CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Synthesis Polythiophene

In the polymerization process of polythiophene, these chemicals were used: thiophene (Sigma Aldrich) as a monomer, iron (III) chloride (Ajax Chemicals) as an oxidant, acetylsalicylic acid (Sigma Aldrich) as a dopant. Methanol (AR grade, RCI Labscan), chloroform (AR grade, RCI Labscan), hydrogen peroxide, H_2O_2, (AR grade, RCI Labscan), and distilled water were used as solvents.

3.1.2 Polythiophene/Carrageenan Blend Film Preparation

κ-carrageenan (Thai Food and Chemical Co., Ltd.) was used as the polymer matrix. Acetylsalicylic acid (Sigma Aldrich) was used as the model drug. Barium chloride, calcium chloride, and magnesium chloride (Ajax chemicals) were used as the crosslinking agent. 2-(N-morpholino) ethanesulfonic acid, MES, (Sigma Aldrich) was used as the buffer solution.

3.2 Methodology

3.2.1 Preparation of Acetylsalicylic Acid-Loaded Carrageenan Hydrogels (ASA-loaded Carrageenan Hydrogels)

Carrageenan powder was dissolved in distilled water under stirring at 60 °C to prepare a carrageenan solution at concentration of 1.3 %w/v. Then, 2.5 %wt (based on the weight of carrageenan) of acetylsalicylic acid was added into the carrageenan solution under a constant stirring. The salt solution ($CaCl_2$, $MgCl_2$, and $BaCl_2$) as a crosslinker was added into the solution at various crosslinking ratios (moles of crosslinker to moles of ester sulfated group monomer units) and then cast onto a mold (8 cm diameter) at room temperature.

3.2.2 Preparation of Polythiophene (PTh)

PTh was synthesized via the Fe^{3+}-catalyzed oxidative polymerization according to the method of Sugimoto *et al.* (1986). Thiophene (1 mol) was dispersed into chloroform (50 ml) at constant stirring for 45 min. $FeCl_3$ (5 mol) in 30 ml

chloroform was added to the monomeric solution. The polymerization was allowed to proceed for 24 h with stirring at room temperature. The collected sample was washed with methanol in order to remove the excess $FeCl_3$ and then, the sample was dried at 80 °C for 24 h.

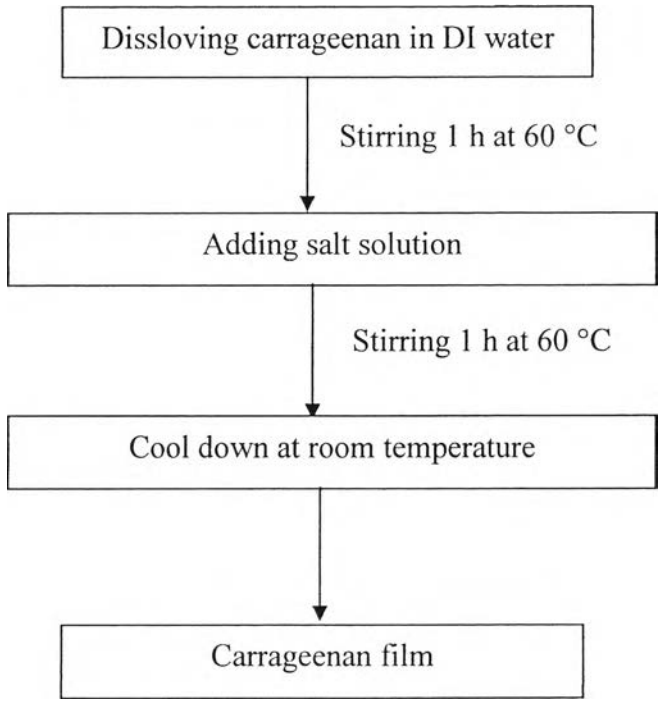
3.2.3 Preparation of Acetylsalicylic Acid-Doped Polythiophene (ASA-doped PTh)

The acetylsalicylic acid-doped polythiophene was prepared by the acid-assisted redox doping reaction according to the method of Sanden *et al.* (1997). 1 g of PTh was stirred with 100 ml of ASA solution and 50 ml H_2O_2 for 24 h ASA-doped PTh particles were filtered and vacuum dried for 24 h.

