CHAPTER III

EXPERIMENTAL METHODS

In this chapter, experimental details include the chemical used, the methods employed for the silver nanoparticles preparation as well as the polyelectrolyte multilayers formation are listed. The sensing of the silver nanoparticles in solution and assembled into thin films is also detailed. Instrumental methods used in the characterization of the samples are also briefly overviewed.

3.1 Chemicals

3.1.1 Silver nitrate

For the preparation of silver nanoparticle, AR graded silver nitrate ($AgNO_3$) purchased from Mallinckrodt, Thailand was used.

3.1.2 Sodium borohydride

The reducing agent used was sodium borohydride ($NaBH_4$) purchased from Sigma-Aldrich.

3.1.3 Capping agents

The following polyelectrolytes used as capping agents for stabilizing the nanoparticles were all purchased from Aldrich. Their chemicals structure are summarized in Figure 3.1.

- O poly(acrylic acid, sodium salt), (PAA) (MW ~ 5.1×10^3)

- O poly(4-styrene sulfonic acid-*co*-maleic acid), sodium salt, (CoPSS) with a molar ratio of sulfonic to maleic group in the polyelectrolyte of 2:1, (MW ~ 2×10^4).

- O Alginic acid, sodium salt.

- O poly(methacrylic acid, sodium salt), 30 wt. % solution in water, (MW ~ 5.4×10^3)

- O Humic acid, sodium salt (HA)

3.1.4 Organic compounds

AR graded organic compounds including methanol, ethanol, propanol, acetone, were purchased from Aldrich and used as received without any further purification.

3.1.5 Primer coatings

Poly(diallyldimethylammonium chloride) (PDADMAC), medium molecular weight, 20 wt. 20% (MW= 3.69×10^5, DPI = 2.09) and poly(sodium 4-styrene-sulfonate) (PSS) (MW = 6.89×10^4, DPI= 1.07) used as primer coatings were purchased from Aldrich. Analytical graded sodium chloride was purchased from Carlo Erba.
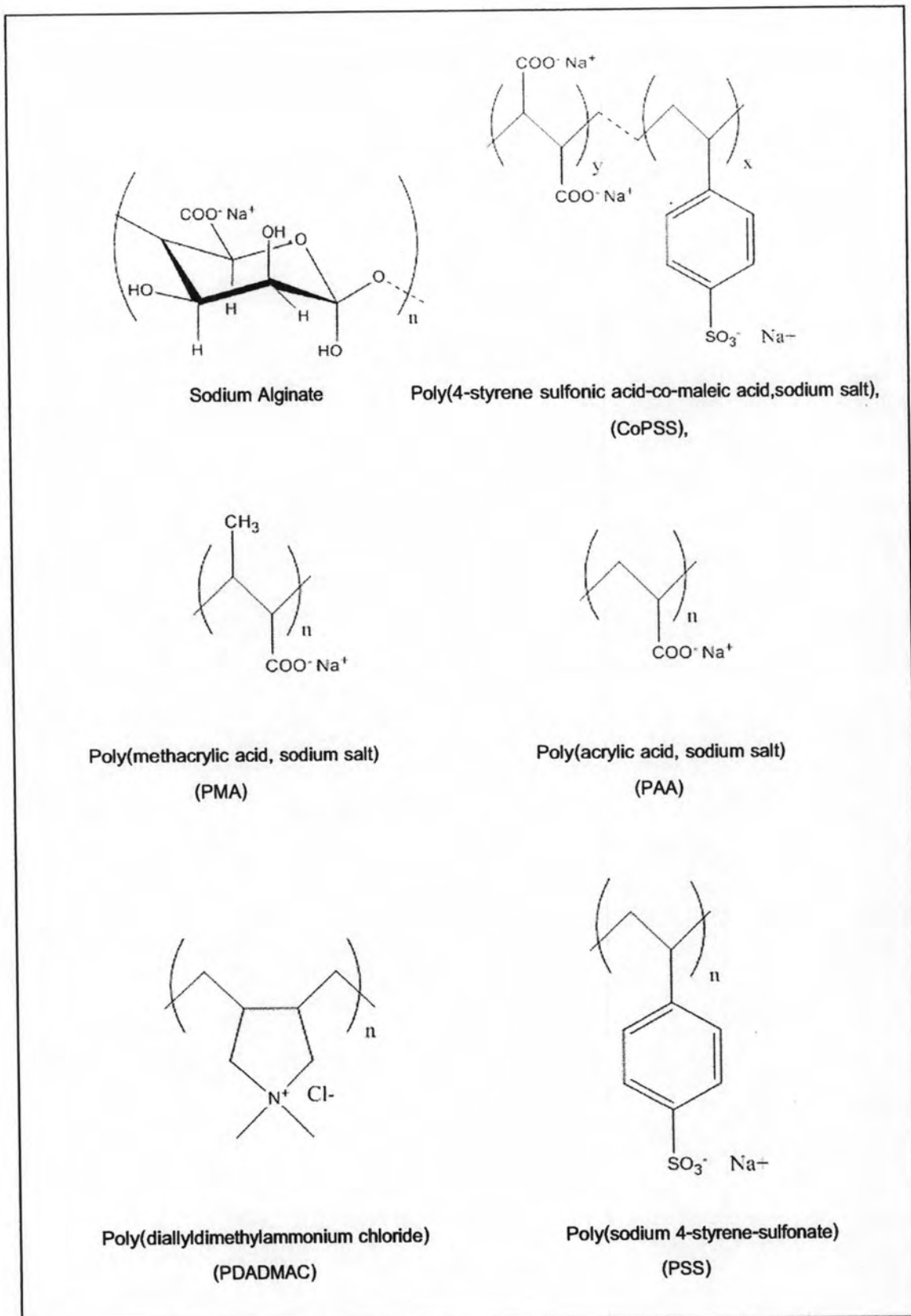
Figure 3.1: Chemical structures of the polyelectrolytes

