# CHAPTER III
# EXPERIMENTAL

## 3.1 Materials

Poly(vinyl alcohol) (PVA) (degree of polymerization ≈1600 and degree of hydrolysis ≈ 97.5 to 99.5 mol% with $\bar{M}_n$ = 72000 ) was supplied from Fluka. 5-sulfosalicylic acid was purchased from Fluka and used as the model drug. Glutaraldehyde (50% in water) was purchased from Fluka and used as the crosslinking agent. Sodium acetate (Ajax Chemicals, Australia), sulfuric acid (Merck), methanol (Carlo Erba Reagent), and glacial acetic acid (Merck) were of analytical reagent grade and used without further purification.

## 3.2 Methodology

### 3.2.1 Preparation of Drug-loaded PVA Hydrogels

A weighed amount of PVA powder was dissolved in distilled water at 80 $^0$C for 3 h to prepare a PVA solution at a fixed concentration of 10% w/v. After the solution was cooled down to room temperature, the model drug was loaded at10 wt% (based on the weight of PVA powder) into the PVA solution under constant stirring for 1 h. In order to crosslink PVA, glutaraldehyde was used as the crosslinking agent at various crosslinking ratios. The crosslinking ratio, *X*, is defined as the ratio of moles of crosslinking agent to moles of PVA repeating unit. In addition to adding 25% solution of glutaraldehyde, other solutions used were a 10% solution of sulfuric acid (the catalyst), a 50% solution of methanol (the quencher), and a 10% solution of acetic acid (the pH controller). They were added to the PVA solution in a 2: 1: 2: 3 ratio, respectively. The solution was mixed very slowly to prevent the formation of air bubbles (Peppas *et al.*, 1998). Immediately after mixing the solution, the mixture was cast on the mold (diameter 9 cm, film thickness 0.45-0.50 mm) in a dust-free atmosphere at 60 $^0$C for 3h and then cooled to room temperature.

