

CHAPTER III EXPERIMENTAL



3.1 Materials and Chemicals

3.1.1 Materials

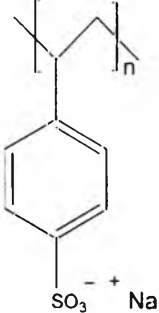
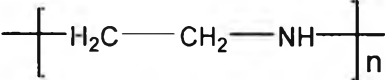
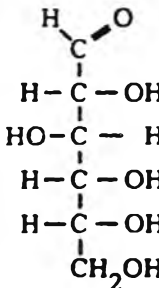
- Glass slide
- Silicon wafer
- Silk fiber
- Nylon fiber

3.1.2 Chemicals

- Sodium chloride
- Sodium hydroxide
- Sodium acetate trihydrate
- Acetic acid

Chemicals	Chemical structure	Properties	Company
Poly (diallyldimethyl ammonium chloride) (PDAD)		<ul style="list-style-type: none"> - M_w 200.000-350.000 - bp 100°C - mp -2.8- 0 °C - density 1.04 	Aldrich
Poly(methacrylic acid, sodium salt) (PMA)		<ul style="list-style-type: none"> - 30wt% solution in water - viscosity 110.00-375.000cps (25°C) - M_n 5,400 (GPC) - M_w 9,500 (GPC) 	Aldrich

Chemicals	Chemical structure	Properties	Company
Silver nitrate(AgNO_3)		- bp 444°C - mp 212°C - M_w 169.87	Aldrich
Poly(4-styrenesulfonic acid-co-maleic acid),sodium salt (CoPSS)		- viscosity 30.000 cps - M_w 20,000 - Density 0.94	Aldrich
Alginic acid (Alginate)		- viscosity 200.000-400.000 cps	Aldrich
Chitosan			

Chemicals	Chemical structure	Properties	Company
Poly(sodium 4-styrene sulfonate) (PSS)		<ul style="list-style-type: none"> - M_w 70,000 - Density 0.801 - viscosity 15,000 – 55,000 cps (20% solution, 25 °C) 	Aldrich
Poly(ethyleneimine, PEI)			
Glucose			

3.2 Equipments

3.2.1 Automatic dipping machines

3.2.2 Macbeth COLOR EYE® 7000 spectrophotometer

3.2.3 UV-Vis Spectrophotometer (SPECORD S 100, Analytikjena)

3.2.4 JEOL scanning electron microscope, JSM-6400, Japan

3.2.5 JEOL transmission electron microscope, JEM 2100, Japan

3.2.6 Holder supports

3.2.7 pH/ion meter

3.2.8 Transonic 460/H from Elma®

3.3 Procedure

3.3.1 Synthesis Silver Nanoparticles Solutions

3.3.1.1 The best searching polyelectrolyte can be reacting with silver nitrate

The PMA, PDAD, Alginate, Chitosan, PSS, PEI, CoPSS, Glucose and AgNO_3 were dissolved in deionized water to form aqueous solution of PMA ($1 \times 10^{-3} \text{M}$), PDAD ($1 \times 10^{-3} \text{M}$), Alginate ($1 \times 10^{-3} \text{M}$), Chitosan (0.1%w/w), PSS ($1 \times 10^{-3} \text{M}$), PEI ($1 \times 10^{-3} \text{M}$), CoPSS ($1 \times 10^{-3} \text{M}$), Glucose ($1 \times 10^{-3} \text{M}$) and AgNO_3 ($100 \times 10^{-3} \text{M}$), respectively. Then, the aqueous solution of each polyelectrolytes was mixed with AgNO_3 solution in tube at volume ratio 20:1 respectively. Their was observed in two day.

