CHAPTER III
METHODOLOGY

3.1 Materials

3.1.1 Chemicals

1. 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione, curcumin
2. Poly(D-glucosamine), Chitosan, low molecular weight, ALDRICH, China
3. Poly(β-D-mannuronic acid)-co-poly (α-L-guluronic acid), Alginic acid sodium salt, ALDRICH, USA
4. Poly(diallydimethylammonium chloride), PDADMAC, medium molecular weight, 20 wt.% in water, Mw=200,000-350,000, ALDRICH
5. Poly(sodium 4-styrene-sulfonate), PSS, ALDRICH, Mw=70,000
6. Sodium Acetate, CH_3COONa, CARLO ERBA, 99.0-100.5%
7. Sodium Chloride, NaCl, CARLO ERBA, 99.5%
8. Sodium Dodecyl Sulfate, SDS, $C_{12}H_{25}O_4SNa$, Fw=288.38 ALDRICH, Japan
9. Hexadecyltrimethylammonium bromide, CTAB, $C_{19}H_{42}BrN$, ALDRICH, Denmark

3.1.2 Solvents

1. Acetic Acid, CH_3COOH, glacial, 2.5L, CARLO ERBA, >99.9%
2. Acetone, AR
3. Ethanol, EtOH, C_2H_5OH, AR
4. Methanol, MeOH, CH_3OH, AR

3.2 Equipment

3.2.1 Atomic Force Microscope (AFM)

Atomic force microscope was used to measure the PEM film thickness and roughness of surface by doing a scratch on the glass slide with a sharp needle and measuring the step height.

3.2.2 Contact Angle

Contact angle analysis use to study the hydrophobicity by wettability of film surface. This is especially useful in processes like coating and painting where good wettability required ensuring good adhesion. This technique can be measured by producing a drop of water on a film surface. The angle formed between the film/water interface and the water/air interface is referring as a contact angle.

3.2.3 Ellipsometry

Ellipsometry is an optical technique for investigating the dielectric properties of PEM thin films. This technique used to measure the thickness when increasing the number of layers.

3.3.4 Fluorescence Spectroscopy

Fluorescence spectroscopy is a type of electromagnetic spectroscopy that excites the electrons in molecules of curcumin and causes them to emit light. This technique used to measure the amount of curcumin in the film.

3.2.6 UV-Vis Spectroscopy

UV-Vis spectrophotometer use to find out the amount of curcumin in the PEM films because curcumin is a natural pigment that show yellow color about 440 nm to state the amount of curcumin in PEM thin films.

3.3 Experimental Procedures

3.3.1 Parameter Controlling the Loading of Curcumin into PEM

3.3.1.1 Effect of the Number of Layers on the Thickness of PEM

Prepare a stock solution of 100mM PDADMAC and PSS in distilled water. Dilute the stock solution to reach the concentration of 10mM and adding 1M NaCl to adjust the ionic strength. After dipping the glass slide in each solution for 1 min, rinse with water for 1 minute (1 time) and finally dry at air conditions.

The PEM were fabricated by increasing the layers of 10mM PDADMAC/PSS containing 1M NaCl on quartz slide. The deposition time of each layer is 1 min and then, rinses the PEM in water 1 min for 1 time. The growth of the PEM thin films were characterized by UV-Vis spectrophotometer to confirm that the film growth with increasing the number of PEM layers.

