CHAPTER III
EXPERIMENTAL

3.1 Materials

3.1.1 Polypyrrole Synthesis

In the polymerization process of polypyrrole, these chemicals were used: pyrrole (Fluka) as a monomer, ammoniumpersulfate (MERCK) as an oxidant, 5-sulfosalicylic acid (Fluka) as a dopant. Methanol (AR grade, Fluka), Acetone (AR grade, Fluka), and distilled water were used as solvents.

3.1.2 Polypyrrole /Poly(acrylic acid) Blend Film Preparation

Acrylic acid (Aldrich) was used as the polymer matrices. 5-sulfosalicylic acid (Fluka), a model drug, was used in the symptomatic management of painful and inflammatory conditions. Ethylene glycol dimethacrylate, EGDMA (Aldrich) was used as the crosslinking agent. Sodium acetate (Ajax Chemicals, Australia) and glacial acetic acid (Fluka) were of analytical reagent grade and used without further purification.

3.2 Methodology

3.2.1 Preparation of Drug-Loaded Poly(acrylic acid) Hydrogels (Peppas and Wright, 1996)

We mixed distilled acrylic acid (AA) and water in a 1:1 ratio. Ethylene glycol dimethacrylate used as crosslinking agent, was added to the solutions at various amounts of 0-2.5% and thoroughly mixed. A quantity of 1% azoisobuthylonitrile (AIBN) was added to initiate the reaction. The model drug was added into the PAA solution under constant stirring for 1 hour. Their solutions were cast on a mold (diameter 9 cm) and dried in a vacuum oven at 60 °C for 12 hours.

3.2.2 Preparation of Polypyrrole (Prissanroon *et al.*, 2000)

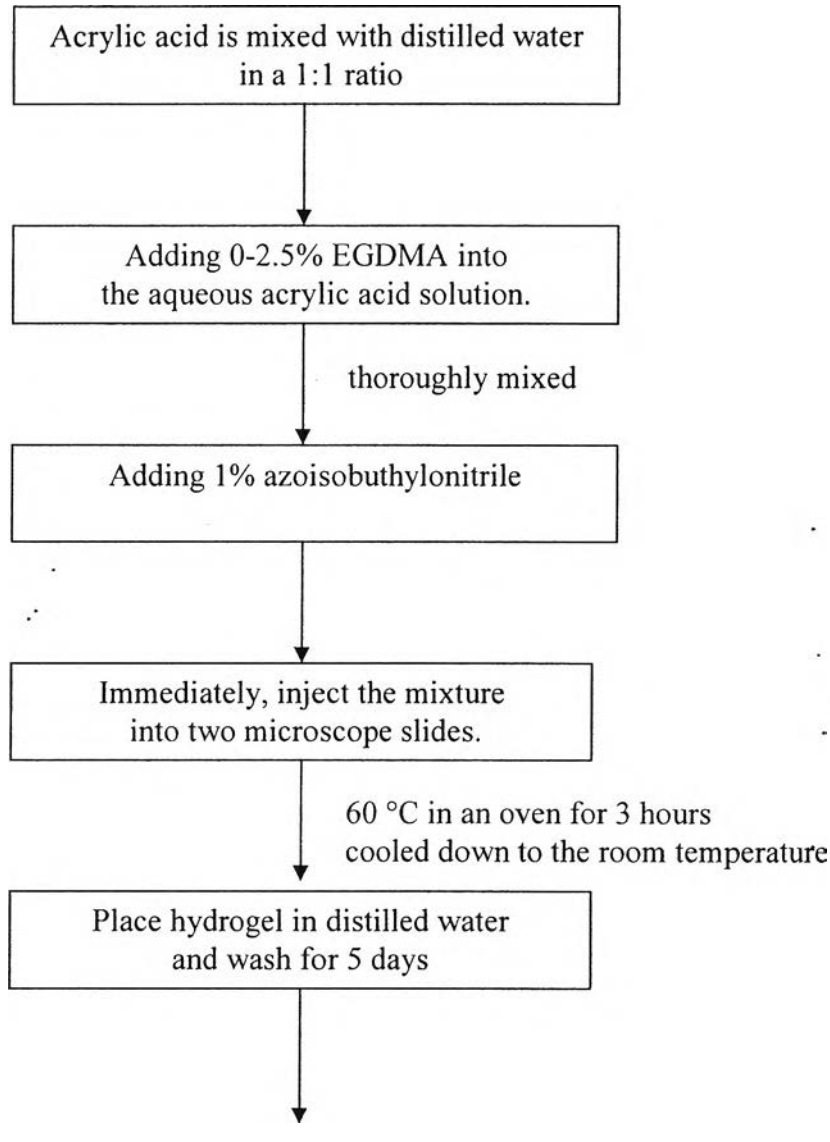
Pyrrole monomer was dried with CaH_2 at the ratio of 100 g of CaH_2 per litre of pyrrole for 24 hours and purified by distillating pyrrole under the reduced