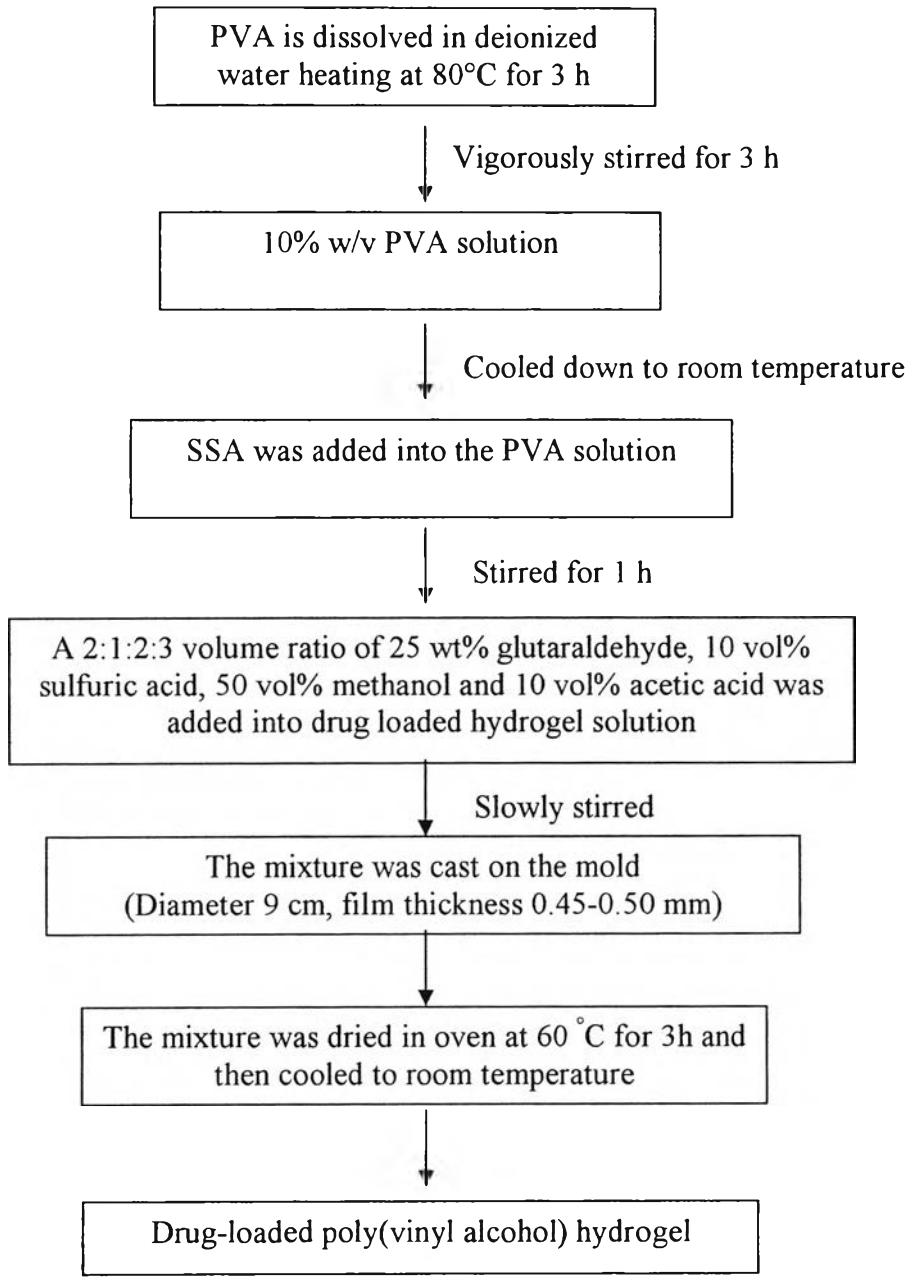


**Figure 3.1** Preparation of drug-loaded PVA hydrogel.

3.2.2 Characterization

*3.2.2.1 Fourier-Transform Infrared Spectrometer (FT-IR)*

A ATR-FTIR spectroscopy (Thermo Nicolet) in the absorption mode with 32 scans at a resolution of 4 $cm^{-1}$ was used to investigate the polymer/drug interaction in the drug-loaded PVA hydrogel. The sample was placed on the crystal and spectra were taken to determine any interactions between the drug and polymer. The observed spectra were in the range of 400-4000 $cm^{-1}$.

*3.2.2.2 Thermogravimeteric Analyzer (TGA)*

The thermal behavior of the PVA hydrogel, the drug, and the drug-loaded PVA hydrogel were determined by a thermalgravimetric analyzer (TG-DTA, Perkin Elmer). Sample was weighted at 5-10 mg and put in a titanium pan. The TGA thermogram was obtained during heating from 30 to 600 $^0C$ at a rate of 10 $^0C\ min^{-1}$ under nitrogen purge (200 ml $min^{-1}$).

*3.2.2.3 Differential Scanning Calorimeter (DSC; Mettler Toledo 822e/400)*

The thermal behavior of the PVA hydrogel, the drug, and the drug-loaded PVA hydrogel were determined by a differential scanning calorimeter (DSC; Mettler Toledo 822e/400). The DSC thermogram (equilibrated with an indium standard; each sample weighed 3–5 mg) was obtained during heating from 25 to 350 $^0C$ at a heating rate of 10 $^0C\ min^{-1}$ under nitrogen purge (60 ml $min^{-1}$).

*3.2.2.4 Scanning Electron Microscope (SEM)*

Scanning electron micrographs were taken with a JEOL, model JSM-5200 scanning electron microscope to determine the morphology and surface appearance of each crosslinked PVA hydrogel. The hydrogel was immersed in distilled water at 37 $^0C$, under the electric field of 0, 1 and 5 V before it was rapidly frozen in liquid nitrogen then dried it in the vacuum chamber at -50 $^0C$. Figure 3.2 shows the schematic diagram of swelling of hydrogel under the action of electric field. After Freeze-Dry process, a piece of sample was placed on the holder with an adhesive tape and coated with a layer of gold by using a JFC-1100E ion-sputtering device for 4 min. The scanning electron micrographs of crosslinked PVA hydrogels were obtained by using an acceleration voltage of 15 kV with a magnification of 350 and1500 times.