3.3.1.2 Effect of Polymer Type on the Final Dipped Coating on PEM

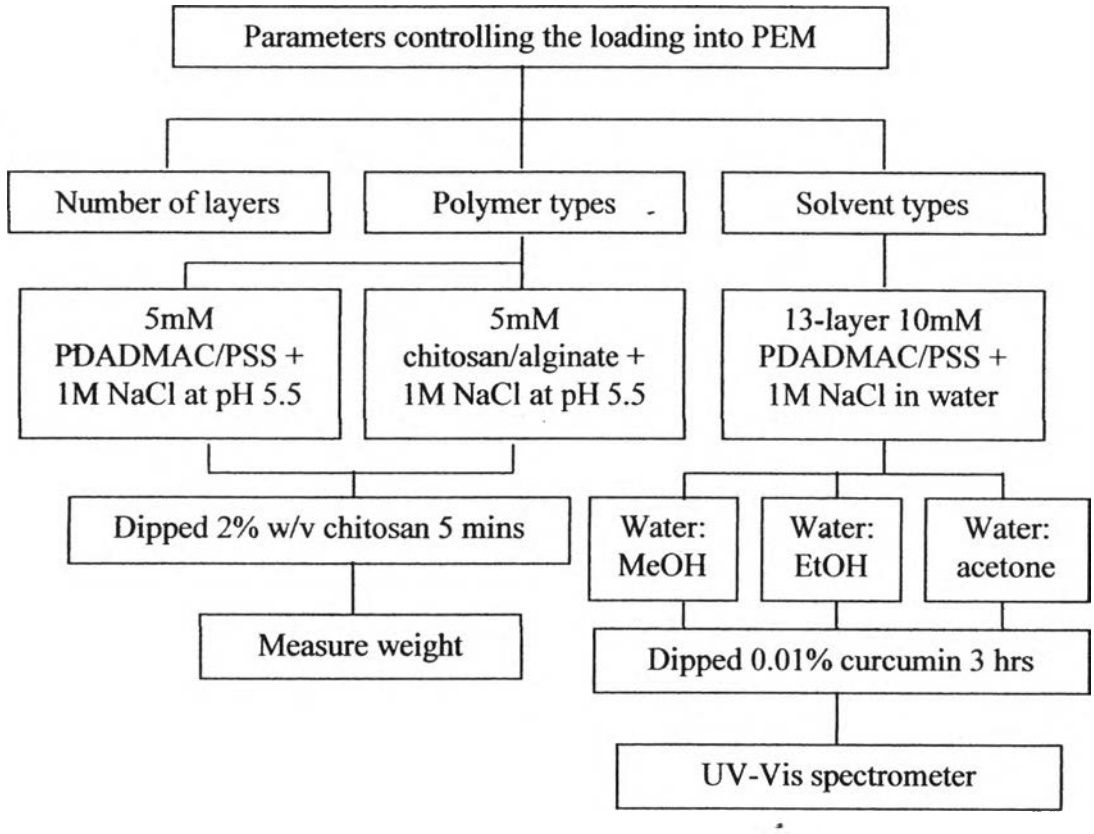
The PEM primers were prepared with two different solution systems: 5mM chitosan/alginate in acetic-acetate buffer pH 5.5 and 5mM PDADMAC/PSS in acetic-acetate buffer pH 5.5 which was coated on the surface of substrate by increasing the number of layers 4 and 8 layers using LbL deposition process. These materials and their order were chosen based on the polyelectrolyte interaction between opposite charges to obtain a stable and uniform coating. Then, adding 1M NaCl into solution and stir to homogeneous. The substrate were dip in to each coating solution for 1 minute and excess coating was allow to drip off for 20 sec (3 times) before submerging the substrate into the next solution.

Prepare 2% w/v chitosan (low molecular weight) solution in acetic-acetate buffer containing dye (Lanaset Bordeaux B.). Chitosan was add to the 0.5% acetic acid while stirring on a magnetic stirrer until total dissolution of the compositions was reached, then adjust the solution to has pH 5.5 by acetic-acetate buffer. Dipping the substrate coating with PEM into 2% w/v chitosan for 5 min and dry in air condition. The obtained PEM layers coating with chitosan were characterized by measuring the weight of 2% chitosan dipped on PEM.

3.3.1.3. Effect of Solvent Type on the Loaded of Curcumin into PEM

The PEM primer was prepare with 10mM PDADMAC/PSS in water that coated on glass slide for 13 layers by LbL deposition process. Then, adding 1M NaCl into solution and stir to homogeneous. The glass slide were dip in to each coating solution for 1 minute and excess coating was allow to drip off for 1 minute (1 time) before submerging the substrate into the next solution. Finally, prepare the 0.01% w/v curcumin was disperses in EtOH to get the curcumin solution (keep in dark).