3.2 Substrate cleaning steps

3.2.1 Dipping in piranha solution

Prior to the monolayer and layer-by-layer deposition, glass slide substrates were cleaned from organic contaminants by a 15 min dipping in an oxidizing "piranha solution" prepared by mixing a 2:1 volume ratio of concentrated sulfuric acid with concentrated hydrogen peroxide (30%). The substrates were then rinsed thoroughly with de-ionized water. This cleaning step resulted in a hydrophilic, organic free surface. (Warning: piranha is a very oxidizing solution and should not be stored in a closed container)

3.2.2 Dipping in hot ammonia solution

The cleaned substrates from 3.2.1 were then dipped in hot ammonia solution in order to deprotonate the surface to increase the electrostatic charge density and hydrophilicity. The solution was prepared by mixing a 5:1:1 ration of water, hydrogen peroxide (30%) and concentrated ammonia. The solution was heated to $60^{\circ}C$ and the substrates were immersed in this solution for 20 min. A final rinse step and blow dry with a stream of nitrogen gas resulted in cleaned substrates ready for deposition.

3.3 Preparation of silver nanoparticle solutions

Silver nanoparticles were prepared by the well-described method using sodium borohydride as a reducing agent. For the stabilization of these particles, various polyanionic polymers were used as the capping agents in water. The preparation steps can be summarized as shown in Figure 3.2. 20 ml of a 10-0.1 mM solution of a polyanionic polymer was mixed with an equal volume of a 1 mM silver nitrate solution and stirred for 5 min. Then, a 20 ml of a freshly prepared 10 mM sodium borohydride solution was then quickly added into the silver/polyelectrolyte mixture under vigorous stirring. The experimental conditions for the preparation method are summarized in Table 3.1. The solution immediately turned dark yellow/orange confirming the formation

of silver nanoparticles. The solution was stirred overnight and then stored in the dark.