3.3.1.2 Case study of polyelectrolyte concentration effect, it's base on absorbance with controlling pH 7

Sodium acetate trihydrate ($1 \times 10^{-3} \text{M}$) was used for buffer solutions pH7. The PMA and AgNO_3 were dissolved in buffer solutions to form aqueous solution of PMA ($0.1 \times 10^{-3} \text{M}$, $1 \times 10^{-3} \text{M}$, $5 \times 10^{-3} \text{M}$, $10 \times 10^{-3} \text{M}$) and AgNO_3 ($10 \times 10^{-3} \text{M}$), respectively. Then, the aqueous solution of each polyelectrolyte concentration was mixed with AgNO_3 solution in tube at volume ratio 1:1 respectively. Then, the mixed solutions of each polyelectrolyte concentration were taken in quartz cuvette to measure UV-Vis. The mixed solutions were left one day before taking UV-Vis.

3.3.1.3 Case study of silver nitrate concentration effect, it's base on absorbance with controlling pH 7

Sodium acetate trihydrate ($1 \times 10^{-3} \text{M}$) was used for buffer solutions pH7. The PMA and AgNO_3 were dissolved in buffer solutions to form aqueous solution of PMA ($10 \times 10^{-3} \text{M}$) and AgNO_3 ($0.1 \times 10^{-3} \text{M}$, $1 \times 10^{-3} \text{M}$, $5 \times 10^{-3} \text{M}$, $10 \times 10^{-3} \text{M}$), respectively. Then, the aqueous solution of each polyelectrolyte concentration was mixed with AgNO_3 solution in tube at volume ratio 1:1 respectively. Then, the mixed solutions of each polyelectrolyte concentration were taken in quartz cuvette to measure UV-Vis. The mixed solutions were left one day before taking UV-Vis.

3.3.1.4 Find relation of the time and absorbance

The PMA and AgNO₃ were dissolved in deionized water to form aqueous solution of PMA ($10 \times 10^{-3} \text{M}$) and AgNO₃ ($10 \times 10^{-3} \text{M}$), respectively. The both solutions were mixed in beaker at volume ratio 1:1 respectively. Then, the mixed solutions were taken in quartz cuvette to measure UV-Vis.