The 13-layer of 10mM PDADMAC/PSS containing 1M NaCl on glass slide loaded with 0.01% w/v curcumin in difference solvent composition 85:15, 90:10 and 95:5 water:solvent which using EtOH, MeOH and acetone as solvent with loading time 3 hours. The final amount of curcumin in PEM was characterized by UV-Vis spectrophotometer (Scheme 3.1).

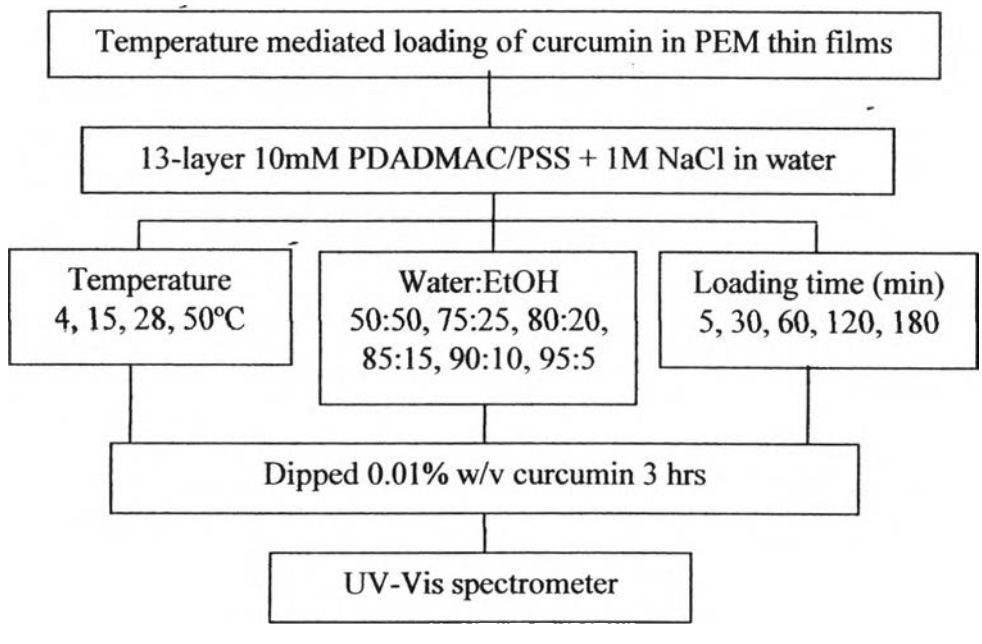


Scheme 3.1 Parameters controlling the loading into PEM.

3.3.2 Temperature Mediated Loading of Curcumin in PEM Thin Films

Prepare the stock solution 100mM PDADMAC and PSS in distilled water. Dilute the stock solution to reach the concentration 10mM and adding 1M NaCl to obtain the ionic strength. After dipped the substrate in each solution for 1 minute, rinse with distillated water for 1 minute (1 time) and finally dry at air conditions.

The PEM were fabricated by 13-layer of 10mM PDADMAC/PSS containing 1M NaCl on glass slide with deposition time 1 minute and rinse in water 1 minute for 1 time. To study the loading of 0.01% curcumin in water:EtOH solvent by various the solvent composition to 50:50, 75:25, 80:20, 85:15, 90:10 and 95:5. The PEM films were dipped in each solution at various temperature as 4, 15, 28 and 50°C. Finally, measure the absorbance at 440 nm at various loading time begin with 5, 30 minutes, 1, 2 and 3 hours to study the kinetic of loading curcumin in PEM. The effect of water:EtOH composition and loading time at increasing temperature were study by UV-Vis spectrophotometer (Scheme 3.2).