Solutions prepared by this method were found to remain stable for several months.
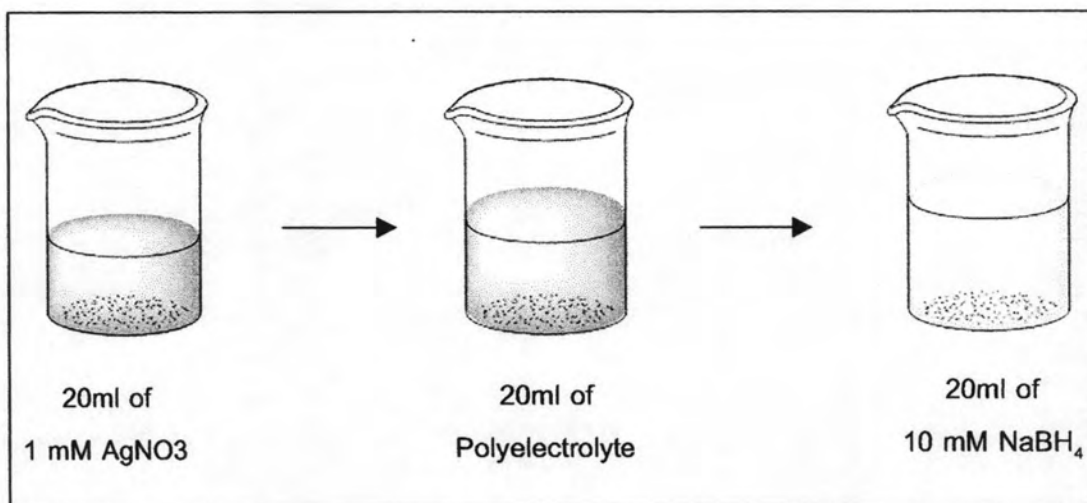

Figure 3.2: Schematic diagram depicting the steps used in the preparation of silver nanoparticles by encapsulation with polyelectrolytes and reduction by sodium borohydride.

Table 3.1: Experimental conditions used in the preparation of silver nanoparticles

Type of polyelectrolyte·	Polyelectrolyte Concentration	Polyelectrolyte (ml)	Silver nitrate (ml)	NaBH$_4$ (ml)
PMA	10, 5, 1, 0.5, 0.1 mM	20	20	20
PAA	10, 5, 1, 0.5, 0.1 mM	20	20	20
Alginate	10, 5, 1, 0.5, 0.1 mM	20	20	20
CoPSS	10, 5, 1, 0.5, 0.1 mM	20	20	20
Humic acid	0.1, 0.05, 0.01, 0.005, 0.001 %	20	20	20

3.4 Preparation of silver nanoparticle thin films by layer-by-layer assembly

For PEM thin film buildup, the cleaned substrates were first immersed for 2 min in a solution containing 10 mM of polycationic PDADMAC and 0.1M sodium chloride. The substrates were then rinsed three times using de-ionized water for 1 min. The purpose of the rinse bath was to remove the excess and loosely bound polyelectrolytes from the substrate surface. These steps resulted in the adsorption of a thin layer of polycationic polymer which consequently reversed the charge at the substrate surface from negative to positive. The surface with polycationic polymer top layer was then immersed for 2 min in a solution containing 10 mM of polyanionic polymer and 0-1 M sodium chloride followed by three times of water rinsing. pHs of the polyelectrolytes and all rinse solutions were adjusted accordingly to each experiment and will be discussed in Chapter 4. These steps resulted in the deposition of a bi-layer of polyelectrolytes as shown in Figure 3.3 and were repeated as many times as needed. It can be seen that