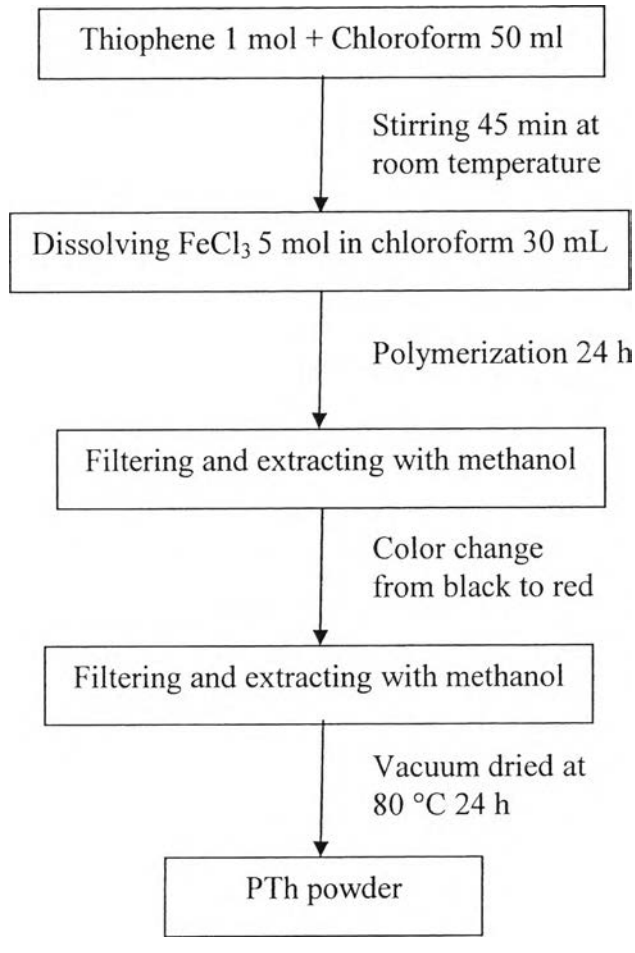
3.2.4 Preparation of Acetylsalicylic Acid-Doped Polythiophene/Carrageenan Hydrogel

The ASA-doped PTh was dispersed into the carrageenan solution, and the mixture was stirred for 30 min. The mixture was cast on the mold (8 cm diameter) and dried at room temperature.

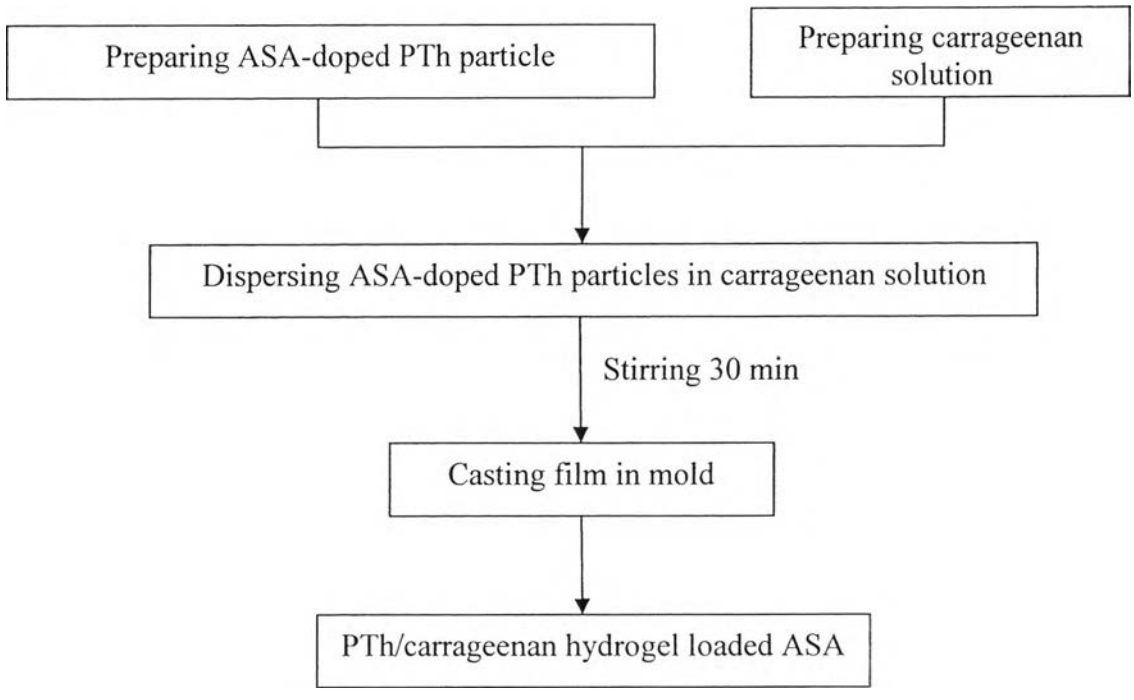
Flow chart of fabricating carrageenanhydrogel



