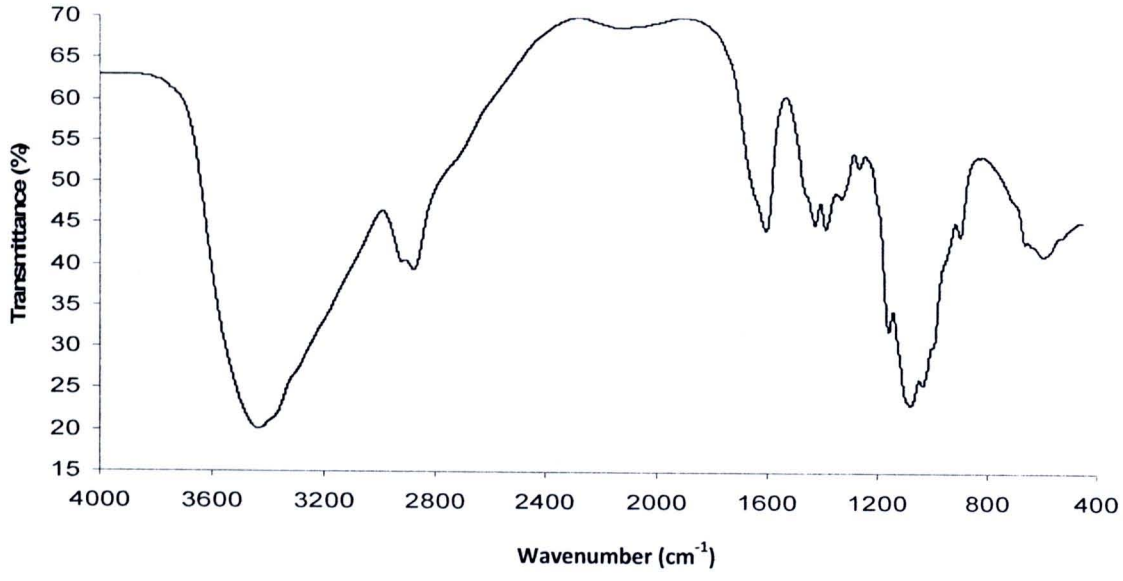


Figure D-3 FT-IR spectra of the chitosan hydrolyzed for 24 h.

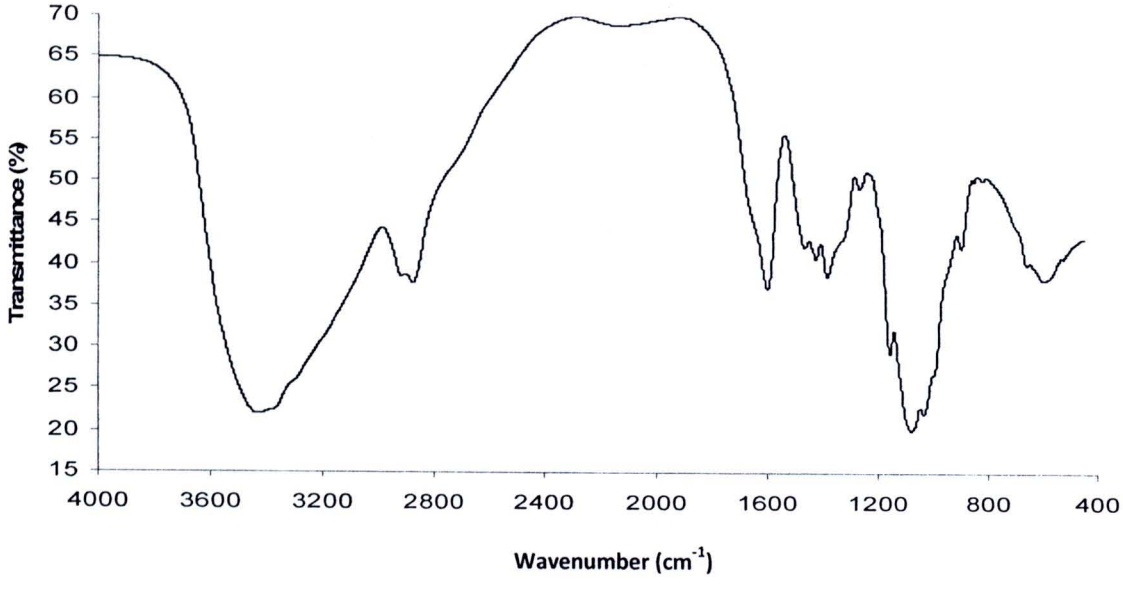


Figure D-4 FT-IR spectra of the chitosan hydrolyzed for 36 h.

