CHAPTER 3

Materials and Methods

3.1 Chemicals, Materials, Apparatus and Instruments

3.1.1 Chemicals and Materials

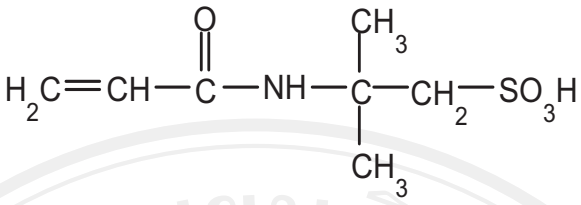
The various chemicals and materials used in this research were as listed in Table 3.1. Their chemical structures are shown in Figure 3.1.

Table 3.1 : Chemicals and materials used in this research

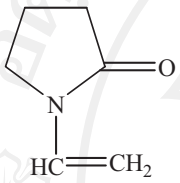
Chemical / Material	Usage	Grade	Supplier
2-Acrylamido-2-methylpropane sulfonic acid (AMPS)	Monomer precursor	98%	Fluka
4,4'-Azo-bis(4-cyanopentanoic acid)	Photoinitiator	98%	Fluka
Ethylene glycol dimethacrylate (EGDM)	Crosslinking agent	97%	Fluka
1-Hydroxycyclohexyl phenyl ketone	Photoinitiator	99%	Sigma-Aldrich
Poly(ethylene glycol) diacrylate 600	Crosslinking agent	98%	Sigma-Aldrich
1-Vinyl-2-pyrrolidone (or *N*-Vinyl pyrrolidone)	Comonomer	97%	Fluka
Glycerol	Humectant	98%	Sigma-Aldrich

Table 3.1 : Chemicals used in this research (continued)

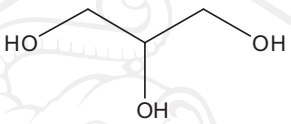
Chemical / Material	Usage	Grade	Supplier
Potassium persulfate ($K_2S_2O_8$)	Redox/thermal initiator	98%	BDH
Potassium metabisulfite ($K_2S_2O_5$)	Redox coinitiator	96%	Fluka
Ferrous sulphate ($FeSO_4$)	Redox coinitiator	99%	Carlo Erba
Poly(ethylene glycol) 10000	Humectant	98%	Sigma-Aldrich
Sodium hydroxide	Neutralizing agent	98%	Carlo Erba
Potassium hydroxide	Eluent	-	Sigma-Aldrich
Potassium chloride	Salt bridge	98%	Sigma-Aldrich
Poly(ethylene terephthalate) (PET) film	Release liner	-	3M
Polyethylene mesh	Reinforcement	-	Thai Pipe Industry
Polyurethane	Backing sheet	Opsite Flexifix	Smith & Nephew
Teflon® adhesive sheet	Mould covering	-	Creative Polymers



2-Acrylamido-2-methylpropane sulfonic acid
(AMPS)

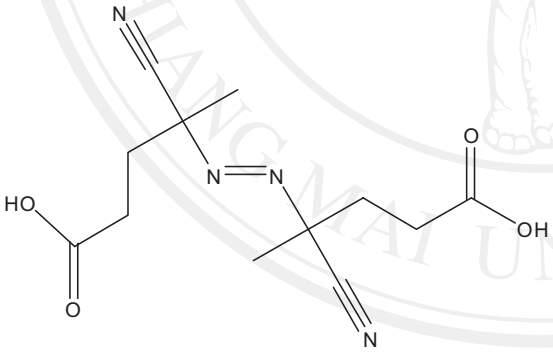


N-Vinyl pyrrolidone
(NVP)

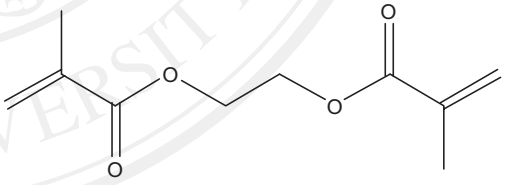


Glycerol

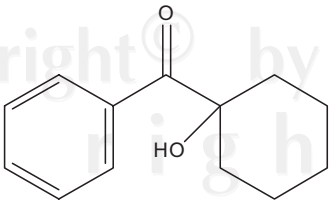
Poly(ethylene glycol) 10000
(PEG 10000)