Flow chart of chemical synthesis for PTh



Flow chart of prepare of ASA-loaded carrageenan hydrogel



3.3 Characterizations

3.3.1 Fourier Transforms Infrared Spectrometer (FTIR)

The FTIR spectrometer (Thermo Nicolet, Nexus 670) was used to identify the functional groups of synthesized PTh and doped PTh and an ATR-FTIR spectrometer was used to investigate interactions between the drug-loaded carrageenan and drug-PTh loaded carrageenan, the interaction can be observed from the shifts in the function groups. FTIR was carried out in the transmission mode with 64 scans between 4000-400 cm^{-1} at a resolution of 4 cm^{-1}.

3.3.2 Thermo Gravimetric Analyzer (TG-DTA)

Thermal gravimetric analyzer (TG-DTA, Perkin Elmer) was used to investigate weight loss of volatile molecules, the amount of residual water, and the degradation temperatures of the PTh, doped PTh, and the drug with a temperature scan from 30 to 800 °C and with a heating rate of 10 °C/min under nitrogen atmosphere. The samples were weighed in the range of 4-10 mg and loaded into a platinum pan.

3.3.3 Scanning Electron Microscope (SEM)

A Scanning Electron Microscope or SEM (JEOL, model JSM-5200) was used to investigate surface morphology of PTh, doped PTh, and morphology of crosslinked carrageenan hydrogels. The hydrogels were immersed in distilled water at 37°C, before it was rapidly frozen in liquid nitrogen then dried it in the vacuum chamber at -50°C. After a freeze-dry process, the samples were placed on the holder with an adhered tap and coated with thin layer of platinum prior measurement.The scanning electron micrographs of crosslinked carrageenan hydrogels were obtained by using an acceleration voltage of 5 kV at magnifications of 120x.

3.3.4 Ultraviolet-Visible Spectrophotometer (UV-VIS)

A UV-VIS spectrophotometer (TECAN, Infinite M200) was used to determine the spectra of the acetylsalicylic acid at wavelength 230 nm in order to obtain the calibration curve to determine the amount of drug release.

3.3.5 Two-point Probe Meter

The specific conductivity, which is the inversion of specific resistivity (ρ) of undoped PTh and doped PTh pellets was measured by using the two-point probe meter connected with a voltage supplier (Keithley, Model 6517A) whose constant voltage can be varied and the current was measured. The thickness of pellets was measured by a thickness gauge. The regime where responsive current is linearly proportional to the applied voltage, called the linear Ohmic regime, which can be identified by plotting the applied voltage against the current. The voltage and the current in the regime were converted to the electrical conductivity of the polymer by using Eq. (3.1) as follows:

$$\sigma = 1/\rho = 1/(R_s \times t) = I/(K \times V \times t) \qquad (3.1)$$

where σ = specific conductivity (S/cm.)

ρ = specific resistivity (Ω.cm.)

R_s = sheet resistivity (Ω)

I = measured current (A)

K = geometric correction factor = 4.29×10^{-4}

V = applied voltage (voltage drop) (V)

t = pellet thickness (cm.)

The geometric correction factor was taken into account geometric effects, depending on the configuration and probe tip spacing. The geometric correction factor was determined by using the standard materials with known specific resistivities. The sheet resistivity obtained from the two-point probe meter was used to calculate the geometric correction factor by the following equation:

$$K = \rho_{ref} / (R_s t) \qquad (3.2)$$

where ρ_{ref} = known specific resistivity from the chemical handbook (Ω.cm.).

