CHAPTER 2
EXPERIMENT AND METHODS

2.1. Reagents, Apparatus and Instruments

2.1.1. Reagents

The various chemicals used in this research project were shown in Table 2.1.

Table 2.1. The reagents used in the experimental work.

Chemicals	Usage	Supplier
2-acrylamido-2-methylpropane sulfonic acid (AMPS-H^+)	Monomer	Fluka, AG Switzerland and Lubrizol company (United state of America)
N-vinylpyrrolidone (NVP)	Monomer	Sigma-Aldrich
Methacrylic acid (MAA)	Monomer	Fluka, AG Switzerland
N,N′-methylene-bis-acrylamide (NMBA)	Crosslinking agent	Fluka, AG Switzerland
Ethylene glycol dimethacrylate (EGDM)	Crosslinking agent	Fluka, AG Switzerland
4,4′-Azo-bis(4-cyanopentanoic acid)	Initiator	Fluka, AG Switzerland
Sodium hydroxide	Neutralize	Carlo Erba

2.1.2. Apparatus and Instruments

The major items of equipment used in this project were shown in Table 2.2.

Table 2.2. Apparatus and instruments used in this work.

Apparatus and instruments	Model	Company
UV Lamp	MD1-15	Philips
Oxygen Permiometer	210T	Rehder Development Co., USA
Hounsfield Tensometer	HIOKS/0368	Hounsfield
Dionex Ion Chromatograph	DX600	Dionex (UK)
Fourier-Transform Infrared (FT-IR) Spectrometer	510	Nicolet
pH meter	713	Metrohm
Incubator	BM 400	Memmert
Drying oven	FCO-100	Whatman
Vacuum oven	VOS-300SD	EYELA, Tokyo Rikakai Co., Ltd.
Analytical balance	BA 210s	Sartorius Basic
Water vapour transmission test set	-	Yasuda
Hot plate / magnetic stirrer	MR 3001	Heidolph

2.2. Procedures

2.2.1. Preparation of Monomers

2.2.1.1. AMPS-Na^+ Monomer

AMPS-H^+ crosslinking polymerization was carried out in water, used as the solvent and medium, at room temperature at various concentrations ranging from 30-50% w/v. In the case of AMPS-Na^+ at a concentration of 30% w/v, the stock solution was prepared by dissolving 30 g of AMPS-H^+ in about 30 ml of distilled water. Then, the solution was neutralized by titration with 1 M NaOH to pH 7.00 with cooling to prevent of self polymerization [69]. Finally, the volume of the solution was adjusted to 100 ml with distilled water. The 40% w/v and 50% w/v monomer concentrations were prepared using the same procedure simply by changing the weight of the solid monomer AMPS-H^+ to 40 g and 50 g respectively. The apparatus used for preparation of AMPS-Na^+ solution are shown in Fig. 2.1.

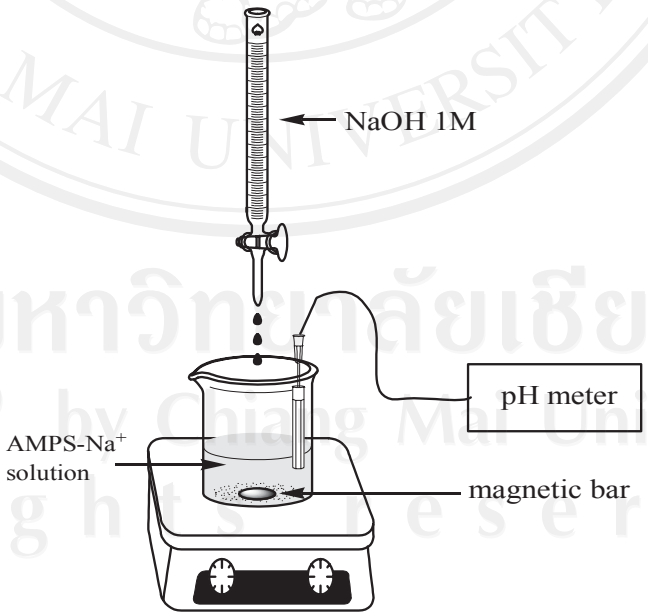

Figure 2.1. Apparatus used in the neutralization of monomer.

2.2.1.2. Purification of *N*-vinylpyrrolidone (NVP) and methacrylic acid (MAA) monomers

Vacuum Distillation of Monomers

The main reason for using vacuum distillation for the purification of the monomers used in this project was that both the NVP and MAA can thermally self-polymerize at their normal boiling points at atmospheric pressure. The vacuum distillation apparatus are shown in Fig. 2.2.

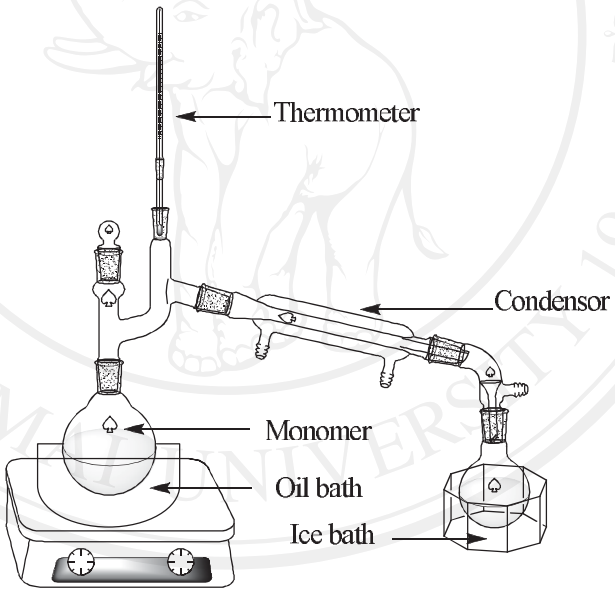

Figure 2.2. Vacuum distillation apparatus used in the purification of monomers.