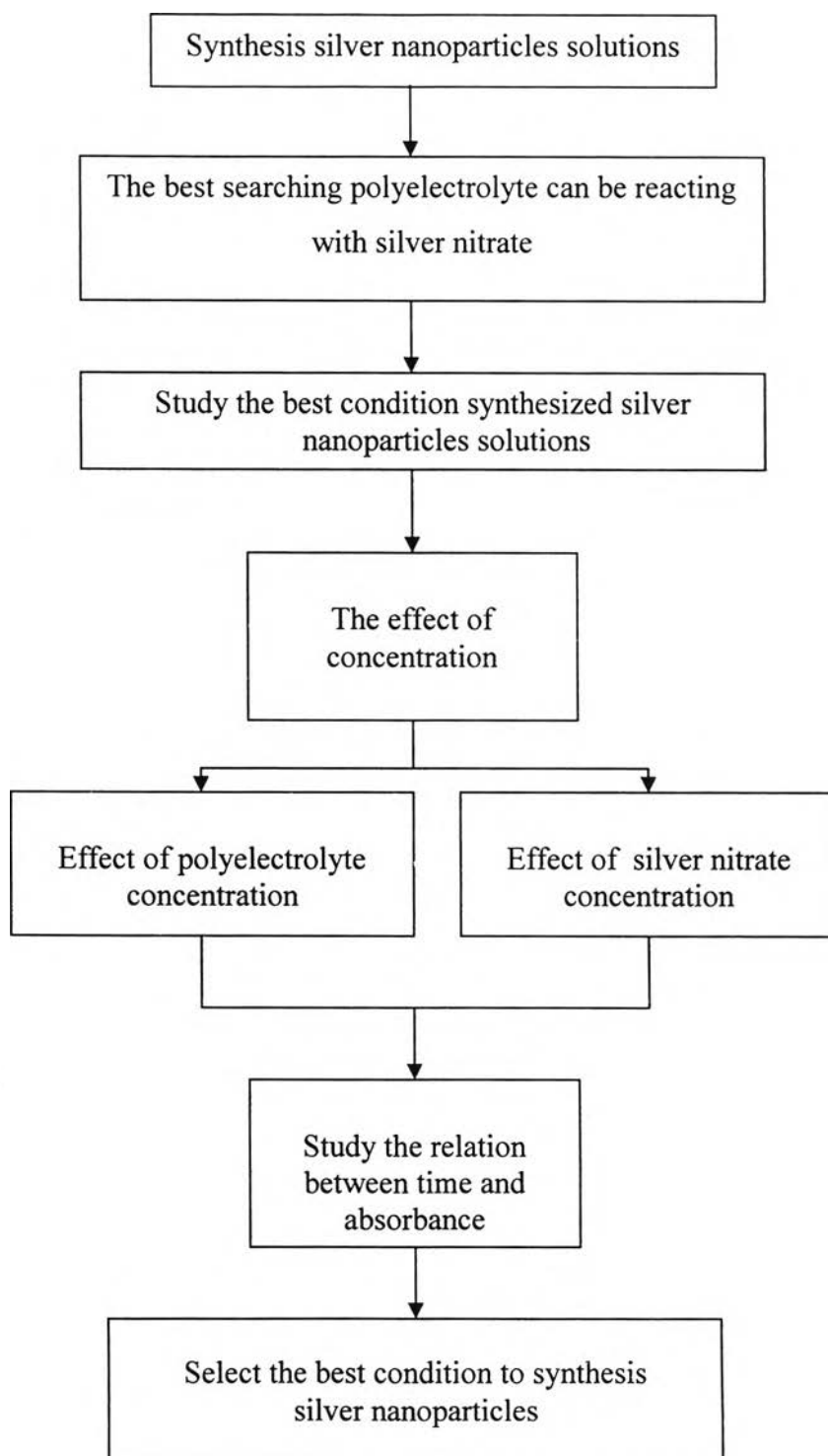


Figure 3.1 Schematic diagram of synthesis silver nanoparticles solutions.

3.3.2 Preparation of Polyelectrolyte Multilayer Thin Films

3.3.2.1 Substrate onto holder

The resin holder was accessory equipment, the resin holder as shown in Figure 3.2. It was used for supported the substrate in order to the solution. The fibers were wrapped onto this holder before dipping in the solutions.

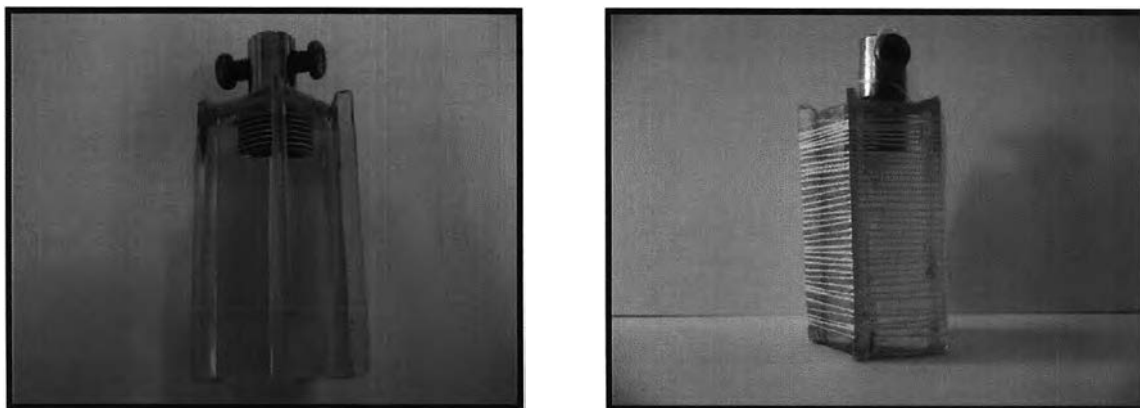


Figure 3.2 Resin holder supports.

3.3.2.2 Solution Preparation

In this system, various solutions were added into eight 100 ml beakers. One beaker was dilute cationic polyelectrolyte solution, one was anionic polyelectrolyte solution. The others were rinse water. Solutions of anionic were used by silver nanoparticle solutions. In cationic polyelectrolyte solutions, sodium chloride salt was added, then sonicated in order to dissolve.