during the deposition, some excess polyelectrolytes still adsorbed at the surface of the film and can only be removed by extensive rinsing in water. When completed, PEM thin films were dried with a stream of nitrogen gas and stored in a closed container. A homemade robot was used to improve reproducibility and to allow the facile deposition of up to 20 layers. All samples were spun with a homemade sample holder while in solution to accelerate the deposition process and render PEM to be more uniform.
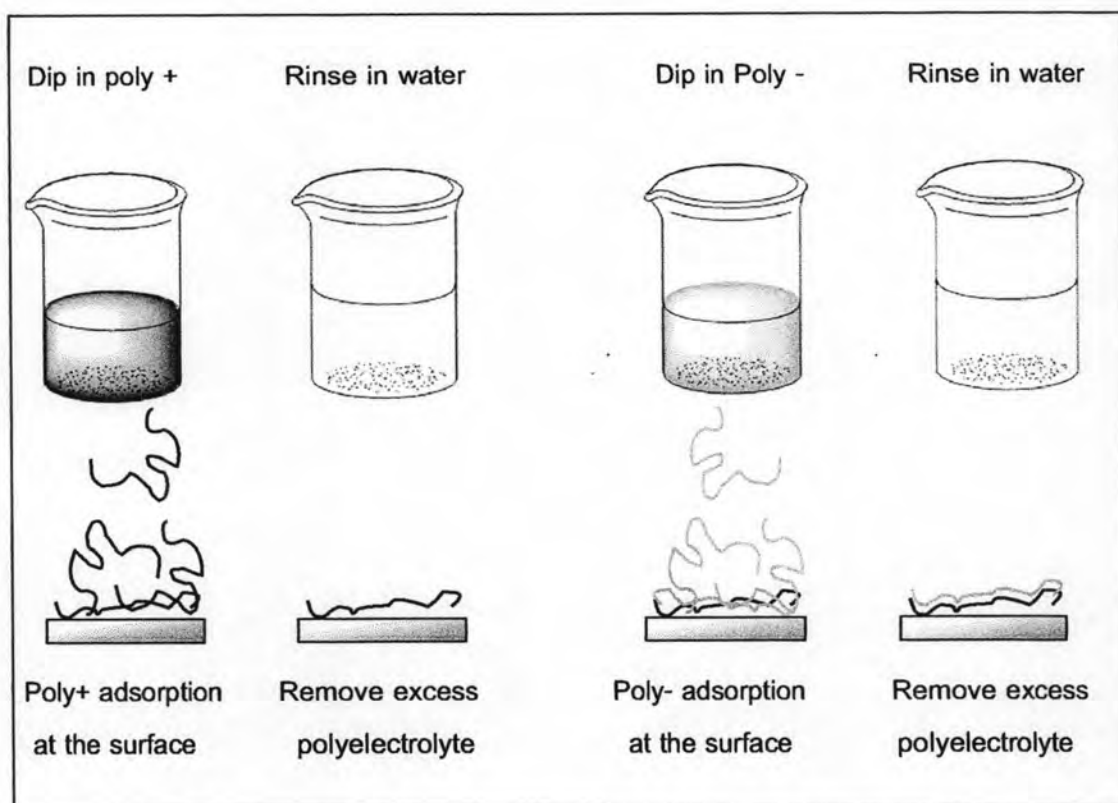

Dip in poly +	Rinse in water	Dip in Poly -	Rinse in water
Poly+ adsorption at the surface	Remove excess polyelectrolyte	Poly- adsorption at the surface	Remove excess polyelectrolyte

Figure 3.3: Steps involving in the layer by layer deposition of polycationic and polyanionic species onto a substrate.