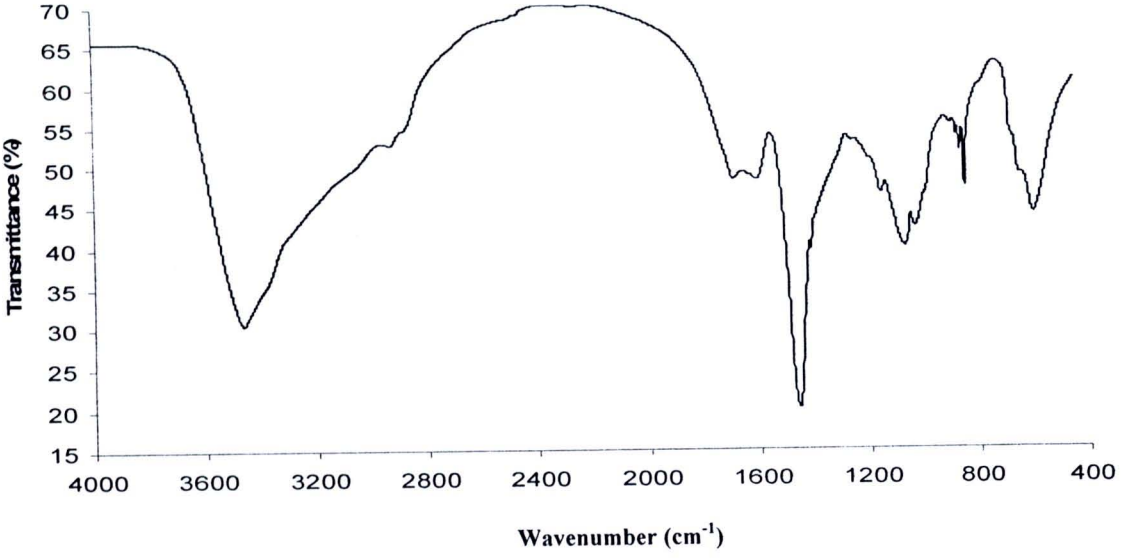


Figure D-5 FT-IR spectra of the chitosan hydrolyzed for 48 h.

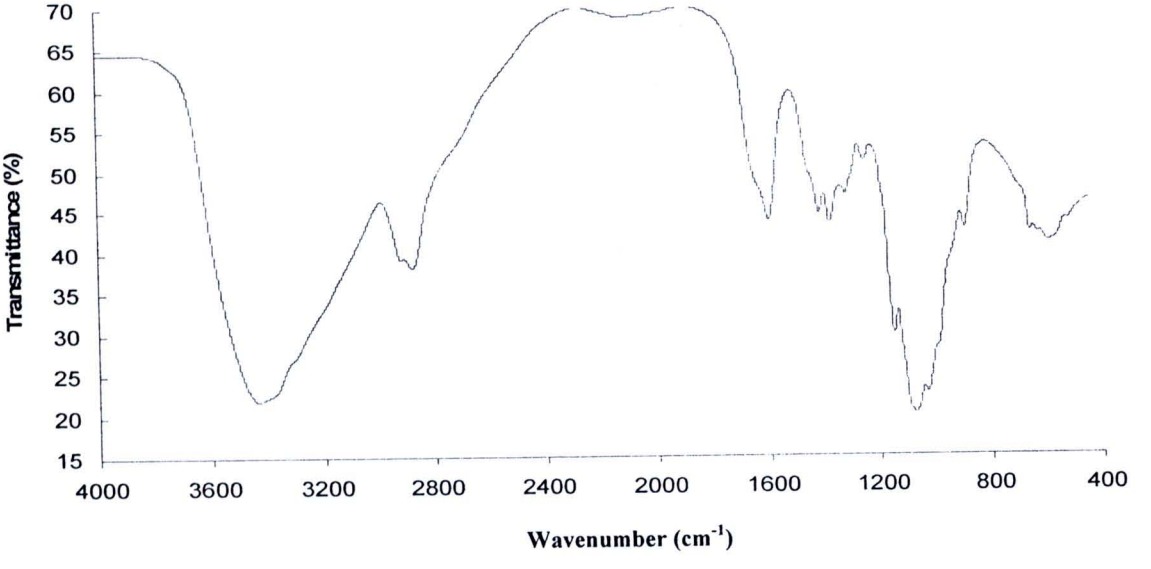


Figure D-6 FT-IR spectra of the chitosan at molecular weight 100 kDa.