_N_-vinylpyrrolidone (NVP)

The NVP monomer (Sigma, assay = 95%), as supplied, was first dried with anhydrous sodium sulphate. After occasional tumbling, the monomer was allowed to stand over the drying agent for at least one hour before vacuum distillation.

In the vacuum distillation that followed, a multi-limb receiver adapter was used which allowed more than one fraction to be collected without disturbing the system. After decanting the NVP monomer away from the drying agent, about 1 g/L of copper (I) chloride was added to the monomer as stabilizer, and the monomer was distilled under vacuum. Pure NVP was collected as the constant boiling fraction at 65°C / 2-3 mmHg pressure (cf. lit [93] b.p. 87°C / 5-8.5 mmHg). The initial 10% volume fraction of distillate was discarded. The distilled NVP was then stored in the refrigerator (freezer compartment) until required for use in polymerization.

Methacrylic Acid (MAA)

The MAA monomer (Fluka, assay = 97%), was also purified by vacuum distillation. The procedure used was similar to that for NVP, as described previously. The constant boiling fraction at 90°C / 2-3 mmHg pressure was collected (cf. lit [93] b.p. 60°C / 5-8 mmHg).

2.2.2. Methods of Polymerization

2.2.2.1. UV Initiated Polymerization

Partially hydrated skin adhesive hydrogels were prepared in the presence of an appropriate photoinitiator. In this research work, 4,4′-azo-bis(4-cyanopentanoic acid) was employed as an photoinitiator. In the presence of UV light this chemical dissociates to form free radicals. A typical formulation used to prepare skin adhesive hydrogels consists of hydrophilic monomers. Various concentrations of crosslinker ranging from 0.1-2.5% mole were added as a crosslinking agent and the mixtures stirred to give homogeneous solutions. Then added photoinitiator of 0.1% mole to each solution and poured into a vertical plastic mould with Teflon® release liners. Thickness of hydrogel sheet was controlled by the spacer. Photopolymerization was carried out at room temperature for 10 mins in aluminium cabinet using a commercially available UV lamp (254 nm), as shown in Fig. 2.3. The distance between surface of the mould and UV lamp is approximately 10 cm.

Figure 2.3. Photopolymerization apparatus with aluminium cabinet and UV-lamps.

2.2.2.2. Copolymerization Procedure

The hydrogel sheets were prepared by polymerization of the monomer mixture in the mould. The aqueous AMPS-Na^+ solutions with NVP or MAA were mixed with EGDM or NMBA as crosslinking agent. Then, 0.1% mole of 4,4′-azo-bis(4-cyanopentanoic acid) per mole of monomer was added as photoinitiator and the mixture stirred until homogeneous solutions were obtained [94]. In this project, the various comonomer feed compositions employed are shown in Table 2.3. Finally the hydrated hydrogel sheet, of approximately 1 mm thickness, was removed from the mould and its water absorption properties were studied at 35.0±1.0°C [95].

Table 2.3. Comonomer feed compositions.

Polymers	Comonomer Feed Compositions	
	*AMPS-Na^+ (% mole)	NVP or MAA(% mole)
poly(AMPS-Na^+)	100	0
poly(AMPS-Na^+-co-NVP) (or MAA)	25	75
poly(AMPS-Na^+-co-NVP) (or MAA)	50	50
poly(AMPS-Na^+-co-NVP) (or MAA)	75	25

* AMPS-Na^+ used as 40% w/v

The rationale used in the choice of comonomer feed compositions was that the AMPS-Na^+ monomer is intended to be the major component. The NVP and MAA are the minor component intended to modify the properties of poly(AMPS-Na^+).

2.3. Mould Design

The mould consisted of two square acrylic plates (15 cm x 15 cm) covered with Teflon® sheet as release liners. Polytetrafluoroethylene (PTFE) covered gasket as spacer, which used to control the sheet thickness. The two plates were held tightly together by clip with a small inlet at the top for injection of the polymerization mixture. The mould is shown in Fig. 2.4.

Figure 2.4. Mould used for polymerization in the form of thin sheet.

2.4. Multicomponent of Hydrogel

The prepared aqueous solution was poured into a vertical mould where the polymer mesh was already inserted in the middle to improve mechanical strength of hydrogel and handling property. Photopolymerization was carried out at room temperature using a commercially available UV lamp (254 nm).

The hydrogel sheet was removed from the mould. One side of the hydrogel sheet was covered with a polyurethane (PU) film with adhesive as backing sheet, impermeable to liquids and bacteria but allows for gas exchange. The other side was covered with a polyethylene (PE) film as release liner which is removed before use, as shown in Fig. 2.5 below.

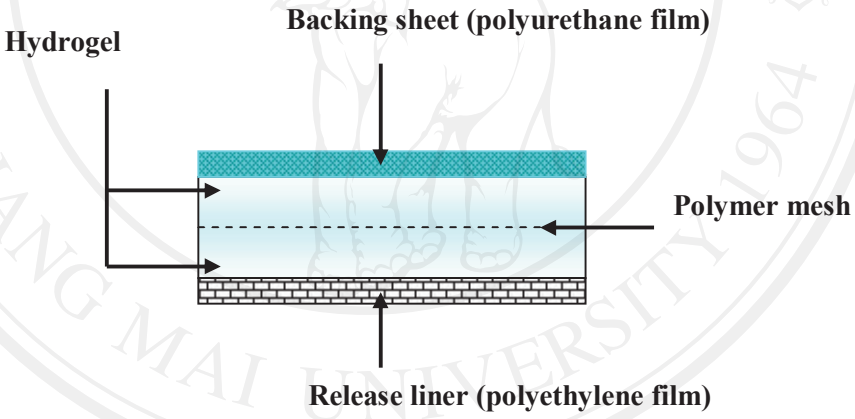

Figure 2.5. Prototype hydrogel wound dressing showing its multilayer construction.