3.3.6 Swelling Studies

The carrageenan hydrogels were analyzed for swelling immediately after the crosslinking process, according to the method of Gudeman and Peppas (1995). To determine the molecular weight between crosslinks, $\bar{M}_c$, the mesh size, ξ, and the crosslinking density, ρ_x, the sample of carrageenan film was cut immediately after crosslinking (1 cm^2). This sample was weighted in air and heptane. The sample was then placed in distilled water at 37 °C for 5 days and allowed to swell to equilibrium, then weighted in air and heptane again. Before weighting, the sample was blotted with tissue paper to remove residue surface water. Finally, the sample was dried at 25°C in a vacuum for 5 days. Once again, it was weighted in air and heptane. The equilibrium swelling ratio, the polymer volume fraction in the relaxed and swollen states were calculated using the weights measured.

The membranes were prepared and their polymer volume fraction in the relaxed was calculated using Eq. (3.3). After each membrane swelling to equilibrium at 37 °C, the polymer volume fraction of the swollen polymer was calculated using Eq. (3.4):

$$\upsilon_{2,r} = \frac{V_d}{V_r} \tag{3.3}$$

$$\upsilon_{2,s} = \frac{V_d}{V_s} \tag{3.4}$$

where V_d = the volumes of the polymer sample in the dry states

V_r = the volumes of the polymer sample in the relaxed states

V_s = the volumes of the polymer sample in the swollen states

$\upsilon_{2,r}$ = the polymer volume fractions of the relaxed polymer gel

$\upsilon_{2,s}$ = the polymer volume fractions of the swollen polymer gel

The volumes of the polymer sample in the dry, relaxed, and swollen states are calculated using Eqs. (3.5) - (3.7), respectively:

$$V_d = \frac{W_{a,d} - W_{h,d}}{\rho_h} \tag{3.5}$$

$$V_r = \frac{W_{a,r} - W_{h,r}}{\rho_h} \tag{3.6}$$

$$V_s = \frac{W_{a,s} - W_{h,s}}{\rho_h} \tag{3.7}$$

where W_d = the weights of the dry polymer in air and heptane

W_r = the weights of the relaxed polymer in air and heptane

W_s = the weights of the swollen polymer in air and heptane

ρ_h = the density of heptane

The swelling ratio (Q) was determined from the weight measurement using Eq. (3.8):

$$Q = \frac{1}{\upsilon_{2,s}} \tag{3.8}$$

The molecular weight between crosslinks, $\overline{M}_c$, was calculated from the swelling data using Eq. (3.9):

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{\frac{\bar{v}}{V_1}\left[\ln(1-v_{2,s}) + v_{2,s} + \chi v_{2,s}^2\right]}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \tag{3.9}$$

where $\overline{M}_n$ = the number-average molecular weight of the polymer before crosslinking

$\bar{v}$ = the specific volume of carrageenan = 0.49 ml/g

V_1 = the molar volume of water (18.1 cm^3/mol)

x = the Flory interaction parameter of carrageenan = 0.44 (Wan *et al., 2009*) and the dissociation constant is pKa = 4.7.

In general, the presence of carrageenan leads to a more open network structure and results in a higher $\overline{M}_c$ value. The hydrogel mesh size, ξ, was calculated using Eq. (3.10) (Peppas and Wright, 1996):

$$\xi = \upsilon_{2,s}{}^{-1/3}\left[C_n\left(\frac{2\bar{M}_c}{\bar{M}_r}\right)\right]^{1/2} \cdot l \qquad (3.10)$$

where C_n = the Flory characteristic ratio for carrageenan = 33 (Marcelo *et al.*,2004)

l = the carbon-carbon bond length of the monomer unit = 5.5 Å

The crosslinking density of the hydrogel, ρ_x, was calculated by using Eq. (3.11) (Peppas *et al.*, 1996).

$$\rho_x = \frac{1}{\bar{v}\bar{M}_c} \qquad (3.11)$$

The degree of swelling and the weight loss of carrageenan hydrogel were measured in a MES buffer solution at 37 °C for 2 days, using the following Eqs. (3.12) and (3.13):

$$\text{Degree of swelling (\%)} = \frac{M - M_d}{M_d} \times 100 \qquad (3.12)$$

and

$$\text{Weight loss (\%)} = \frac{M_i - M_d}{M_i} \times 100 \qquad (3.13)$$

where M = the weight of the sample after immersing in the buffer solution

M_d = the weight of the sample after immersing in the buffer solution in its dry state

M_i = the initial weight of the sample in its dry state

3.4 Drug Release Experiments

3.4.1 Preparation of MES buffer

An MES buffer solution was chosen to simulate the human skin pH condition of 5.5. To prepare 200 ml of MES buffer solution, 0.1 M of MES pH 5.5 was poured into the receptor chamber of a modified Franz-diffusion cell.