3.3.2.3 Layer-by-layer Deposition on Substrate

Multilayer thin films were deposited using a home-built automated dipping machine, it as shown in Figure 3.3. Multilayer thin films of silver nanoparticle and PDAD (Ag/PDAD)_n were prepared by repeating the following step on the nylon fiber substrate n times;

(1) immersing in the PDAD solution for 1 min ; (2) 3 times washing with buffer solution pH 7 to remove excess material (3) immersing in silver solution for 1 min. (4) 3 times washing with buffer solution pH 7.

Self-assembled films on silk fiber substrates were fabricated by similar sequential deposition but silver solution was diluted 10 times.

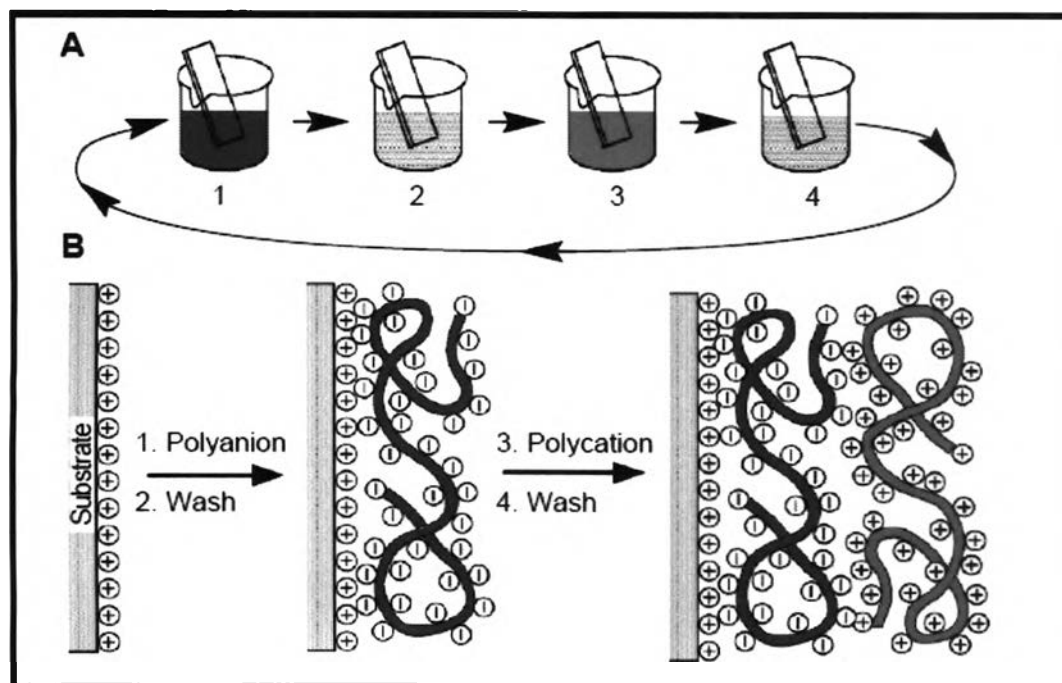


Figure 3.3 Schematic of the layer-by-layer deposition technique.

Table 3.1 Experimental condition of the comparison the PEM between primer and no-primer on silk and nylon fiber

Code		Positive charge	Negative charge	Salt concentration (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 1	Concentration (mM)	Primer		0.1	Silk, Nylon	80	1	7	4
		PDAD	PSS						
		1	1						
	Concentration (mM)	Layer-by-layer		0.1 (Only PDAD)		80	1	7	20
PDAD		PMacap Ag							
		10	10						
PEM 2	Concentration (mM)	No-primer		0.1 (Only PDAD)	Silk, Nylon	80	1	7	20
		PDAD	PMacap Ag						
		10	10						

Table 3.2 Experimental condition of constructed PEM on silk at various number of layer

