

# CHAPTER III

## EXPERIMENTAL

### 3.1 Materials

The following materials were obtained from commercial suppliers as follows:

- Chitosan, food grade, Mw = M.W. 50,000 – 300,000, Deacetylation 90% (Bonafides, Thailand)
- Poly(acrylic acid), 35 wt% solution in water, Mw=250,000 (Aldrich, USA.)
- Acetic acid glacial, AR grade (Scharlau, Spain)
- Sodium chloride, AR grade (Merck, Germany)
- Glutaric acid, reagent grade (Aldrich, Hong Kong)
- Glutaraldehyde, reagent grade (Aldrich, USA.)
- Citric acid, reagent grade (Aldrich, Hong Kong)

### 3.2 Instruments

The instruments used in this study are listed in Table 3.1

**Table 3.1** Instruments

Instrument	Manufacture	Model
Fourier transform infrared spectrometer	Nicolet	Impect 4.1
Universal testing machine	Hounsfield	H10KM
Differential scanning calorimetry	Perkin-Elmer	DSC 7
Cyclic voltammetry	Autolab	PGSTAT 30
Digital Multimeter	Pro's KiT	303-150NCS

**Table 3.1** Instruments (continued)

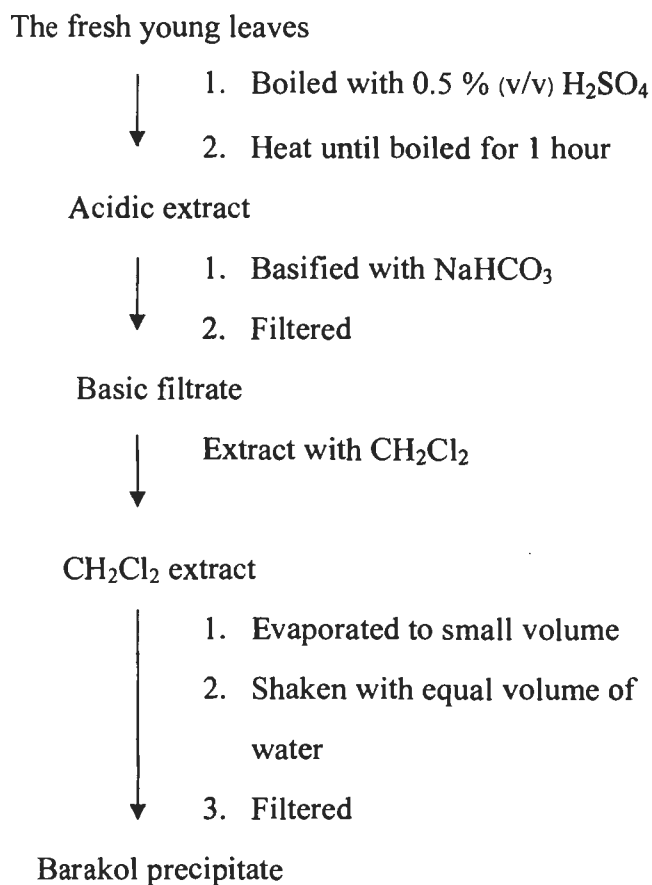
Instrument	Manufacture	Model
Micropipette	Mettler Toledo	Volumate (100-1000 $\mu$ l)
Power Supply	Kenji	Regulated 0-30V 3A
Platinum Wire	Goodfellow Cambridge Limited	Diameter 1 mm Purity 99.95% Temper-Hard
Digital camera	Canon	A80

### 3.3 Procedures

#### 3.3.1 Extraction of Barakol<sup>[41]</sup>

Fresh young leaves of Khilek (*Cassia siamea* Lamk.) were obtained from Bangkok, Thailand. The barakol extraction and purification procedures of *Cassia siamea* Lamk. are presented in Figure 3.1. Two kilograms of fresh young leaves and flowers of *Cassia siamea* Lamk. were sliced into small pieces and boiled with 4 liters of 0.5 % (v/v) aqueous sulfuric acid for 1 hour. It was then cooled to room temperature and filtered. The marc was re-boiled with 2 liters of 0.5 % (v/v) aqueous sulfuric acid, cooled and filtered. The filtrates were combined and basified with sodium hydrogen carbonate to pH 8-9. The basic solution was extracted with dichloromethane (300 ml) three times. The volume was one-fourth of the starting volume, and mixed with the equal volume of distilled water. The mixture was shaken strongly by hand to allow precipitation of the yellow lemon needle crystals, then left the solution in a refrigerator overnight for complete precipitation. The yield was approximately 5 g.