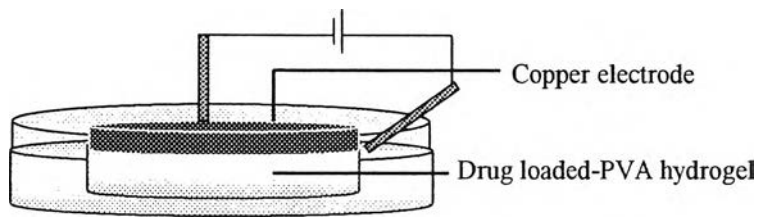


**Figure 3.2** Schematic diagram of swelling of hydrogel under the action of electric field.

3.2.3 Swelling Behavior

The degree of swelling and weight loss of PVA films were measured in acetate buffer solution at 37 $^0$C for 5 days according to the following equations (Taepaiboon et al., 2006):

$$\text{Degree of swelling (\%)} = \frac{M - M_d}{M_d} \times 100 \qquad (1)$$

and

$$\text{Weight loss (\%)} = \frac{M_i - M_d}{M_i} \times 100 \qquad (2)$$

where M is the weight of each sample after submersion in the buffer solution, $M_d$ is the weight of sample after submersion in the buffer solution in its dry state, $M_i$ is the initial weight of the sample in its dry state.

3.2.4 Determinaion of Mc, Mesh Size and Crosslinking Density

To determine the molecular weight between crosslinks, $\bar{M}_c$ the mesh size, $\xi$, and the crosslinking density, a sample of PVA film was cut immediately after crosslinking. This sample was weighted in air and heptane. The sample was then placed in distilled water at 37 $^0$C for 5 days to allow it to swell to equilibrium, and weighted in air and heptane. Finally, the sample was dried at 25 $^\circ$C in vacuum oven for 5 days. Once again, it was weighted in air and heptane. These weights were used

to calculate the polymer volume fraction in the relaxed and swellen states, $\upsilon_{2,r}$ and $\upsilon_{2,S}$, respectively (Peppas *et al.*, 1998).

$$\upsilon_{2,r} = \frac{V_d}{V_r} \tag{3}$$

$$\upsilon_{2,s} = \frac{V_d}{V_s} \tag{4}$$

where $V_d$, $V_r$, and $V_s$ are the volumes of the polymer sample in dry, relaxed, and swollen stated, respectively. The volumes were calculated using following equation which utilize the weights of the dry polymer, $W_d$, the relaxed polymer, $W_r$, and the swollen polymer, $W_s$, in air and heptane and $\rho_h$ is the density of heptane .

$$V_d = \frac{W_{a,d} - W_{h,d}}{\rho_h} \tag{5}$$

$$V_r = \frac{W_{a,r} - W_{h,r}}{\rho_h} \tag{6}$$

$$V_s = \frac{W_{a,s} - W_{h,s}}{\rho_h} \tag{7}$$

The molecular weight between crosslinks, $\bar{M}_c$, was calculated from the swelling data using Eq (8) (Peppas *et al.*, 1998).

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\frac{\bar{\upsilon}}{\bar{V}_1}[\ln(1-\upsilon_{2,S}) + \upsilon_{2,S} + \chi\upsilon_{2,S}^{\,2}]}{\upsilon_{2,r}[(\frac{\upsilon_{2,S}}{\upsilon_{2,r}})^{1/3} - \frac{1}{2}(\frac{\upsilon_{2,S}}{\upsilon_{2,r}})]} \tag{8}$$

where $\bar{M}n$ is the number-average molecular weight of the polymer before crosslinking, $\bar{\upsilon}$ is the specific volume of polymer, $\bar{V}_1$ is the molar volume of the solvent, $\upsilon_{2,r}$ is the volume fraction of the polymer in the relaxed state, $\upsilon_{2,S}$ is the

volume fraction of the polymer in the swollen state, and $\chi$ is the interaction parameter of the polymer-solvent system in water.

The hydrogel mesh size, $\xi$, defines the linear distance between consecutive crosslinks. It indicates the diffusional space available for solute transport and can be calculated using Eq (9) (Hickey *et al.*, 1995).

$$\xi = \upsilon_{2,s}^{-1/3} [C_n(2\bar{M}_c/\bar{M}_r)]^{1/2} l \quad (9)$$

where $C_n$ is the Flory characteristic ratio, $l$ is the carbon-carbon bond length, $\bar{M}_r$ is the molecular weight of the repeating unit of polymer, and $\bar{M}_c$ is the molecular weight between crosslinks.