Code		Positive charge	Negative charge	Salt concentration in positive charge solution (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 3A-3G	Concentration (mM)	PDAD	PMacap Ag	0.1	Silk	80	1	7	2-20
		10	10						

Table 3.3 Experimental condition of constructed PEM on silk at various number of layer compared with dipped no PDAD solution

Code		Positive charge	Negative charge	Salt concentration in positive charge solution (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 4A-4F	Concentration (mM)	PDAD	PMAcap Ag	0.1	Silk	80	1	7	2-20
		1	1 (dilute 10 times from 10 mM)						

Table 3.4 Experimental condition of constructed PEM on nylon at various number of layer compared with dipped no PDAD solution

Code		Positive charge	Negative charge	Salt concentration in positive charge solution (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 5A-5F	Concentration (mM)	PDAD	PMAcap Ag	0.1	Nylon	80	1	7	2-20
		1	10						

Table 3.5 Experimental condition of constructed on silk with AgNO₃ and NaCl

Code		Positive charge	Negative charge	Salt concentration in positive charge solution (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 6A-6F	Concentration (mM)	AgNO ₃	NaCl	-	Silk	80	1	7	2-20
		5	10						

Table 3.6 Experimental condition of constructed PEM on silk with CoPSS solution at various number of layer

Code		Positive charge	Negative charge	Salt concentration in positive charge solution (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 7A-7F	Concentration (mM)	PDAD	CoPSS capAg	-	Silk	80	1	5	2-20
		1	10						

Table 3.7 Experimental condition of constructed PEM on silk with Alginate solution at various number of layer

Code		Positive charge	Negative charge	Salt concentration in positive charge solution (M)	Substrate	Volume	Dipping time (min)	pH	Number of layer
PEM 8A-8F	Concentration (mM)	PDAD	Alginate capAg	-	Silk	80	1	6.7	2-20
		1	10						

3.3.3 Characterization

3.3.3.1 Macbeth COLOR EYE® 7000 spectrophotometer

The degree of dye fixation was determined spectrophotometrically with an Instrumental Color System (I.C.S) spectrophotometer (Macbeth 700), Macbeth COLOR EYE® 7000 spectrophotometer as shown in Figure 3.4, which was used to measure the reflectance values of the color samples in the 360-750 nm range.

The color strength of fibers were expressed as K/S values calculated by the Kubelka-Munk equation.

$$\frac{K}{S} = \frac{(1 - R)^2}{2R}$$

Where K is the absorption coefficient

S is the scattering coefficient

R is the reflectance of the fiber at the wavelength of maximum absorption (λ_{\max})



Figure 3.4 Macbeth COLOR EYE® 7000 spectrophotometer

3.3.3.2 UV-Vis Spectrophotometer (SPECORD S 100, Analytikjena)

Prior to any UV-Vis measurements, UV-Vis Spectrophotometer as shown in Figure 3.5, a bare clean glass slide was first introduced and recorded as a background. For the measurements, the PEMs coated glass slide was measured. The absorbance of the thin film was measured in the 400 to 700 nm visible range.

The deionize water was first introduced and recorded as a background. For the measurements, The solutions in quartz cuvette were measured in the 180 to 800 nm UV-VIS rang



Figure 3.5 UV-Vis Spectrophotometer (SPECORD S 100, Analytikjena)

3.3.3.3 JEOL scanning electron microscope, JSM-6400, Japan

The SEM samples were cut from silk fiber coated with silver nanoparticles. The samples were mounted on stub with double-side adhesive tape and coated with a thin layer of gold. Images were taken using a JEOL scanning electron microscope, JSM-6400 as shown in Figure 3.6, using an accelerating voltage of 15 kV, and a magnification 5,000 times, 30,000 times and 35,000 times of original specimens size.