2.5. Preparation of Synthetic Body Fluid (SBF)

SBF is known to be a metastable buffer solutions [96-97] undesired variance in both of the preparation steps and the storage temperatures. It prepared in accord with concentrations nearly equal to those of the inorganic constituents of human blood plasma, was first used by Kokubo *et al* [98]. SBF solutions [99-103] were prepared by dissolving appropriate quantities of the chemicals in distilled water [104-105].

Reagents were added, one by one after each reagent was completely dissolved in 700 ml of water, in the order given in Table 2.4. A total of 40 ml of 1 M hydrochloric acid (HCl) solution was consumed for pH adjustments during the preparation of SBF solutions.

The 15 ml aliquot of this amount was added just before the addition of the 6th reagent, i.e., $CaCl_2.2H_2O$. Otherwise, the solution would display slight turbility. The second portion of the HCl solution was used in the reminder of the titration process. Following the addition of the 8th reagent (tris(hydroxymethyl)aminomethane), the solution temperature was raised from the ambient to 37°C. This solution was then appropriately titrated with 1 M HCl to a pH value of 7.4 at 37°C [106-109]. During the titration process, the solution was also continuously diluted with consecutive additions of distilled water to make the final volume equal to 1 L. It was observed in this study that the prepared SBF solutions can be stored at 5°C for a month without degradation.

Table 2.4. Chemical compositions of SBF solutions.

Order	Reagents	Amount (g/L)
1	NaCl	6.547
2	$NaHCO_3$	2.268
3	KCl	0.373
4	$Na_2HPO_4.2H_2O$	0.178
5	$MgCl_2.\ 6H_2O$	0.305
6	$CaCl_2.\ 2H_2O$	0.368
7	Na_2SO_4	0.071
8	$(CH_2OH)_3CNH_2$	6.057

2.6. Polymer Characterization

2.6.1. Infrared Spectroscopy (IR) [110]

In this research project, a Nicolet FT-IR 510 Infrared Spectrometer was used to characterize the chemical structure of the monomers. In addition to their qualitative analysis for structural characterization, infrared spectra may also be analyzed quantitatively for following microstructural changes by determination of the absorbance ratio of functional group through their characteristic absorption frequencies.

To analyze NVP and MAA, a drop of each monomer was placed between two NaCl plates without a spacer, and mounted on a sample holder located in the instrument. Scans at time intervals over the range 4000 to 700 cm^{-1} were taken. The spectrum was the average of 30 scans per second at resolution of 4 cm^{-1}. The monomer AMPS and poly(AMPS-Na^{+}) were analyzed by KBr disc method.

2.6.2. Water Content (WC)

This part of the work covers the determination of the relative rates of water absorption by thin sheets immersed in distilled water. The water absorbed by a hydrogel network is quantitatively expressed in terms of the "Water Content" (WC) of the polymer.

The hydrated hydrogel sheet was cut into small pieces with size 1.5 cm x 1.5 cm and allowed to be equilibrated in an incubator at 35°C for 24 hrs. before testing water absorption properties. The experiments were conducted at 35.0±1.0°C by immersing the hydrogel in distilled water or SBF solutions. The weights of samples were recorded by periodically removing them from the swelling media, blotting them with absorbent tissue and weighing. The experiments were performed until each of the samples reached it equilibrium swollen state.

Water absorption is arguably the most important single property of a hydrogel. The hydrogels synthesized from each set of conditions were compared in terms of

their swelling ratio. When a sample of a dehydrated or water-deficient hydrogel is immersed in water, there will be an osmotic driving force for the water to enter the free volume within the hydrogel, as shown in Fig. 2.6. After the osmotic force driving water into the system is balanced by the force exerted by the polymer chains in resisting further expansion, such as from the constraints imposed by crosslinking, an equilibrium will be reached. The water content of the hydrogel at this equilibrium is termed the "Equilibrium Water Content" (EWC) [111].

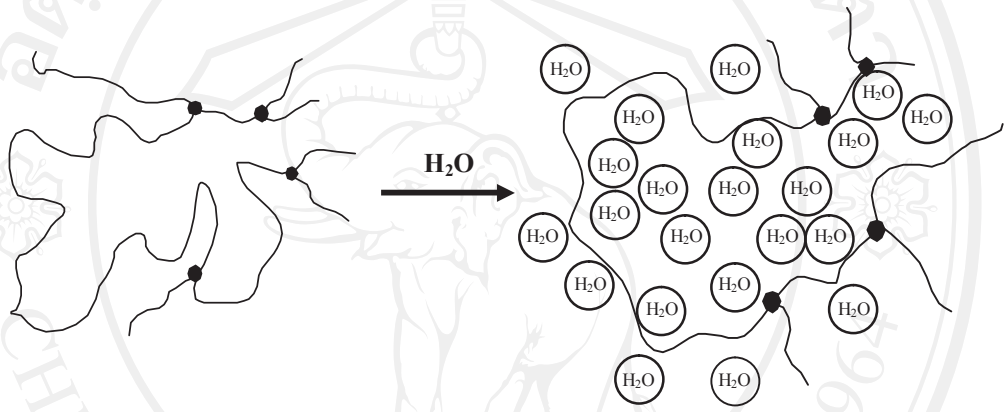

Figure 2.6. Absorption of water into a crosslinked hydrogel chain segment.
(• crosslinking units)

EWC is defined mathematically as the ratio of the partial weight of water in the hydrogel to the total weight of the swollen hydrogel at equilibrium hydration, expressed as equation 1.1. The swelling ratio of a hydrogel is given by equation 1.1 as shown in section 1.4.1.