3.5 Sample characterization

3.5.1 UV–Vis spectroscopy

The silver nanoparticle thin films were analyzed using a UV–Vis spectrophotometer (SPECORD S 100, Analytikjena). The absorbance of the film was measured in 188 to 1100 nm range, which includes the absorbance peak of the silver nanoparticles centered at 400 nm. UV-Vis absorbance was used to monitor the film deposition due to the relationship between absorbance and the amount of silver nanoparticles present on the surface of the film. To monitor their growth, the multilayer thin films were assembled on fused quartz plates (2 mm thick, 4cm long and 2cm wide) which are transparent to UV-vis region. The plates were pretreated with "piranha" and then in H_2O_2/ ammonia / water (1:1:5) then rinsed with distilled water. PEMU buildup can be monitored with this method since the amount of polymer deposited is proportional to the absorbance according to Beer's law:

$$Abs = \varepsilon \times b \times c \qquad (3.1)$$

where A is the absorbance , ε e is the molar absorptivity ($L\ mol^{-1}\ cm^{-1}$), b is the absorbing medium path length (cm) and c is the concentration (mol L^{-1}).

3.5.2 Transmission electron microscopy

Transmission electron microscopy is a vacuum technique in which a sample is bombarded by high energy electrons. The electrons are focused onto the sample and produce and image which is projected onto a fluorescent panel. The obtained image is function of the crystal nature and density of the material. This technique is suitable especially for metallic nanoparticle. It produces image with excellent resolution down to the nanometer. A transmission electron microscope model (Jeol JEM 100SX) was used to analyze the particles size of the freshly prepared silver nanoparticles. For sample preparation, a drop of silver nanoparticles solution was dropped on the copper grid and left to dry overnight in desiccators. The samples were measured without any further

treatment on the following day. Particle size distributions were analyzed using a free software ImageJ version 1.38 available on-line at "http://rsb.info.nih.gov/ij/index.html".

3.5.3 Atomic Force Microscopy

Atomic force microscope, firstly described by Binning et al. in 1986 has become one of industry's most popular scanning probe tool. Atomic Force Microscopy (AFM) is a valuable technique used for studying surface topographical, mechanical and electrical properties. The principle is based on using sharp tip that is scanned over the surface at a constant force (for height information) or height (for force information) using a feed-back loop mechanism. Tips are typically made from silicon or silicon nitride and extend down to the end of a reflective cantilever. A diode laser is focused onto the back of this reflective cantilever. As the tip scans the surface of the sample, moving up and down with the contour of the surface, the laser beam is deflected off the attached cantilever into a dual element photodiode. The photodetector measures the difference in light intensities between the upper and lower photodetectors, and then converts to voltage. Feedback from the photodiode difference signal, through software control from the computer, enables the tip to maintain either a constant force or constant height above the sample.

AFM analysis was performed in air using a SPM multimode unit (Digital Instruments Inc., Santa Barbara, CA) with a type RTESP silicon tip with a 125 μm length and 300 KHz resonance in tapping Mode. The tapping mode was used since it causes less damage to soft surface and polymeric materials by minimizing the contact between the tip and the sample.

3.6 Sensing experiments

3.6.1 Organic compound sensing of silver nanoparticles solution

The prepared nanoparticles were tested for their sensing properties in solution when exposed to different types and concentrations of organic compounds. For sensing experiments involving nanoparticles dispersed in solutions, the testing of their sensing properties was performed using the conditions given in Table 3.2. In a test tube 2.7 ml of an organic compound and 300 µl of the nanoparticles dispersed in water were mixed. The mixture was allowed to react for 5 min or as long as needed for the color of the solution to be stable and then added into a quartz cuvette for UV-Vis spectroscopic analysis.

Table 3.2 Experimental conditions used in the testing of organic compound sensing.

