CHAPTER III
EXPERIMENTAL

3.1. Materials

3.1.1. Chemicals

1) Alginic acid sodium salt, ALDRICH, U.S.A
2) Boric acid, H_3BO_3, EMSURE
3) Calcium chloride, $CaCl_2$, ERBA
4) Chitosan, CHI, low molecular weight, ALDRICH, U.S.A
5) Curcumin, MERCK
6) Glutaraldehyde, MERCK
7) Poly(diallydimethylammonium chloride), PDADMAC, medium molecular weight, 20 wt.% in water, Mw=200,000-350,000, ALDRICH
8) Poly(sodium 4-styrenesulfonate), PSS, Mw=70,000, ALDRICH
9) Potassium Dihydrogen Phosphate, KH_4PO_4, CARLO ERBA
10) Poly(vinyl alcohol), PVA, ALDRICH 89%
11) Sodium Chloride, NaCl, CARLO ERBA, 99.5%
12) Sodium Hydroxide, NaOH, CARLO ERBA

3.1.2 Solvents

1) Acetic Acid, CH_3COOH, glacial, 2.5 L, CARLO ERBA, >99.9%
2) Ammonia, APPLICHEM PANREAC, 30%
3) Ethanol, VRBIOSCIENCE
4) Hydrochloric Acid, HCl, LAB SCAN, 37%
5) Hydrogen Peroxide, aqueous solution, CHEM-SUPPLY, 35%
6) Sulfuric Acid, CARLO ERBA, 96%

3.2. Equipment

1) UV-vis spectrophotometer
2) Scanning Electron Microscope

3.3. Experiment Procedures

3.3.1. Cleaning the Substrate

Glass slides were cleaned from organic contaminants by soaking in hot ammonium for 20 minutes. Hot ammonia was prepared by mixing DI water: NH_3:H_2O_2 with the ratio 5:1:1 and solution was heated to 60℃. Next, the glass slides are then soaked in the hot ammonia for 20 minutes. Finally, glass slides were rinse in ethanol (EtOH) and then dried.

3.3.2. Chemical Preparation

3.3.2.1. Preparation of PDADMAC and PSS solutions

Poly(diallyldimethyl ammonium chloride) (PDADMAC) and poly(sodium 4-styrenesulfonate) (PSS) are used as polycations and polyanions respectively. Both 10mM PDADMAC and PSS were dissolved in DI water followed by adding 1 M NaCl; the solution was stirred until it becomes a homogeneous solution.

3.3.2.2. Preparation of Chitosan and Alginate

5mM chitosan was dissolved in acetic acid and 5 mM Alginic acid was dissolved in DI water, followed by adding 1 M NaCl; the solutions were adjusted to pH 5.5 using hydrochloric acid (HCl) and EtOH and stirred until it becomes a homogeneous solution.

3.3.2.3. Preparation of PVA

6 grams of PVA was dissolved in 100ml DI water overnight i.e. 6% wt./v PVA was used in the process.

3.3.2.4. Preparation of Curcumin

Prepare various concentrations of curcumin stock in ethanol (0.01%, 0.05%, 0.1%, 0.2% and 0.3% wt./v). Then, add 1ml curcumin stock to DI water: ethanol mixed solutions where the ratios of the solution were varied.

3.3.3. Part A: PEMU build-up using LbL Deposition

PEMUs are built by following the LbL deposition theory. First, the substrates were dipped into a polycation solution for 1 minute, and then rinsed in three beakers of DI water for 30 seconds each. Second, the substrates were dipped into PSS solution for 1 minute and then rinsed in three beakers of DI water for 30 seconds each as well. The process was repeated to obtain the number of desired layers. Note* All solutions for CHI/ALG PEMUs has to be adjusted to pH 5.5.

3.3.3.1. Loading of Curcumin on PEMU

In this experiment, PDADMAC/PSS-coated glass slides will be dipped in the curcumin solution without pH adjustment for 2 hours to allow adsorption on to the PEMUs. CHI/ALG thin films will be dipped in curcumin solution adjusted to pH 5.5 for 2 hours as well.

3.3.3.2. Investigated Parameters

First, the loading of curcumin was investigated to obtain the most suitable conditions to load curcumin into PEM which includes the solvent composition, loading time, curcumin concentration and number of layers. Second, the pH sensitivity of curcumin loaded thin films were investigated using 10 mM potassium phosphate buffer (KH_2PO_4) adjusted to various pH. All conditions are as shown in Table 1.

Table 3.1 Parameters used in loading of curcumin on PEM and testing pH sensitivity

Parameters (Unit)	Condition
Loading time (minutes)	15, 30, 60, 90, 120, 150, 180, 210, 240
Curcumin concentration (% wt./v)	0.01, 0.05, 0.1, 0.2, 0.3
Number of Layers	5-20
Solvent composition (%water in ethanol)	100, 90, 80, 70, 60, 50, 40, 30, 20, 10
pH of KH_2PO_4	6, 7, 8, 8.5, 9, 9.5, 10

3.3.4. Part B: Constructing PVA/Curcumin Electrospun Fibers

In this part, 6%wt/v PVA was used as the polymer mixed and using various concentrations of curcumin at 0.05%, 0.1%, 0.15%, 0.2%, 0.25% and 0.3%. PVA has hydroscopic property that absorbs water vapor helps keep curcumin moist. In addition, with the ability to absorb surrounding vapors, it could absorb ammonia vapor which increase contact with curcumin; therefore, could increase the pH sensitivity of curcumin. The mixture then undergoes electrospinning process where the conditions are of the following:

Table 3.2 Parameters used in electrospinning process

Parameters	Conditions
Gap Distance	30 cm
Voltage	15kV
Flow rate	0.3ml/hr
Syringe	3ml
Needle size	24G
Spin Time	2hrs

3.3.5. Characterization

UV-Visible spectroscopy would be used to determine the films' optical properties. Scanning electron microscopy (SEM) was used to observe the electrospun fiber morphology.