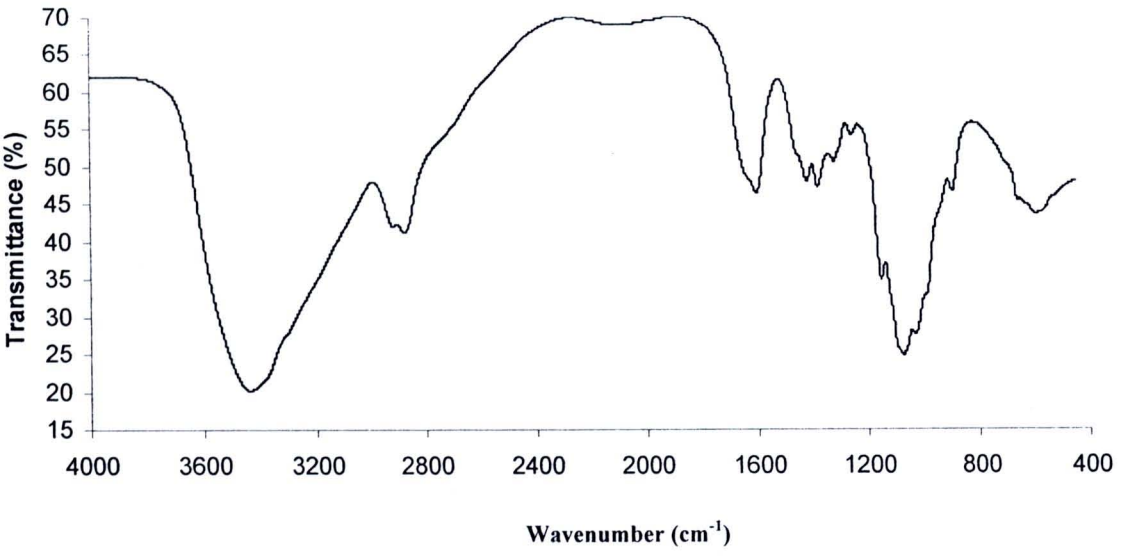


Figure D-7 FT-IR spectra of the chitosan at molecular weight 400 kDa.