2.6.3. Water Retention (WR)

The physical interaction between a hydrogel network and the water incorporated in it leads to a gel-like structure containing firmly bound water [112]. This ability to bind water is an important property of hydrogels in their use as wound covering materials. Lamke *et al*. [113] showed that the average surface temperature of

injured skin is 35°C and hence the water loss from hydrogels is usually measured in experiments carried out at this temperature. In this research project, AMPS-Na^+, NVP and MAA were studied with respect to their water retention. The water retention was calculated according to equation 2.1.

The test specimens used in this determination were cut into 1.5 cm x 1.5 cm and a thickness of about 0.5 mm. Each specimen was immersed in distilled water to hydrate until equilibrium state for at least 24 hrs. The hydrate hydrogel specimen was then placed in an incubator at 35°C and the water loss by evaporation followed by measuring the decrease in weight at various time intervals.

$$\text{Water Retention} = \frac{\text{Water Content (at time t)}}{\text{Initial (Equilibrium) Water Content}} \times 100 \qquad (2.1)$$

2.7. Diffusion Kinetics [114]

When a glassy hydrogel is brought into contact with water, water diffuses into the hydrogel and the network expands resulting in swelling of the hydrogel. Diffusion involves migration of water into pre-existing or dynamically formed spaces between chains. Swelling of the hydrogel involves larger segmental motion resulting, ultimately, in increased separation between hydrogel chains. Analysis of the mechanisms of water diffusion into swellable polymeric systems has received considerable attention in recent years, because of important applications of swellable polymers in biomedical, pharmaceutical, environmental, and agricultural engineering.

The following equation 2.2 is used to determine the nature of diffusion of water into hydrogels;

$$F = M_t / M_s = kt^n \quad (2.2)$$

where F = the fractional uptake at time t.
M_t = the mass uptake of the water at time t.
M_s = the mass uptake of the water at equilibrium.
n = the diffusional exponent.
k = a constant incorporating characteristic of the macromolecular network system and the penetrant.

Equation 2.2. is valid for the first 60% of the fractional uptake. Fickian and non-Fickian diffusion are defined by n values of 0.5 and 1.0, respectively [115]. The values of diffusional exponents n, and diffusion constant k, are calculated from the slopes and intercepts of the plot of ln F against ln t, respectively. The study of diffusion phenomena of water in hydrogels is of value in that it clarifies polymer behavior. For hydrogel characterization, the diffusion coefficients can be calculated by various methods. The diffusion coefficient D, of the water was calculated using the following equation 2.3 [116]:

$$D = \pi r^2 (k/4)^{1/n} \quad (2.3)$$

Where D = diffusion coefficient ($cm^2\ s^{-1}$).
r = the radius of a cylindrical polymer sample.
n = the diffusional exponent.
k = a constant incorporating characteristic of the macromolecular network system and the penetrant.

2.8. pH-Sensitive Hydrogels [117]

This research has studied the dynamic swelling of pH-sensitive networks. Poly(AMPS-Na^+-co-MAA) and poly(MAA) were selected to be characterized for their sensitivity to external conditions, because they showed pH-sensitive swelling due to ionization of the pendant carboxylic groups in the polymers. There were tested in different buffer solutions (pH range from 1.2 to 9.0) with constant ionic strength of 0.1 M at 35.0±1.0°C, a certain amount of KCl was introduced into the buffer solution, as shown in Table 2.5. The experiments were performed in the same procedure as in section 2.6.1.

Table 2.5. Lists of buffer solutions.

Reagents	pH
KCl/HCl	1.2
$NaOH/KH_2PO_4/Na_2HPO_4$	6.0-7.2
H_3BO_3/NaOH	9.0

2.9. Water Vapour Transmission Rate (WVTR)

An *in vitro* testing procedure recommended by the American Society for the Testing of Materials has been commonly used to evaluate the WVTR of skin substitutes. The WVTR is then determined by weighing the cup assembly at different time intervals. Measurements are usually performed in a chamber maintained at 35.0±1.0°C which mimics the average temperature of the wound surface and relative humidity of 55-60%.

Circular-shaped test specimens were cut from the hydrogel sheets using a template with an overall diameter of 7 cm. When mounted in the cup assembly, the

effective surface area of the specimen was 3.068 x 10^{-3} m^2. The test dish was then filled with distilled water to a level 3/4 ± 1/4 inch (19 ± 6 mm) from the specimen. According to the test procedure, the water depth should be not less than 1/8 inch (3 mm) to ensure coverage of the dish bottom throughout the test, as shown in Fig. 2.7.

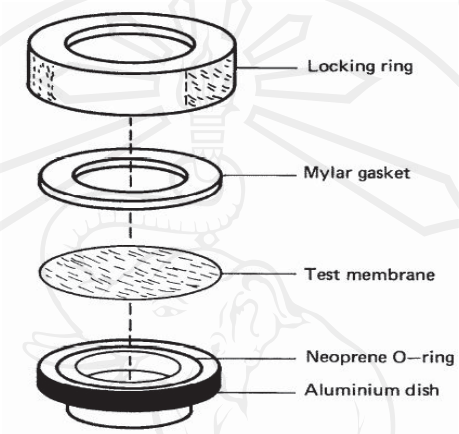

Figure 2.7. Aluminium cup with test membrane in position used for water vapour transmission measurements.