The crosslinking density of the hydrogel was calculated using Eq (10) (Peppas *et al.*, 1996).

$$\rho_x = \frac{1}{v\bar{M}_c} \quad (10)$$

3.2.5 Drug Release Experiments

*3.2.5.1 Preparation of Acetate Buffer*

Acetate buffer was chosen to simulate human skin pH condition of 5.5. To prepare 1000 ml of the acetate buffer solution, 150 g of sodium acetate was dissolved in distilled water. 15 ml of glacial acetic acid was then added very slowly into the aqueous sodium acetate solution.

*3.2.5.2 Skin Preparation*

Transdermal diffusion experiments were performed using fresh pig skins from the abdominal part of pig. The skin used in this work was about 1-1.5 mm thick. The whole pig skins were surgically removed and cleaned with sterile normal saline. The subcutaneous fat, tissue, blood vessel, and epidermal hair were carefully removed by blunt section. The skin was free of obvious holes or defects. The full thickness skin was cleaned with saline and finally with distilled water, cut into circular shape, wrapped with an aluminium foil, and stored frozen before use.

*3.2.5.3 Spectrophotometric Analysis of Model Drug*

A UV/Visible spectrophotometer (Shimadzu, UV-2550) was used to determine the maximum spectra of model drug. Model drug in aqueous solution was prepared for scanning the maximum absorption wavelength. The characteristic peak was observed. The absorbance value at the maximum wavelength of model drug was read and the correspondent model drug concentrations were calculated from the calibration curve with various model drug concentration.

*3.2.5.4 Actual Drug Content*

The actual amount of drug in the drug-loaded PVA hydrogel (circular disc about 2.5 cm in diameter) was quantified by dissolving the sample in 4 ml of dimethylsulfoxide (DMSO) and then 0.5 ml of the solution was pipetted and added into 8 ml of the acetate buffer solution. The drug solution was measured for the amount of drug using the UV/Visible spectrophotometer at a wavelength of 298 nm.

*3.2.5.5 Transdermal Transport Studies*

The custom built modified Franz diffusion cell was used for diffusion studies. The diffusion cell consisted of two compartments; a donor compartment, which is exposed to an ambient condition, and a receptor compartment which was filled with the acetate buffer solution pH 5.5 and maintained at 37 $^{0}$C by a circulating water bath. In the study of effect of crosslinking ratio, a unit of drug-loaded PVA hydrogel with various crosslinking ratios (0, 0.5, 2.5 and 5.0) was placed over the pig skin mounted on the receptor compartment. For the study of effect of electric field, the copper plate was used to distribute the electrical potential (V= 0, 0.5, 1.0, 3.0 and 5.0 Volt) to over all position of the hydrogel. The drug diffused through the polymer matrix and the pig skin towards the solution. The drug concentrations in these samples were determined by the UV/Visible spectrophotometer.

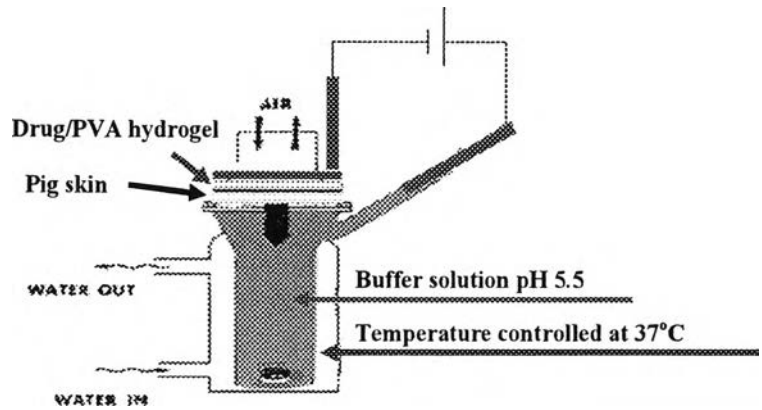


**Figure 3.3** Schematic diagram of experimental set up of transdermal transport studies.