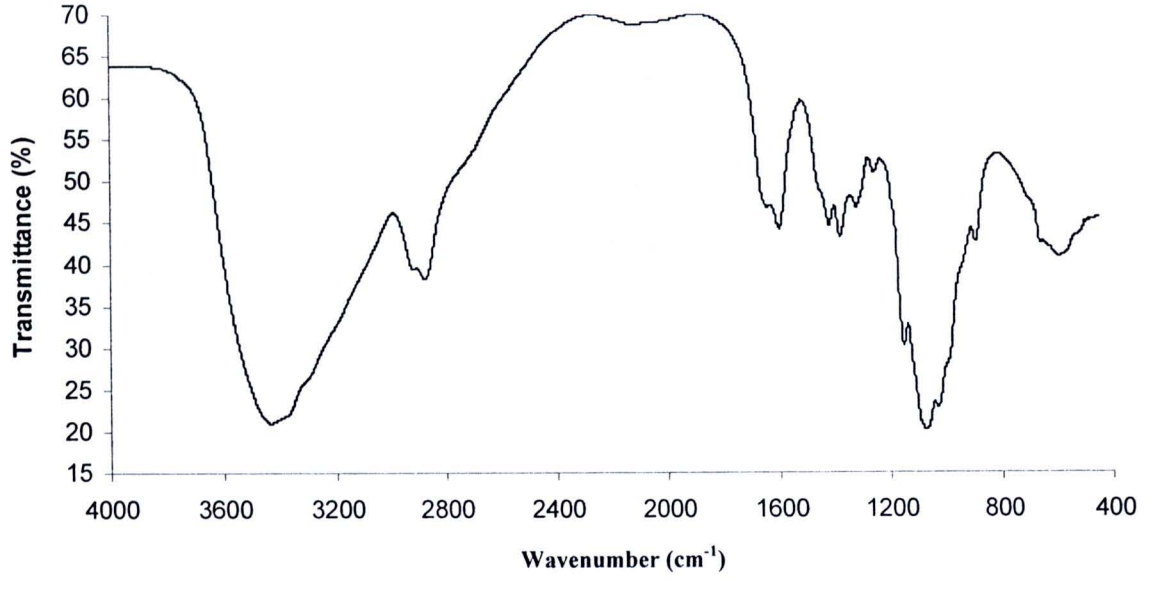


Figure D-8 FT-IR spectra of the chitosan at molecular weight 760 kDa.

APPENDIX E

DETERMINATION OF FIBER PERCENTAGE IN CHITOSAN NANOFIBERS

The percentage of the electrospun nanofibers was measured with SemAfore image analyzing program. For each experiment, were determined from the data of about the randomly selected 5 x 5 μm and 50 measurements from SEM micrograph for 5 times was followed.

For example

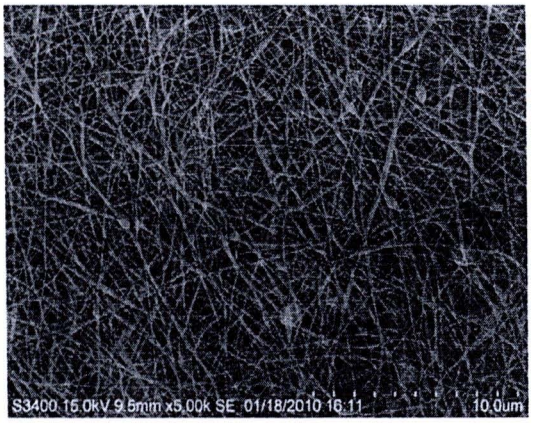


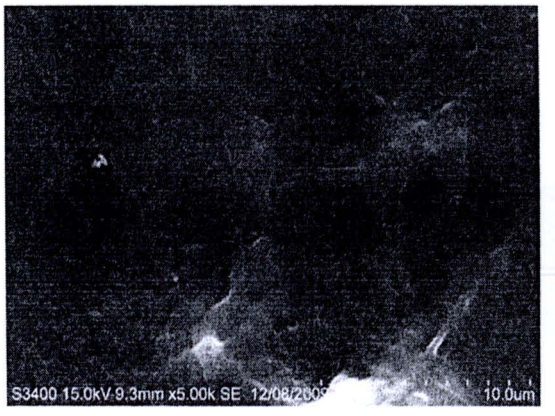
Figure E-1 Example of SEM micrographs of electrospun nanofibers.

Table E-1 Fiber percentage of electrospun fibers in Figure E-1.

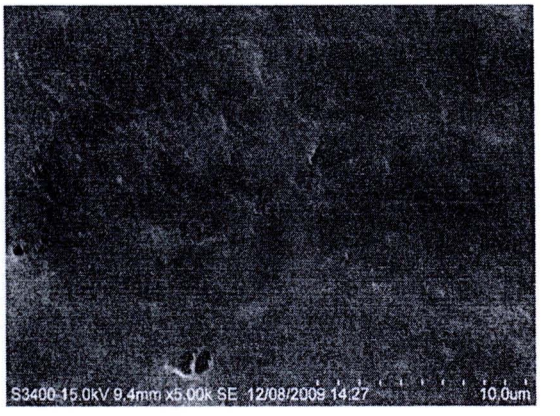
Area (5 x 5 μm)	Measurement		
	Fiber	Bead	Percentage of fiber (%)
1	47	3	94
2	43	7	86
3	44	6	88
4	46	4	92
5	45	5	90
Average			90

APPENDIX F

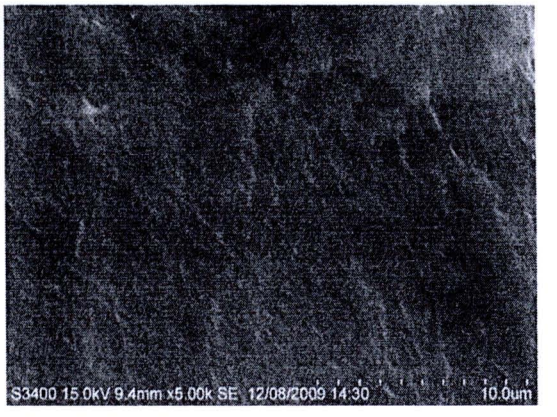
MORPHOLOGY OF BARE FIBERS DIPPED IN WATER