The test specimen was attached to the dish by sealing in such a manner that the dish mouth defined the area of the specimen exposed to the vapour pressure in the dish. The dish assembly was then weighed and placed in a temperature-controlled incubator at 35.0±1.0°C, this being the zero-time (t = 0) for the experiment. The rate of water vapour transmission, WVTR, was calculated from equation 2.4 in which the slope of the plot of weight against elapsed time is divided by the exposed surface area of the specimen.

$$WVTR = G / tA = (G / t) / A \qquad (2.4)$$

where G = weight change, g
t = time, hrs
G/t = rate of weight change, g/hr
A = test area (cup mouth area), m^2
WVTR = rate of water vapour transmission, $g/hr.m^2$

2.10. Mechanical Properties

Peel Tests at 90 Degrees

The adhesion performance of pressure sensitive ashesives can be determined by peel strength. Give that peel strength is comparable in a number of aspects [118]. Peel tests were carried out on the adhesives produced during this investigation. Peel strength testing measures the strength of the adhesive bond between the hydrogel and substrate. The skin on the forearm was used as the substrate due to its uniform area and ease of accessibility. The peel test measures the difficulty of adhesive removal taking into the account that for an adhesive to adhere to a substrate its measured surface energy must be equal to or less than that of the substrate. It is subject to several variables such as variations characteristic of the substrate, contact area, contact pressure and time, the angle of peel and the speed of removal. The shear strength measured by the peel test is a measure of force required to induce adhesive failure between the adhesive and the substrate. It has been suggested [119] that at a peel angle of 90° the test is less sensitive to backing material failures. Thus a 90° peel strength test was used to determine the difficulty of the adhesive's removal from a substrate. This perpendicular peel test consists of a wooden tray sliding over a wooden base. The tray enables the substrate to remain in position at the same time as it slides over the base to allow the angle of peel to remain constant. This test allows the adhesive to leave the substrate directly below the peel grip. The substrate chosen to determine the peel strength of the adhesives synthesized for the purpose of this study was the researchers forearm.

Three strips of skin adhesive hydrogel measuring 10 cm x 2.5 cm (4″x1″) were tested and the average result was recorded and used in further sections. A strip was pressed onto the sliding tray with a small amount of sample left off the tray. This was clamped in the jaws of the tensometer. The strip was peeled from the substrate, at a rate of 500 mm/min, as the tray was moved horizontally forward ensuring that the sample was removed at 90°, as shown in Fig. 2.8.

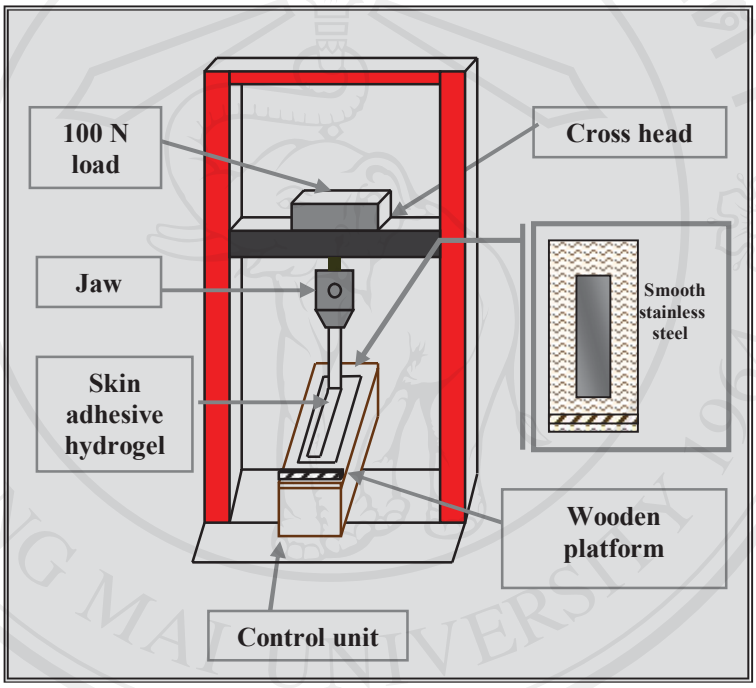

Figure 2.8. Diagram of a Hounsfield Tensometer.

A 100 N load cell was used for all the peel tests and the data was relayed to the computer software, which calculated the peel strength in N/25 mm. A sample trace obtained with peel strength is shown in Fig. 2.9. For each sample at least three peel tests were carried out to establish the degree of reproducibility of the results. The mean average values were used in the results. The forearm was cleaned with methanol in between in each test to remove any residual adhesive as well as to minimize lipid variation.

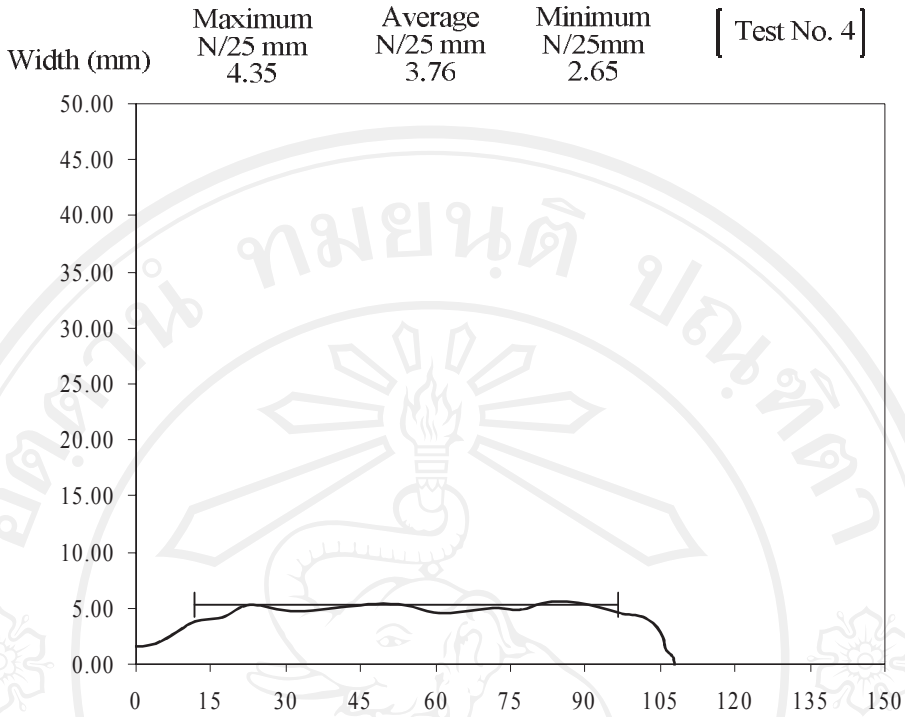

Figure 2.9. A sample trace from a 90° peel test.