pressure before use. The doped polypyrrole (PPy) with various dopant anions was chemically synthesized by the *in situ* doped, oxidative coupling polymerization [6]. 0.3 mole of dried pyrrole monomer and 0.15 mole of model drug were dissolved in 500 ml of distilled water. The mixture was stirred vigorously for 15 min at 0 °C in an ice bath. 0.06 mol of ammonium persulfate (APS) in 100 ml distilled water was slowly added to the mixture solution at a rate of 5 ml/min and the temperature maintained at 0 °C. Reaction was carried out for approximately 40 hours and then terminated by pouring 20 ml of methanol into the mixture. The resultant black polypyrrole powder was filtered and washed sequentially with 50 ml of distilled water, 50 ml of methanol and 50 ml of acetone. The washing procedure described was repeated again followed by filtering and drying in a vacuum oven at 25 °C for 24 hours. Polypyrrole powder synthesized was then stored in a desiccators. For synthesizing the undoped polypyrrole, the procedure was the same as that of the doped polypyrroles except that dopant was not added into the mixture.

3.2.3 Preparation of Drug-loaded Polypyrrole/Poly(acrylic acid) Blend Films

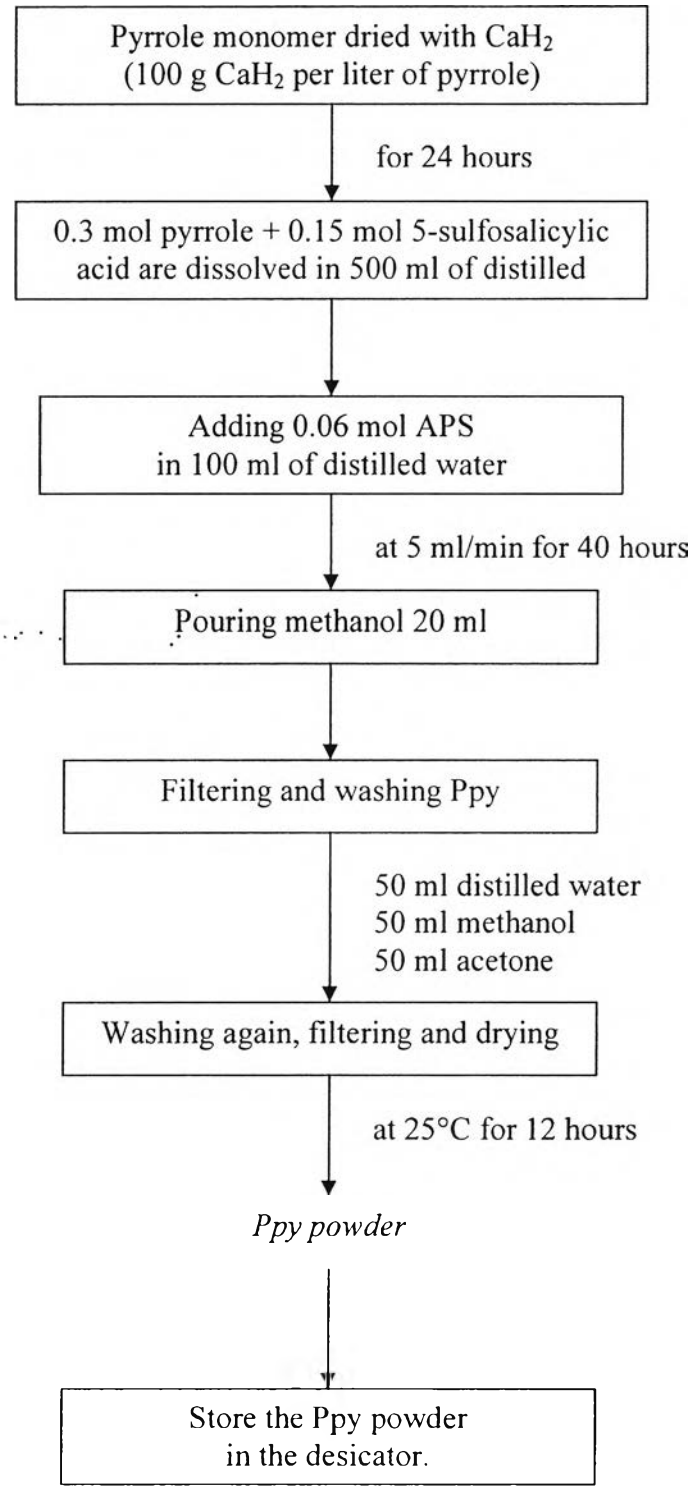
The polypyrrole powder dried at room temperature for 12 hours prior to using. The blends were prepared by mechanical blending of doped synthesized polypyrrole. 0.3 g of polypyrrole and acrylic solution were mixed and the mixture was mechanically stirred for 3 hours to disperse the particles. The mixture was cast on the mold (diameter 9 cm) and specimens were dried in a vacuum oven at 60 °C for 12 hours.