6 h

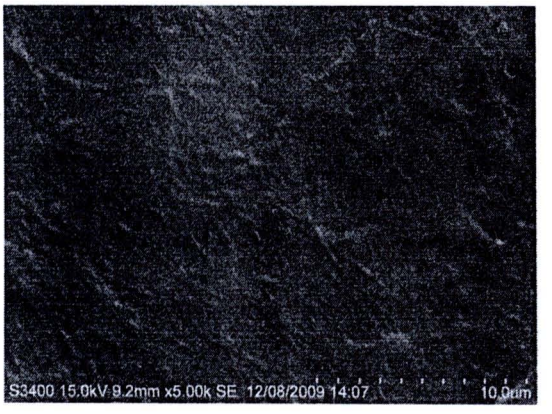


12 h

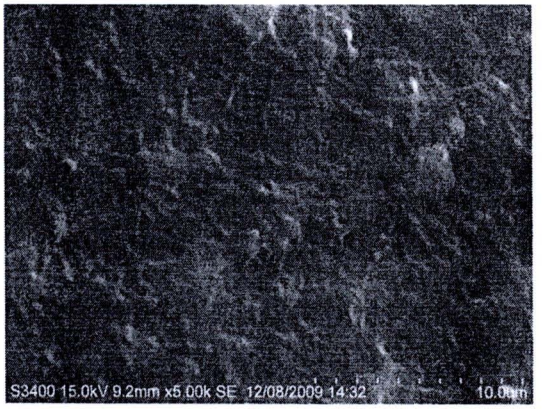


24 h

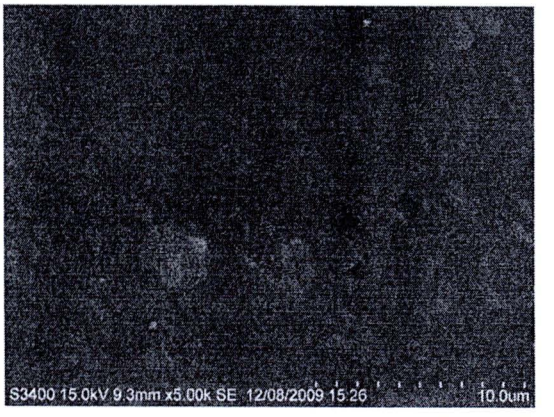
Figure F-1 SEM micrographs of electrospun chitosan/PVA composite nanofibers with 0.04 wt% PVA content, after dipping in water for various periods of time. The molecular weight of chitosan was 400 kDa.