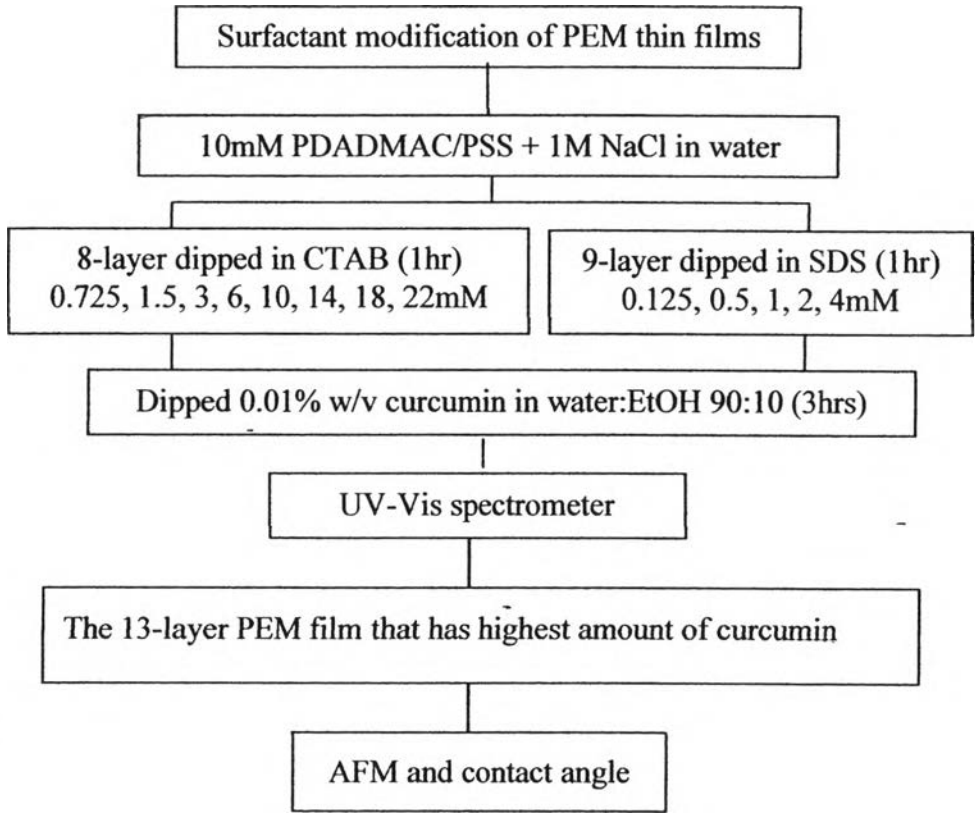
Scheme 3.2 Temperature mediated loading of curcumin in PEM thin films.

3.3.3 Surfactant Modification of PEM

The PEM primer was prepared with 10mM PDADMAC/PSS in distillated water that coated on glass slide. Then, adding 1M NaCl into solution and stir to homogeneous. The substrate were dip in to each coating solution for 1 minute and excess coating was allow to drip off for 1 minute (1 time) before submerging the substrate into the next solution. The 8 and 9 layers PEM were prepared on glass slides to dipped in surfactant solution.

Prepare surfactants solution sodium dodecyl sulfate (SDS) in water in concentration of 0.725, 1.5, 3, 6, 10, 14, 18 and 22mM. (CMC of SDS is about 6-8mM). Dipping the glass slide coating with 9-layer PEM into various concentration of SDS for 1 hour rinse in water and dry at air condition. Prepare surfactants solution hexadecyltrimethylammonium bromide (CTAB) in water in concentration of 0.125, 0.25, 0.5, 1, 2 and 4mM. (CMC of CTAB is about 1mM). Dipping the glass slide coating with 8-layer PEM into various concentration of CTAB for 1 hour rinse in water and dry at air condition.

Prepare stock 0.01% w/v curcumin in ethanol EtOH. Dilute curcumin solutions by add 2ml 0.01% w/v curcumin in 40ml of water:EtOH (90:10). Dipping the glass slide coating with PEM and dipped in surfactant into curcumin solution for 3 hours and dry at air condition. The loading of curcumin in PEM was characterized by using UV-Vis spectrophotometer for study the amount of curcumin in PEM. Then, study the morphology of 13-layer PEM that has highest amount of curcumin by AFM and report the hydrophobicity of film surface by contact angle measurement compare to the PEM and PEM with out surfactant treatment. (Scheme 3.3)



Scheme 3.3 Surfactant modification of PEM thin films.