2.11. Oxygen Permeability

Because of their hydrophilicity, hydrogels as a class of polymers, are in many ways ideal material for wound dressings. In terms of oxygen permeability is a vitally important property to consider in the design of hydrogel polymers for used as wound dressings. Initial studies, hydrogel materials of various water contents which were used for the "dissolved" oxygen permeability measurements were made in sheet form by AMPS-Na^+ monomer.

In this work a permiometer model 201T was used to calculate the oxygen permeability of membranes. This method measures the permeability between gas and liquid. Samples were flat materials of equivalent size. With most methods of oxygen permeability measurement used to date, ether gravimetric (change in sample weight), barometric (change in ambient gas pressure), or volumetric (change in ambient gas volume) measurements are used. These methods quantify gas sorption into, or desorption out of, a polymer sample or gas permeation through a polymer membrane.

Current commercial instruments often rely on modern thermal conductivity, coulometric, or ionization based detectors.

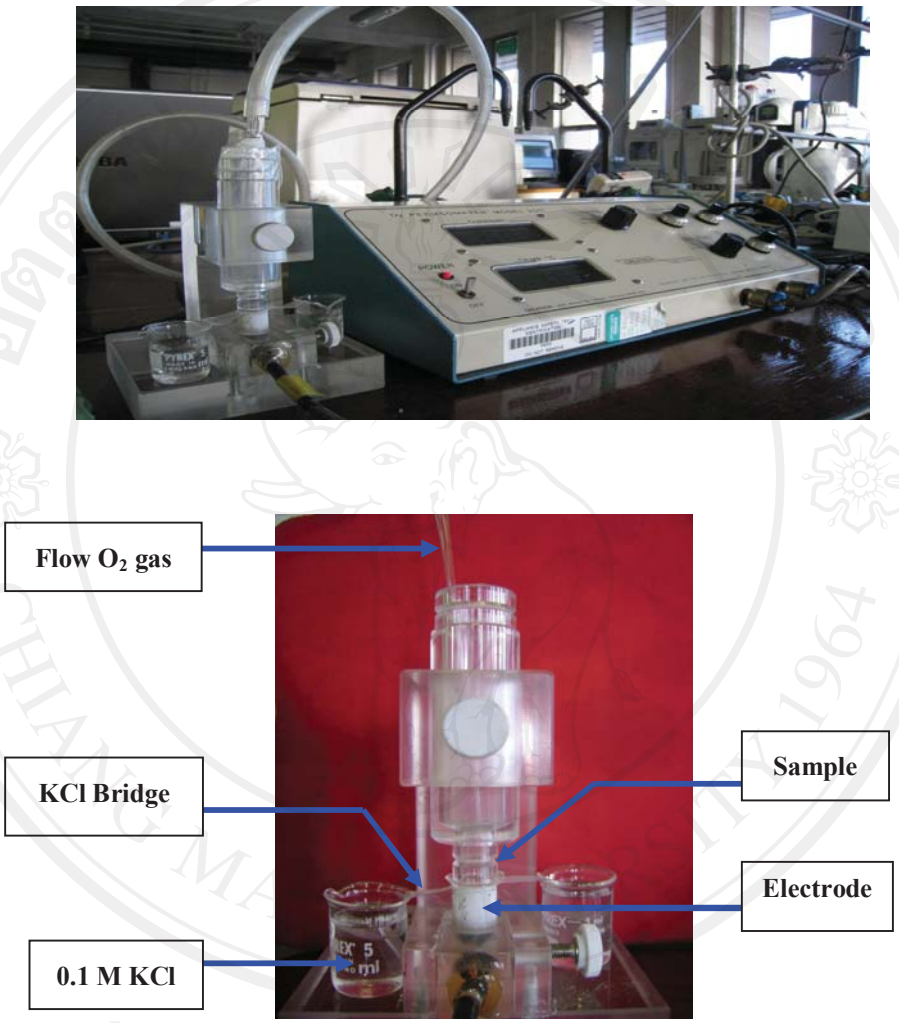

Figure 2.10. Oxygen permeability measurements were made using a 201T permiometer.

Polymer samples were cut to a circle with diameter 0.5 cm and the thickness was measured using a micrometer. Samples were placed over an electrode and a lens tissue, saturated in 0.1 M potassium chloride (KCl) was used as an electrode bridge situated in between the sample and the electrode. A column was placed over the

sample and a slow flow of gas, either nitrogen or oxygen, was passed through the sample, as shown in Fig. 2.10.