Flow chart of synthesis poly(acrylic acid) hydrogel

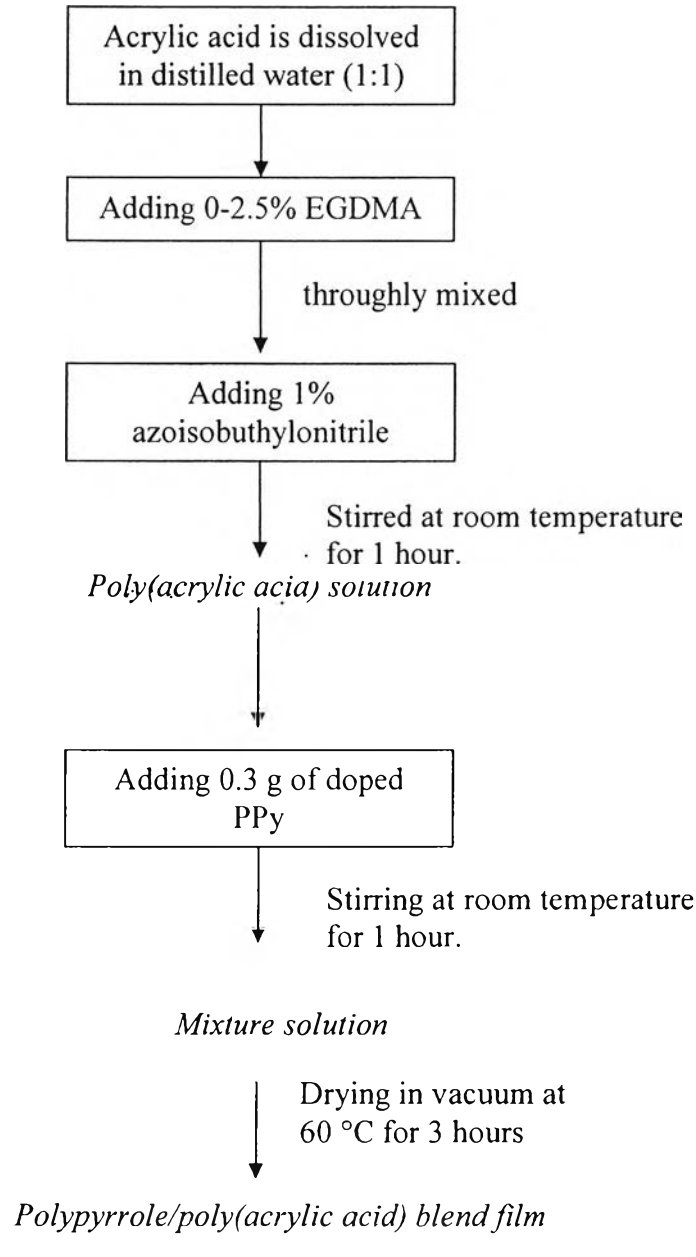


Hydrogel of poly(acrylic acid)

Flow chart of chemical synthesis for polypyrrole



Flow chart of blending process



3.3 Characterizations

3.3.1 Fourier Transforms Infrared Spectrometer (FTIR)

The FTIR spectrometer (Bruker, Equinox 55/FRA 1065) was used to identify the functional group of synthesized PPy and doped PPy and an ATR-FTIR spectrometer was used to investigate interaction between the drugs and the blend films. FTIR is carried out in the transmission mode with 32 scans and a resolution of ± 4 cm^{-1}between 4000-400 cm^{-1} using a deuterated triglycine sulfate detector. Optical grade KBr (Carlo Erba Reagent) was used as the background material.

3.3.2 Thermal Gravimetric Analyzer (TG-DTA)

The weight loss of volatile molecule, the amount of residual water and degradation temperature of the PPy, doped PPy, the drug, PAA hydrogel, the drug-loaded PAA hydrogel and the drug-loaded PPy/PAA blend film can be determined by the thermal gravimetric analyzer (TG-DTA, Perkin Elmer) at the temperature scan from 30 to 600 °C and a heating rate of 10 °C/min under nitrogen atmosphere. The samples were weighed in the range of 7-13 mg and loaded into a platinum pan.

3.3.3 Differential Scanning Calorimeter (DSC)