6 h

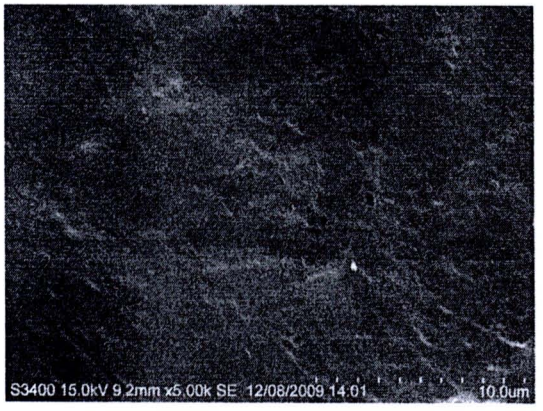


12 h

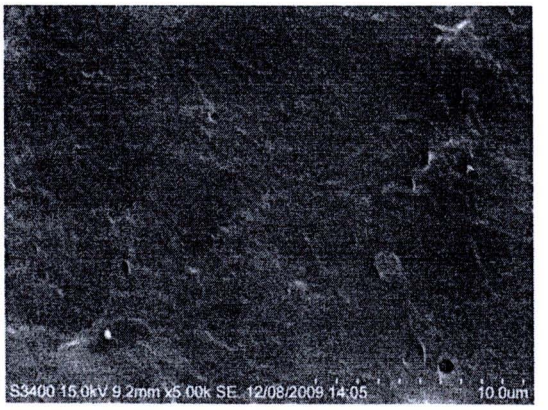


24 h

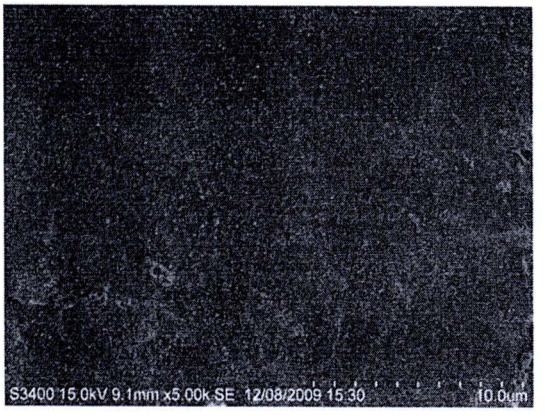
Figure F-2 SEM micrographs of electrospun from chitosan/PVA composite nanofibers with 0.06 wt% PVA content, after dipping in water for various periods of time. The molecular weight of chitosan was 400 kDa.



6 h

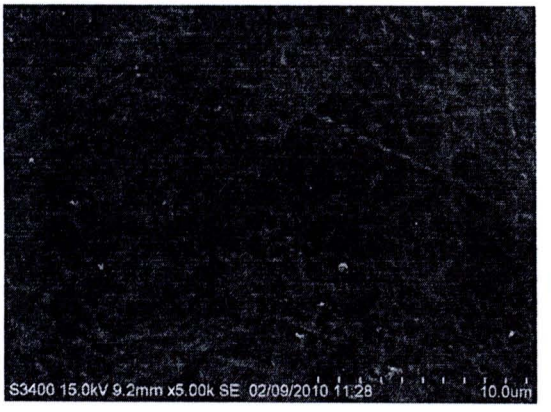


12 h

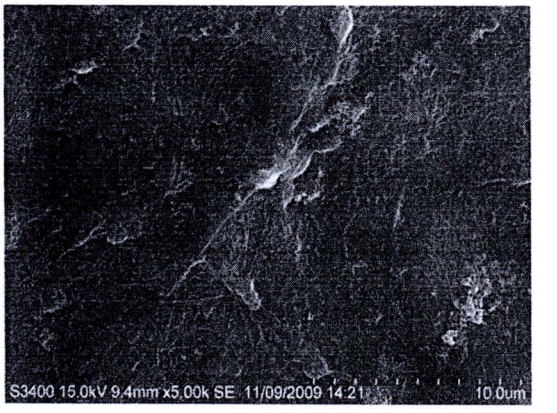


24 h

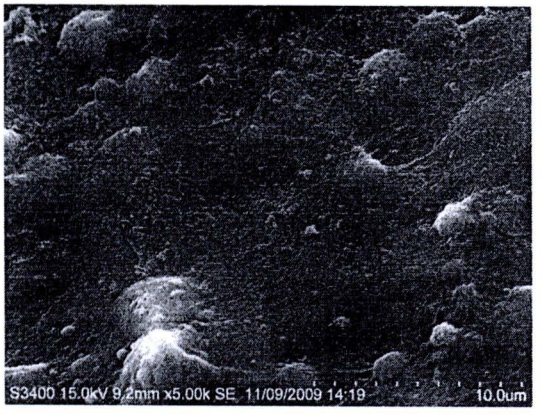
Figure F-2 SEM micrographs of electrospun from chitosan/PVA composite nanofibers with 0.08 wt% PVA content, after dipping in water for various periods of time. The molecular weight of chitosan was 400 kDa.