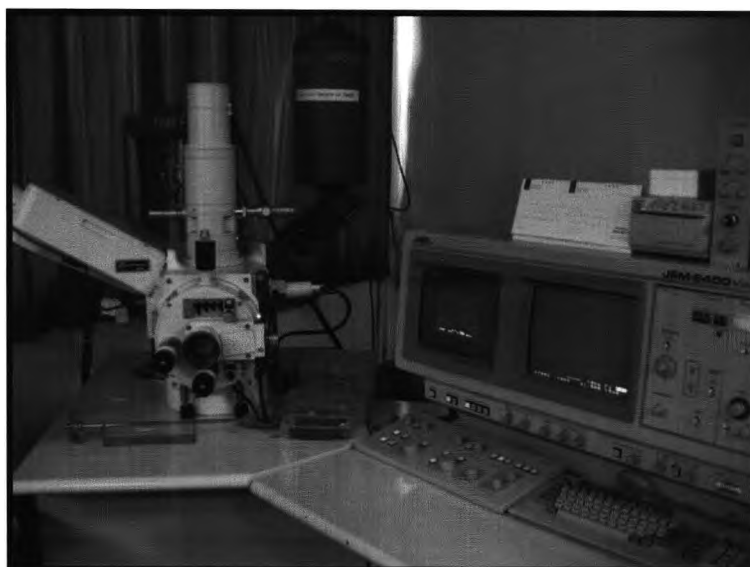


Figure 3.6 JEOL scanning electron microscope, JSM-6400, Japan

3.3.3.4 JEOL transmission electron microscope, JEM 2100, Japan

The silver nanoparticles solutions were measured by JEOL transmission electron microscope. These solutions were prepared by dropping solutions onto Cu grids and dried at ambient temperature over night. TEM images were obtained with JEM 2100 TEM show in Figure 3.7, using an acceleration voltage of 100 kV. High-magnification images were taken at 100,000 and 300,000 times of specimens size.

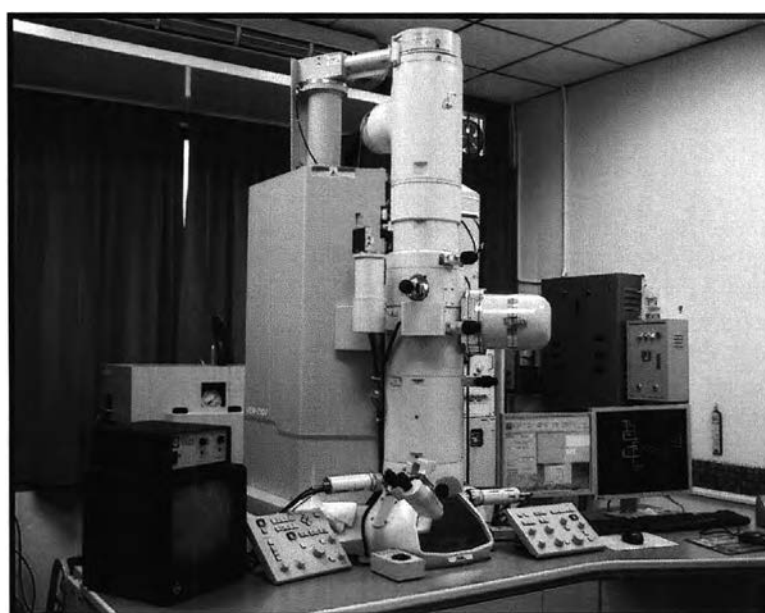


Figure 3.7 JEOL transmission electron microscope, JEM 2100, Japan

3.3.3.5 Antibacteria Test

The fibers were placed in the test tube and 0.5 ml of bacteria (*Staphylococcus aureus*) with 5 ml nutrient broth (media) were pipetted onto the fibers and then the tube was shaken for 1 h. At predetermined time, cell was pipetted out from the tubes and consecutive dilutions were prepared by taking 1 ml of previous solution and mixed with deionized water. From the solution, 0.1 ml was plated onto the solid agar using the spread plate method. After incubating for 24 h., the number of viable bacteria was then counted and results with the dilution factor expressed as mean colony forming units (CFU) per ml.

Calculate percent reduction of bacteria by the specimen treatment by one of the following formula;

$$100(B-A)/B = R$$

R= % Reduction

B= CFU value of Sample.