The melting temperature of the PPy, doped PPy, the drug, PAA hydrogel, the drug-loaded PAA hydrogel and the drug-loaded PPy/PAA blend film were determined by a differential scanning calorimeter (DSC; Mettler Toledo 822e/400) at 25 to 350 °C and a heating rate of 10 °C/min under nitrogen purge (60 ml/min). The samples were weighed in the range of 3-5 mg and equilibrated with an indium standard.

3.3.4 A Particle Size Analyzer (PSA)

A particle size analyzer (Malvern Instruments Ltd., Masterizer X) was used to determine particle sizes of polypyrrole. The lenses used in this experiment were 45 mm. The sample was placed in a sample cell across a laser beam. This instrument measured the average particle size and the standard size distribution.

3.3.5 Scanning Electron Microscope (SEM)

A scanning electron microscope or SEM (JEOL, model JSM-5200) was used to investigate surface morphology of PPy and doped PPy at magnification

x3500 and x1500. The sample was fixed on the holder with an adhesive cabon tape and gold sputtered for 4 min.

3.3.6 Ultraviolet-Visible Spectrophotometer (UV-VIS)

A UV-VIS spectrophotometer (PERKIN ELMER, Lambda 10) was used to determine the spectra of the model drug at wavelength 298 nm in order to obtain the calibration curve for the amount of drug released.