The chemical structure of barakol were characterized by spectroscopic study such as IR, UV-visible,  $^1\text{H-NMR}$ .



**Figure 3.1** The barakol extraction and purification procedures

### 3.3.2 Electrochemical studies

Cyclic voltammograms were obtained in three electrode cell, with a 3.0 mm. diameter glassy carbon working electrode, a platinum wire counter electrode and an  $\text{Ag/AgCl}$  reference electrode. The results were obtained with a GPRES software (Version 4.7). The working electrode was carefully polished before each experiment using a BAS polishing kit with stepwise finer abrasives down to 0.03 and 0.1  $\mu\text{M}$  alumina powder slurry. The electrode was then sonicated in 0.005 M  $\text{H}_2\text{SO}_4$  for 5 minutes and then soaked with acetonitrile. The platinum wire counter electrode was cleaned by immersed in 3 M  $\text{HNO}_3$  for 30 minutes, rinsed with distilled water and wiped to dryness before used. The reference electrode was

cleaned by rinsing with distilled water. Solution of 0.1 M tetrabutyl ammonium hexafluorophosphate (TBAPF<sub>6</sub> in acetonitrile) was used as supporting electrolyte. The background solution contained only 0.1 M TBAPF<sub>6</sub>. For electrochemical investigation of barakol, 1 mM of barakol was prepared by adding dry powder to TBAPF<sub>6</sub> electrolyte solution. Cyclic voltammograms were recorded over of scan rate 0.2 V/s.

### 3.3.3 Preparation of the Hydrogel Films

#### 3.3.3.1 Preparation of the various proportion of chitosan/ poly(acrylic acid) hydrogel films

The hydrogels were prepared with chitosan and poly(acrylic acid) solution in different proportions. The compositions of these formulation studies (A-G) are presented in Table 3.2. The chitosan solutions were prepared by dissolving chitosan in 50 % (w/v) acetic acid to make a 2.5 % (w/v) chitosan solution. Aqueous poly(acrylic acid) solutions (10% (w/v)) were added to the chitosan solutions to make chitosan/poly(acrylic acid) various weight ratio (w/w) of 1/0, 1/1, 2/1, 3/1, 4/1, 5/1 and 0/1. The mixture solution was thoroughly stirred at room temperature until it is a homogeneous, overnight. These mixed solutions were poured into plastic dishes and dried at room temperature for a few days. Transparent, rigid membranes were obtained.

**Table 3.2** Formulation for the various compositions of the chitosan/PAA films

Formulation	Compositions	
	Chitosan	Poly(acrylic acid)
A	1	-
B	1	1
C	2	1
D	3	1
E	4	1
F	5	1
G	-	1

### 3.3.3.2 Preparation of the various amounts of barakol-chitosan/PAA hydrogel films

From the previous study (formulation A-G), it was found that the proportion of chitosan/PAA of 3/1 (w/w) showed the most suitable electrical response behavior. Therefore, the various amounts of barakol were added to 3:1 chitosan/PAA solutions to study the influences of the electrical response behavior.

The preparation of barakol-chitosan hydrogel film is analogous to the procedure as described above (3.3.3.1) except that 0.05-2% (w/w) of barakol was added to the resulting mixture. Table 3.3 shown the formulation (H-N) for the studying the effect of barakol quantities.

**Table 3.3** Formulations for the various compositions of the barakol-chitosan/PAA films

Formulation	Compositions		
	Chitosan	Poly(acrylic acid)	Barakol
H	3	1	0.05
I	3	1	0.1
J	3	1	0.2
K	3	1	0.3
L	3	1	0.4
M	3	1	0.5
N	3	1	2

### 3.3.3.3 Preparation of the cross-linking hydrogel films

From the previous study (formulation H-N), it was found that the proportion of 3/1 (w/w) chitosan/PAA containing 0.3% (w/w) barakol showed the most suitable electrical response behavior. Therefore, the various amounts of crosslinking agents were added to 3/1 (w/w) chitosan/PAA containing 0.3% (w/w) barakol solutions to study the influences of the electrical response behavior.

The preparation of crosslinked chitosan hydrogel film is analogous to the procedure as described above (3.3.3.1) except that various amounts of crosslinking agents were added to the resulting mixture. Table 3.4 shown the formulation (O-U) for the studying the effect of crosslinking agents.