3.4.2 Spectrophotometric Analysis of Model Drug

A UV-Visible spectrophotometer (UV-TECAN infinite M200) was used to determine the spectra peaks of the model drugs. For each model drug, in an aqueous solution, it was scanned for its maximum absorption wavelength. The characteristic peak was observed at 230 nm for acetylsalicylic acid. The absorbance value at the peak wavelength of the model drug can be correlated with the model drug concentration, thus the calibration curves with the various model drugs were generated.

3.4.3 Actual Drug Content

The actual amount of drug in the drug-loaded carrageenan hydrogels (circular disc about 2.5 cm in diameter) and doped PTh was quantified by dissolving the sample in 5 ml of dimethyl sulfoxide (DMSO) and then 0.1 ml of the solution was added into 0.4 ml of DMSO. The acetylsalicylic acid in the solution were measured by used the UV-Visible spectrophotometer at a wavelength of 230 nm.

3.4.4 Transdermal Transport Studies

Diffusion studies were carried out by using the modified Franz-diffusion cells. The modified Franz-diffusion cell is a vertical diffusion cell, consisting of two half-cells. The first half-cell is the donor half which is exposed to room temperature. Another half-cell is the receptor half which is exposed to MES buffer (pH 5.5) and maintained at 37 °C by a circulating water bath. The ASA diffused through a nylon net (mesh size = 2.25 mm^2) which was placed on top the MES buffer solution. The nylon net was allowed to come into contact with the MES buffer in the receptor chamber; the buffer was magnetically stirred throughout the experiment period (48 h) at a thermostatically maintained temperature. In the study of the effect of crosslinking ratio, a unit of ASA-loaded carrageenan hydrogels with various crosslinking ratios (0.4, 0.6, 1.0, 1.4, and 2.0) was placed on top of a similar

nylon net above the receptor compartment. In the study of the effect of electric field strength on the release of the ASA from the crosslinked carrageenan hydrogel, a cathode electrode (aluminum) was connected to a power supply (KETHLEY 1100V Source Meter), which provided various electrical voltages (V = 0, 0.5, 1.0, 3.0, 5.0, and 7.0 V) across the hydrogel, nylon net, and buffer solution. The total duration of the constant applied electric field strength to the experiment setup was 48 h. The drugs diffused through a polymer matrix and the membrane into the solution. A sample of 0.1 ml was withdrawn at various time intervals and simultaneously replaced with an equal volume of the fresh buffer solution. The drug amount in the withdrawn solution sample was determined by UV-Visible spectrophotometer.

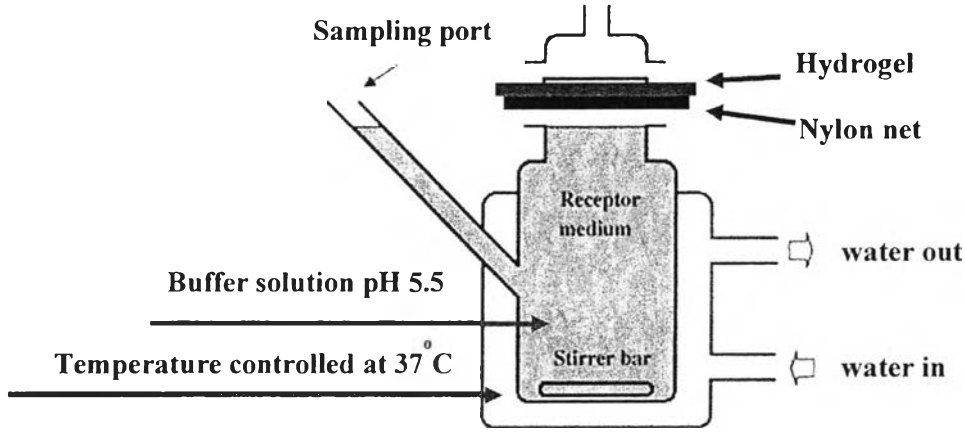


Figure 3.1 Schematic diagram of experimental set up of transdermal transport studies.