3.3.7 Two-Point Probe Meter

The specific conductivity values of the undoped and the doped PPy were measured by a custom-built two point probe (Keithley, Model 6517A). The Electrical conductivity is the inversion of specific resistivity (ρ) which indicates the ability of material to transport electrical charge. The meter consists of two probes for PPy particles and doped PPy. This probe was connected to a source meter for a constant voltage source and for reading current. The applied voltage was plotted versus the current change to determine the linear Ohmic regime of each sample. The applied voltage and the current change in the linear Ohmic regime were converted to the electrical conductivity of polypyrrole using equation (3.1) as follow:

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

where σ = specific conductivity (S/cm.)

ρ = specific resistivity (Ω.cm.)

R_s = sheet resistivity (Ω)

I = applied current (A)

K = geometric correction factor

V = voltage drop (V)

t = film thickness (cm.)

The geometric correction factor will take into account geometric effects, depending on the configuration and probe tip spacing. The geometric correction factor is determined by the standard materials which known the specific resistivities. The sheet resistivity that obtained from the four-point probe meter is used to calculate the geometric correction factor by the follow equation:

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

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

3.3.8 Swelling Studies

The poly(acrylic acid) hydrogels were analyzed by swelling studies immediately after the crosslinking process, according to the method of Gudeman and Peppas (1995). To determine the equilibrium swelling ratio, Q, a sample of the hydrogel (1 cm^2 square) was cut and weighed in air and heptane (a non-solvent). The sample was placed in a stainless steel mesh basket which was suspended with heptane. The sample was then placed in the buffer solutions at 37 °C for 5 days to allow swelling towards equilibrium, and was weighed 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 weighed in air and heptane. The equilibrium swelling ratio and the polymer volume fraction in the relaxed and swollen states are calculated using the weights measured.

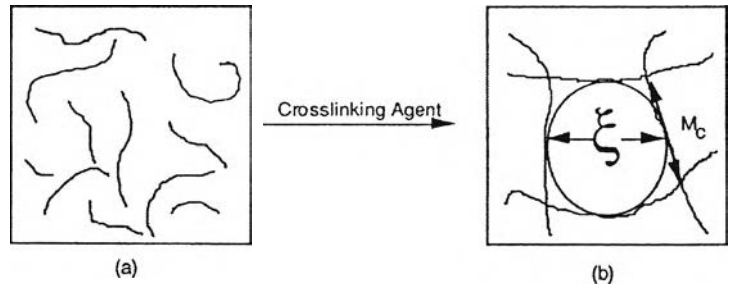


Figure 3.1 Schematic Cross-linking of homopolymers. A cross-linking agent is added to the polymer in solution: (a) to produce a cross-linked network; and (b) with a defined mesh size, ξ, and molecular weight between crosslinks, $\overline{M}_c$ (Peppas *et al.*, 1998).

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

$$\upsilon_{2,r} = \frac{Vd}{Vr} \qquad (3.3)$$

$$\upsilon_{2,s} = \frac{Vd}{Vs} \tag{3.4}$$

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 Eq. (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 $W_{a,d}$, $W_{h,d}$ = the weights of the dry polymer in air and heptane.

$W_{a,r}$, $W_{h,r}$ = the weights of the relaxed polymer in air and heptane.

$W_{a,s}$, $W_{h,s}$ = the weights of the swollen polymer in air and heptane.

ρ_h = the density of heptane.

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

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

The molecular weight between cross-links, $\overline{M}_c$, is calculated from the swelling data using Eq. (3.9) (Peppas and Merrill, 1763):

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\frac{\bar{v}}{\bar{V}_1}[\ln(1 - v_{2,s}) + v_{2,s} + \chi v_{2,s}^2]}{v_{2,r}\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \qquad (3.9)$$

where $\bar{M}_n$ = the number-average molecular weight of the polymer before cross-linking (75000)

$\bar{v}$ = the specific volume of PAA (0.951 cm^3/g)

$\bar{V}_1$ = the molar volume of water (18.1 cm^3/mol)

x = the Flory interaction parameter of PAA (0.5)

and the dissociation constant is p*K*a = 4.7.