6 h



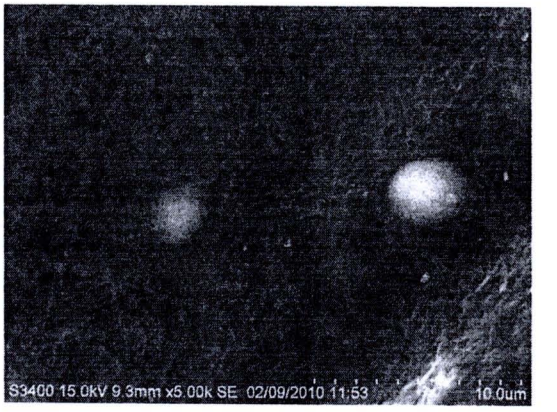
12 h



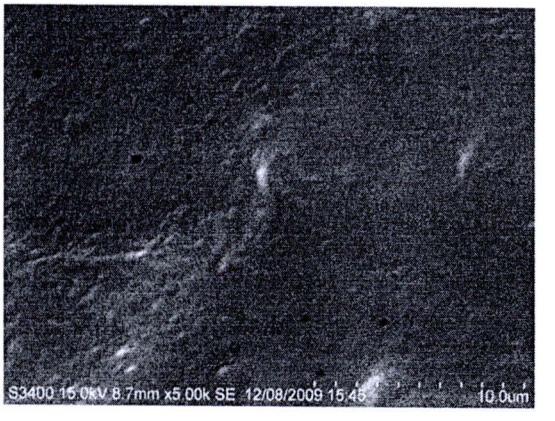
24 h

Figure F-4 SEM micrographs of electrospun from chitosan/PVA composite nanofibers with 0.04 wt% PVA content, after dipping in water for various periods of time. The molecular weight of chitosan was 760 kDa.

6 h



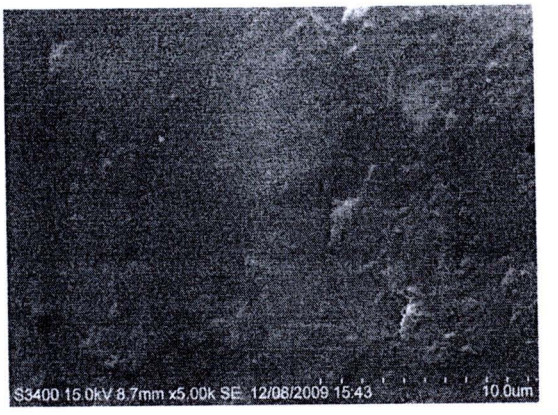
12 h



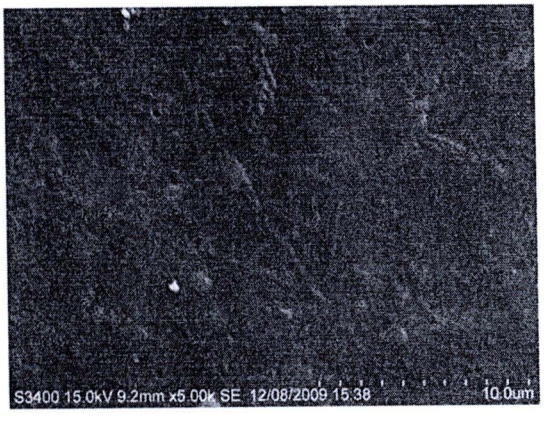
24 h

Figure F-5 SEM micrographs of electrospun from chitosan/PVA composite nanofibers with 0.06 wt% PVA content, after dipping in water for various periods of time. The molecular weight of chitosan was 760 kDa.

6 h

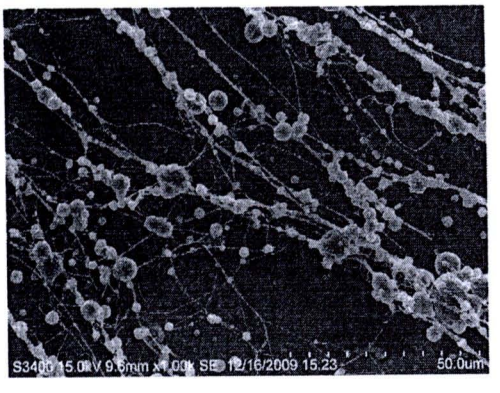


12 h

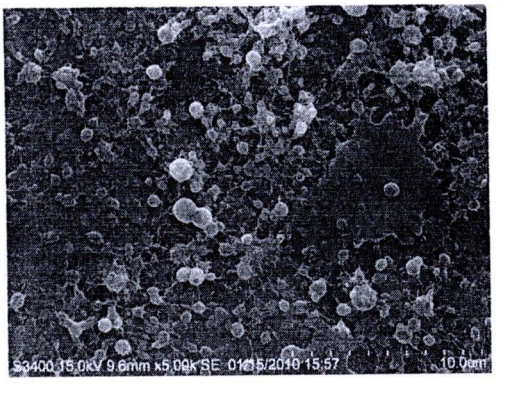


24 h

Figure F-6 SEM micrographs of electrospun from chitosan/PVA composite nanofibers with 0.08 wt% PVA content, after dipping in water for various periods of time. The molecular weight of chitosan was 760 kDa.