**Table 3.4** Formulations of cross-linking 3:1 chitosan/PAA films

Formulation	Barakol	Crosslinking agents		
		glutaraldehyde	glutaric acid	Citric acid
O	0	1		
P	0.3	0.1	-	-
Q	0.3	0.5	-	-
R	0.3	1	-	-
S	0.3	2	-	-
T	0.3	5	-	-
U	0.3	-	1	-
V	0.3	-	-	1

### 3.3.4 Evaluation of the hydrogel films

#### 3.3.4.1 Fourier transform infrared spectroscopy (FT-IR)

Infrared spectroscopy was used to confirm the functional groups of substances and products after preparation process by observing the positions and intensities of IR peaks.

The infrared spectra of all films were recorded using a KBr disc method. The dried sample was mixed with potassium bromide in agate mortar and pestle by geometric dilution technique. The mixture was then transferred to a hydraulic press to a thin disc. The KBr disc was then measured within the wave numbers of 4000-400  $\text{cm}^{-1}$  in transmittance mode.

### **3.3.4.2 Difference scanning calorimetry (DSC)**

Differential scanning calorimetry analysis was used to characterize the thermal behavior of the different films components.

Approximately 3-6 mg of the dried films was weighed into the aluminum pan that was used in the experiments for all the samples. Then it was crimped with the sealed pan and immediately made a few holes for determinations. An empty pan, sealed in the same way as the sample, was used as a reference. All samples were run at a heating rate of 10°C per minute and in the range of 25°C to 350°C

### **3.3.4.3 Mechanical properties**

A universal testing machine (Hounsfield H10KM) was used to measure the tensile strength and elongation (ASTM D 638 M (1999)) of each sample in dry states with an extension rate of 1 mm/min at room temperature

### **3.3.4.4 Swelling studied**

The swelling behavior of the chitosan/PAA films were studied in the different concentration of sodium chloride solutions by measuring the changeable weight of the films. To measure the swelling ratio, preweighed of 2 mm x 2.5 cm dry samples were immersed in NaCl aqueous solutions of 0.3, 0.6, 0.9, 1.2, 1.5 wt% concentrations. After excessive surface NaCl solution was removed, quickly wiped with filter paper to remove droplets on the surface, and weighed, the weight of the swollen samples was measured at various concentrations. The procedure was repeated until there was no further weight increase and then five more times. The swelling ratio was determined according to the following equation:

$$\text{Swelling ratio (\%)} = \left( \frac{W_s - W_d}{W_d} \right) \times 100$$

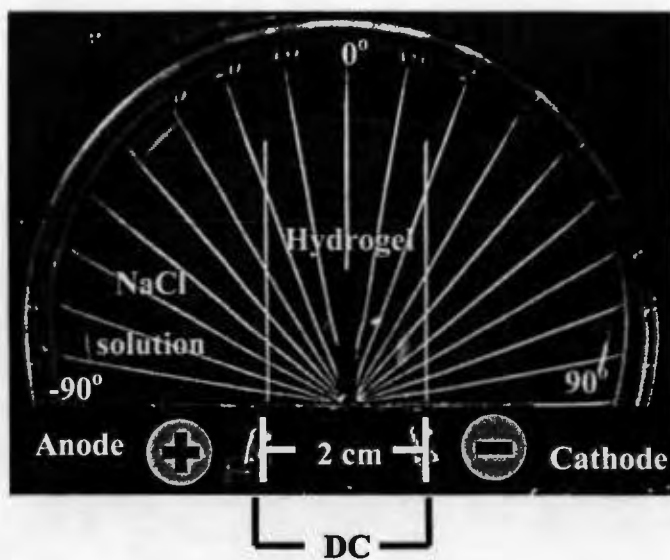
where  $W_s$  and  $W_d$  represent the weight of swollen and dry state samples, respectively.

Experiments were carried out using three replicates, and the swelling degrees are average values.

#### **3.3.4.5 Bending-angle measurement under electrical stimulus<sup>[3]</sup>**

Schematically, the technique for measuring bending angles in the non-contact DC electric field is shown in Figure 3.2. Aqueous NaCl solution was poured into a petri-dish equipped with two parallel platinum electrodes. The space between the electrodes was about 20 mm. The barakol-CS/PAA semi-IPN hydrogels were swelled in NaCl aqueous solutions at room temperature and cut into 25 x 2 mm samples. A hydrogel specimen 25 mm long was placed in the middle of the petri dish filled with 25 ml of solution. To test bending, one end of the specimen was fixed between the two electrodes. A protractor-style scale was drawn on black paper and then placed beneath the petri dish. When the electric stimulus was applied, the angle of bending was measured by reading the deviated angle from the vertical position. The applied electric potentials were set at 25 V. The bath medium were 0.3, 0.6, 0.9, 1.2, 1.5 wt % NaCl solution.





**Figure 3.2** The apparatus for measuring bending angles in the non-contact DC electric field