In general, the presence of PAA leads to a more open network structure and result to higher $\bar{M}_c$ values. The hydrogel mesh size, ξ, is calculated using Eq. (3.10) (Peppas and Wright, 1996)

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

where C_n = the Flory characteristic ratio for PAA (= 6.7).

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

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

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

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

and

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

where M = the weight of each sample after submersion in the buffer solution.

M_d = the weight of sample after submersion 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 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.

3.4.2 Spectrophotometric Analysis of Model Drug

The UV/Visible spectrophotometer (Shimadzu, UV-2550) was used to determine the maximum spectra of the model drug. Model drug in aqueous solution was prepared for scanning the maximum absorption wavelength. The characteristic peak was observed at 298 nm. The absorbance value at the maximum wavelength of model drug was read and the correspondent model drug concentration was calculated from the calibration curve at various model drug concentrations.

3.4.3 Actual Drug Content

The actual amount of drug in the drug-loaded PAA hydrogel(circular disc about 2.5 cm in diameter) and doped PPy were measured by dissolving the sample in 5 ml of dimethylsulfoxide (DMSO) and then 0.1 ml of the solution was added into 0.4 ml of DMSO. The drug solution was measured for the amount of drug by using the UV/Visible spectrophotometer at a wavelength of 298 nm.

3.4.4 Diffusion Studies

Diffusion studies are carried out using the modified franz diffusion cells for in vivo studies. 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 (25 °C). Another half-cell is the receptor half which is exposed to 3 ml acetate buffer (pH 5.5) and maintained at 37 °C by a circulating water bath. The polypyrrole/poly(acrylic acid) blend film filled with drug is placed over the net on the receptor half and pressing the drug with the electric potential passing the membrane into the buffer. The DC current of 0.5 mA/cm^2 or less will be used. In the study of effect of crosslinking ratio, a unit of drug-loaded PAA hydrogel of various crosslinking ratios (0, 0.25, 0.5, 0.75, 1, 1.25, 2.0 and 2.5) was placed over the net on the receptor compartment. To apply effect of electric field, the copper plate was used to distribute the electrical potential (E = 1.0 Volt) to over all position of the hydrogel. The ultraviolet-visible light spectrophotometer is used to measure the absorbance of the samples taken from the receptor half-cell. Samples are collected from the receptor half-cell at every hour and using a calibration curve derived from known concentrations of the drug solutions, the concentration of each sample taken from the receptor half-cell could be determined.

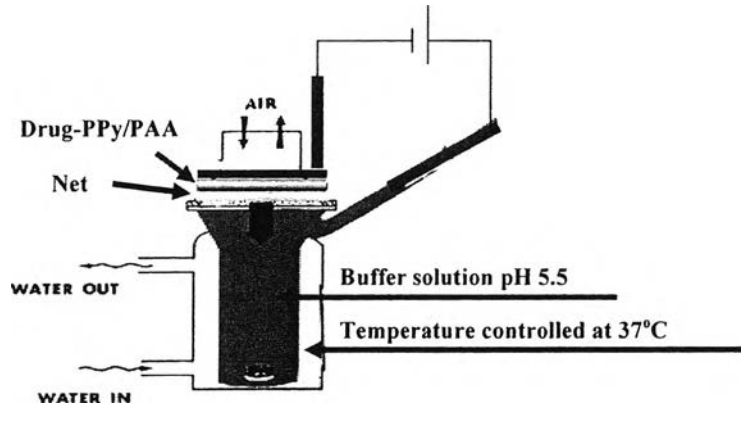


Figure 3.2 Schematic diagram of experimental set up of diffusion studies.