A constant stream of gas was passed through the sample until a steady current was obtained, which was then noted. The steady current obtained when passing nitrogen gas though the sample was denoted as i_o in all cases in this study i_o was found to be zero. The steady current reading obtained when oxygen was passed through the sample was denoted as i. Oxygen permeability were calculated using equation 2.5, given in the form of D_k, the diffusivity of oxygen through a material and the solubility of oxygen in a given material.

$$D_k = L(i - i_o)V/n\,FAP_s \qquad (2.5)$$

Where:
- L = thickness (cm)
- i_o = current obtained for nitrogen
- i = current obtained for oxygen passing through the sample
- F = Faraday constant = 96490 X 10^6 μA s mol^{-1}
- A = area of gold (electrode) = 0.1278 cm^2
- P_s = oxygen tension = 760 mmHg S.T.P.
- N = N^o of electrons involved = 4
- V = standard gas molar volume = 22.415 X 10^3 ml

If the constants are calculated the equation becomes:

$$D_k = I\,(\mu A) \times L\,(cm) \times (6 \times 10^{-9}) \text{ units cc cm / } cm^2 \text{ s cmHg} \qquad (2.6)$$

2.12. Determination of Monomer Residual

2.12.1. Ion Chromatography (IC)

Ion chromatography (IC) is a subdivision of high performance liquid chromatography (HPLC). IC involves separating and quantifying anions and cations using liquid chromatography (LC). LC is an analytical technique based on the separation of a mixture in solution using a liquid mobile phase to carry the mixture through a chromatographic column, which separates the components of the mixture by selective absorption. With IC the ions dissolved in a solvent are injected into a column. The column contains a stationary bed with a surface of opposite charge to the sample ions. The stronger the charge on the sample, the stronger its attraction to the ionic surface thus the longer it takes to elute. Both the pH and ionic strength of the mobile phase control elution time. Once the ions are separated they are detected and quantified by a conductivity detector.

The suitability for using IC to detect anionic monomer was investigated in this study. The ability to detect and quantify anionic monomers would provide a method for determining the amount of residual monomer that remains post curing. This is desired as residual unpolymerized monomers are generally toxic and cause adverse reactions. A Dionex DX600 supported by Chromeleon Client 6.50 software to process the acquired data was used. The main advantages of this system are the online generation of the mobile (eluent) phase as well as its counter ion auto suppression technology. A schematic of the system is shown in Fig. 2.11.

2.12.2. Online Elution Generation

Dionex eluent generators use electrolysis to convert pure water into potassium hydroxide (KOH) eluent for anion separation. The eluent's counter ion (potassium, K^+), which is stored in an EluGen® cartridge, diffuses across a membrane into the cartridge's high-pressure chamber during electrolysis. A degasser built into the cartridge outlet removes by-product gas (hydrogen) from the eluent stream. The concentration of the eluent is delivered as a function of the applied voltage, eluent

flow rate, concentration of the species in the reservoir and other factors; however these are automatically adjusted according to the flow rate and concentration specified. Thus a contaminant-free eluent is delivered on demand at the exact concentration required for the application.

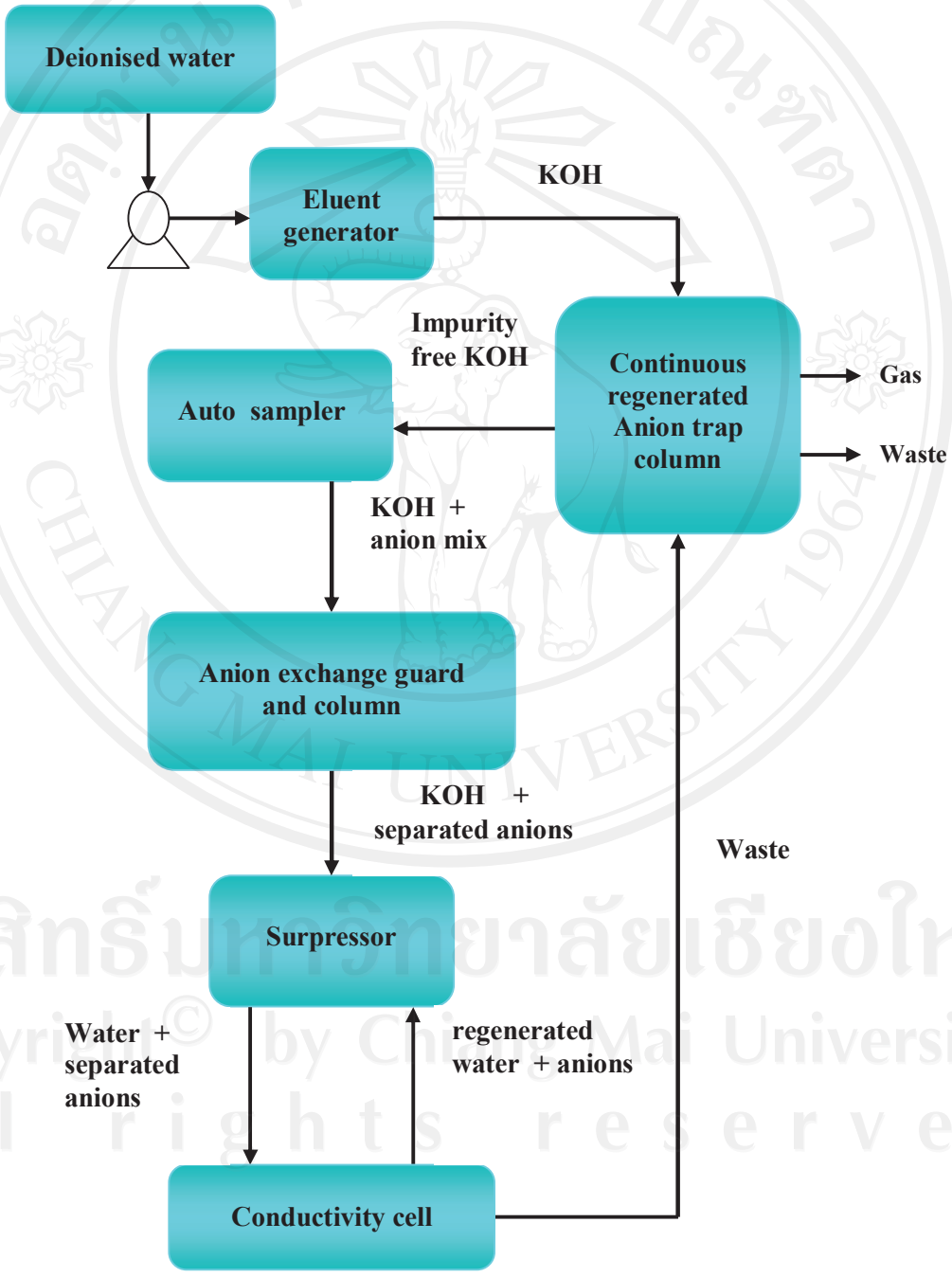

Figure 2.11. Flow diagram of the Dionex DX 600 ion chromatograph.