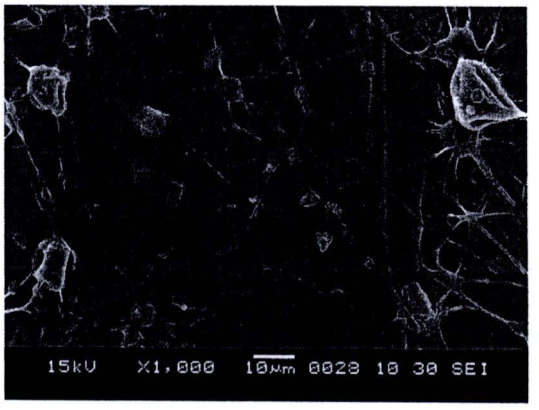


12 h

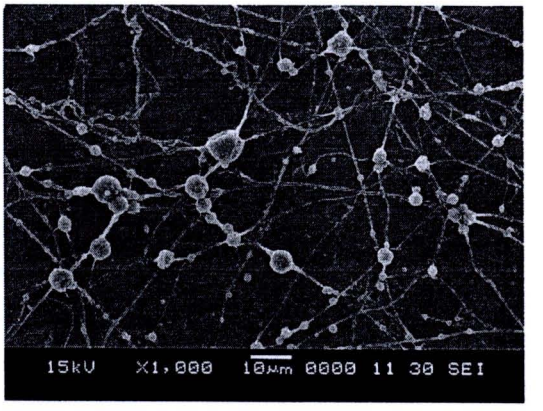


24 h

Figure F-7 SEM micrographs of electrospun from chitosan hydrolyzed for 24 h after dipping in water for various periods of time. The molecular weight of chitosan was 76

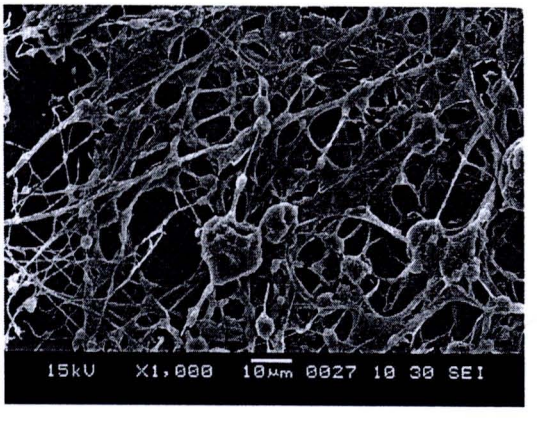


12 h

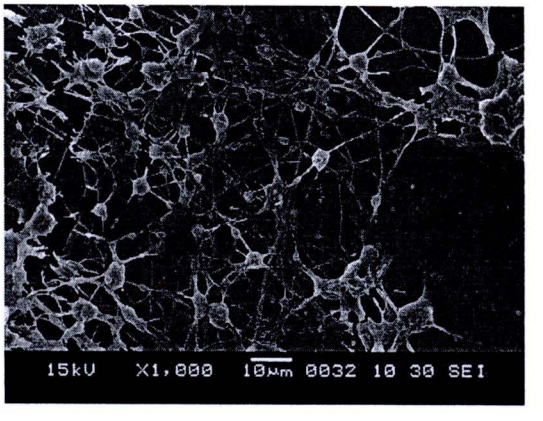


24 h

Figure F-8 SEM micrographs of electrospun from chitosan hydrolyzed for 36 h after dipping in water for various periods of time. The molecular weight of chitosan was 760 kDa.



12 h



24 h

Figure F-9 SEM micrographs of electrospun from chitosan hydrolyzed for 48 h after dipping in water for various periods of time. The molecular weight of chitosan was 760 kDa.

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VITAE

Miss Prissadawan Chumanee was born on December 14th, 1984 in Nakhornsithammarat, Thailand. She attended Wat Chaichumpol for primary school and Princess Chulabhorn's College, Trang for high school. She received a Bachelor Degree of Engineering, Department of Chemical Engineering from Thammasat University, in 2006. Since 2007, she has been a graduate student studying Chemical Engineering as a major course at Chulalongkorn University. During her studies towards the Master's Degree, she won awards on poster presentation at 6th Asian Aerosol Conference in 2009.