Type of polyelectrolyte	Concentration of capping agent	Volume of nanoparticles	Type of organic compound	Volume of solvent
PMA	0.1 mM	300µL	Water, Acetone, EtOH, MetOH, propanol	2.7 ml
PAA	0.1 mM	300µL	Water, Acetone, EtOH, MetOH, propanol	2.7 ml
Alginate	0.1 mM	300µL	Water, Acetone, EtOH, MetOH, propanol	2.7 ml
CoPSS	0.1 mM	300µL	Water, Acetone, EtOH, MetOH, propanol	2.7 ml

3.6.2 Herbicide sensing of silver nanoparticle solution

The prepared nanoparticles were also tested for their sensing properties in solution when exposed to solutions of different herbicide concentrations. The testing of their sensing properties was performed as summarized in table 3.3. In a test tube were mixed 1.5 ml of an herbicide solution containing 1000, 500, 250, 125 or 60 ppm of herbicide and 1.5 ml of the nanoparticles stock solutions. The mixture was allowed to react for 5 min and then added into a quartz cuvette for UV-Vis spectroscopic analysis.

Table 3.3 Experimental conditions used in the testing of herbicide sensing.

solutions	Volume of solutions	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Humic acid capped nanoparticles	1.5ml	0.01mg/l	0.01mg/l	0.01mg/l	0.01mg/l	0.01mg/l
herbicide	1.5ml	1000 ppm	500 ppm	250 ppm	125 ppm	60 ppm

3.6.3 Organic compound sensing of silver nanoparticle thin films

In the case of the film sensors, the nanoparticles were deposited into thin films using the layer-by-layer deposition as described in previous section of this chapter. The glass slide was then cut into 0.5 cm section in order to fit in the cuvette and immersed in a solution containing various types and concentrations of ethanol. As the kinetic of color shift was much slower in film, great care was taken to insure complete color change by recording UV-Vis spectra until no changes in absorbance could be seen.

3.7 Nanoindentation

Nanoindentation experiments were performed using the Digital instrument Atomic Force microscope mounted with a diamond tip attached onto a stainless steel cantilever. The indentation ramp was increase from 0.5, 1, 1.5, 2 and 2.5 V with an indentation distance of 1 micron. In this research, a 90 degree (cube corner) tip was used (Figure 3.4). The cube corner indenter tips are similar to the Berkovich tips in that they are also 3 sided pyramidal tips. The total included angle of the cube corner tips is 90 degrees, which gives them an aspect ratio of 1:1. Because the aspect ratio is higher, it is much simpler to make the radius of curvature smaller. Typical radiuses of curvature measurements for these tips are around 75 nm.
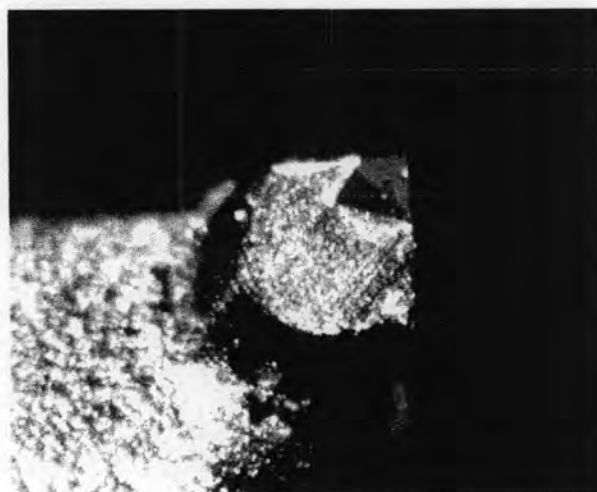

Figure 3.4 Optical image of the cube corner diamond probe tip used in the nanoindentation experiment.

The main advantage that the cube corner tip has over other tips is the sharpness. The radius of curvature can be less than 30 nm. This allows smaller indentations to be performed, while still creating plastic deformation. Because of this, smaller indentations can be made, and an accurate hardness can still be measured. The small indentation impression for a given depth also allows smaller features on a sample or composite material to be measured. The tip was used with the following fitting parameters provided by Hysitron Inc, Nanomechanical Testing Instruments:

Ideal Tip Area Function for 90 degree tip:

$$\text{Area} = 2.598 \, h_c^{\,2}$$

$$\text{Or} \quad C_0 = 2.598$$