By eliminating variability associated with manual eluent preparation and by eliminating the possibility of eluent contamination, online eluent generation enhances reproducibility of results. Furthermore absorption of atmospheric carbon dioxide or ammonia is virtually eliminated; thus eluent contamination is reduced resulting in lower background signal, reduced noise, smaller baseline shifts and better peak resolution.

2.12.3. Counter Ion Auto Suppression

Suppression works to achieve the absolute best sensitivity (signal-to-noise ratio) and corresponding lowest detection limits for inorganic analysis by:

1. decreasing background eluent conductivity (lowers noise).
2. increasing analyte conductivity (increases signal).
3. eliminating sample counterions.

The anion suppressor behaves like a cation exchanger by replacing K^+ from the eluent with hydronium ions (OH_3^+); thus when the analytes leave the suppressor they are in a water solution. Water is only weakly ionized so the background conductivity is very low. The analyte response is also enhanced because the OH_3^+ counterion is about seven times more conductive than the K^+. This water is regenerated back to the surpressor to wet the cells; hence this section of the process has been termed auto-surpression.

2.12.4. Anion Separation

An evaluation of the ability to use ion chromatography to detect and quantify water soluble anionic monomers (AMPS-Na^+) was carried out. Initially, the standard curve for using to compare the results was prepared in this study, as shown in Fig. 2.12.

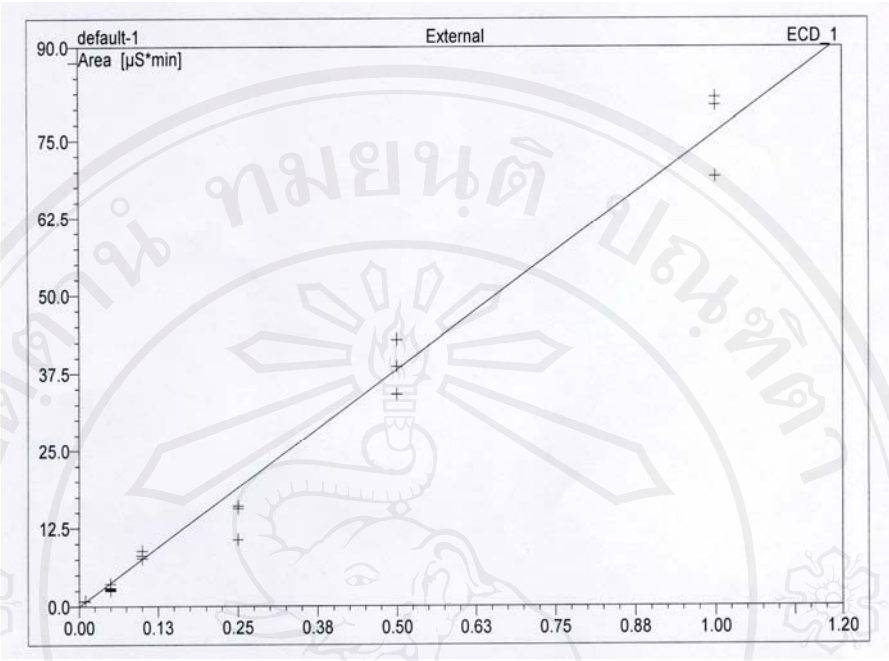

Figure 2.12. Standard curve of various concentrations (%) of AMPS-Na^+ solutions versus area of peak (µs*min).

After that, a sample of 0.058% monomer dissolved in 18.2 MΩ.cm (0.055 µS/cm) water was placed in the autosampler. Then, 2.5 µl of the monomer-water mixture was injected into a 2 mm IonPac AS11 column containing a 13 µm diameter microporous resin bead functionalized with quaternary ammonium groups. The KOH eluent concentration was ramped from 5 mM to 60 mM over 30 minutes with the eluent flow rate set at 0.38 ml/min. Post separation the anions were detected by an electrochemical detector.

2.12.5. Sensitivity Issues

Further exploration of the use of IC for determining anionic monomers or actives was hindered because issues related to sensitivity emerged. Initially there was a steady rise in the baseline suggesting an increase in the signal-to-noise ratio therefore reducing the ability of the software to detect the peaks.

2.13. Cytotoxicity Test [120]

Tested materials, poly(AMPS-Na^+), 50:50% wt poly(AMPS-Na^+-co-NVP) and 25:75% wt poly(AMPS-Na^+-co-MAA) were sterilized by gamma ray with doses 29.4 kGy prior to the biological property assessment. The cell line used in the assay was L929 (ECACC No. 85011425), mouse connective tissue, fibroblast-like cells. The growth medium used was Dulbecco′s modified Eagle′s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), together with penicillin (100 U/ml) and streptomycin (100 lg/ml).

The test specimens were cut into small circular 5 mm diameter discs and saturated with growth medium. The discs were then placed in the middle of a 35 mm dish. L929 cells were seeded onto the dish at a density of 6 x 10^4 cells/dish and incubated for 48 hrs. Cell morphology and the toxic zone were evaluated by inverted phase contrast light microscopy after a 48 hrs. exposure to the cells. The cells were stained with 0.01% neutral red in phosphate buffer saline (PBS) for membrane integrity. High density polyethylene (HDPE) and natural rubber containing carbon black were used as negative and positive controls, respectively. Each sample was tested in triplicate, and the test was repeated twice.