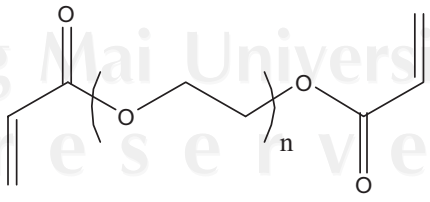
4,4'-Azo-bis(4-cyanopentanoic acid)



Ethylene glycol dimethacrylate
(EGDM)



1-Hydroxycyclohexyl phenyl ketone
(Irgacure 184)



Poly(ethylene glycol) diacrylate 600
(Ebecryl 11)

Figure 3.1 : Chemical structures of the main compounds used in this research

3.1.2 Apparatus and Instruments

The major items of equipment used in this research were as listed in Table 3.2.

Table 3.2 : Apparatus and instruments used in this research

Apparatus and Instruments	Model	Company
Fourier-Transform Infrared (FT-IR) Spectrometer	510	Nicolet
400 MHz ^{1}H-NMR Spectrometer	DRX 400	Bruker
UV Lamp	MD1-15	Philips
Hounsfield Tensometer	HIOKS/0368	Hounsfield
Dionex Ion Chromatograph	DX600	Dionex (UK)
Oxygen Permiometer	210T	Rehder Development Co., USA
pH meter	713	Metrohm
Incubator	BM 400	Memmert
Drying oven	FCO-100	Whatman
Vacuum oven	VOS-300SD	Eyela
Analytical balance	BA210s	Sartorius
Water vapour transmission test set	-	Yasuda
Hot plate / magnetic stirrer	MR 3001	Heidolph

3.2 Monomer Preparation and Characterisation

3.2.1 2-Acrylamido-2-Methylpropane Sulfonic Acid (AMPS)

The Fourier-transform infrared (FT-IR) spectrum of the commercial AMPS (assay 98%) monomer precursor is shown in Figure 3.2 below and can be compared with the reference spectrum in Figure 3.3. The various peaks in the spectrum are assigned to their corresponding bond vibrations in Table 3.3. The AMPS was used as supplied without any further purification.

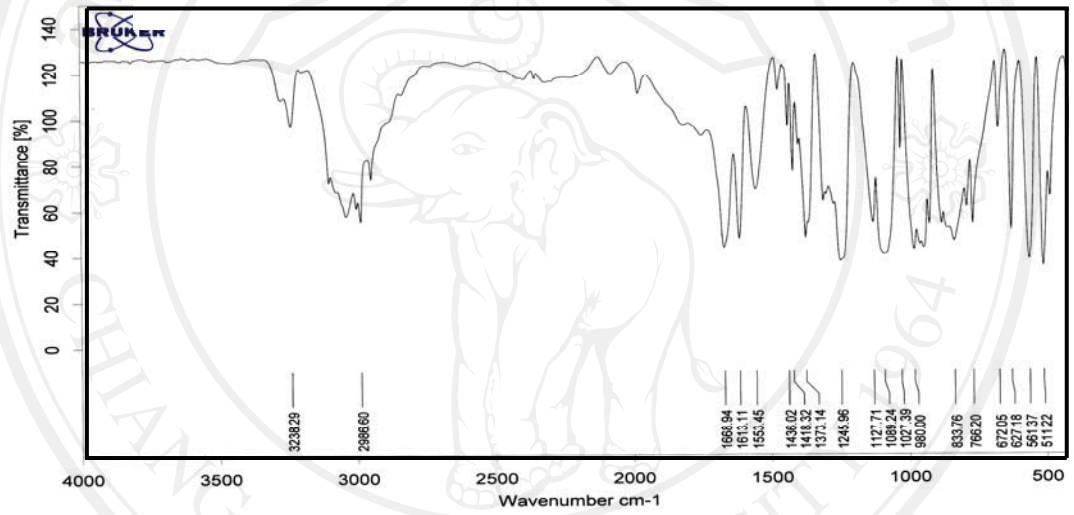


Figure 3.2 : FT-IR spectrum of the commercial AMPS monomer precursor used in this work

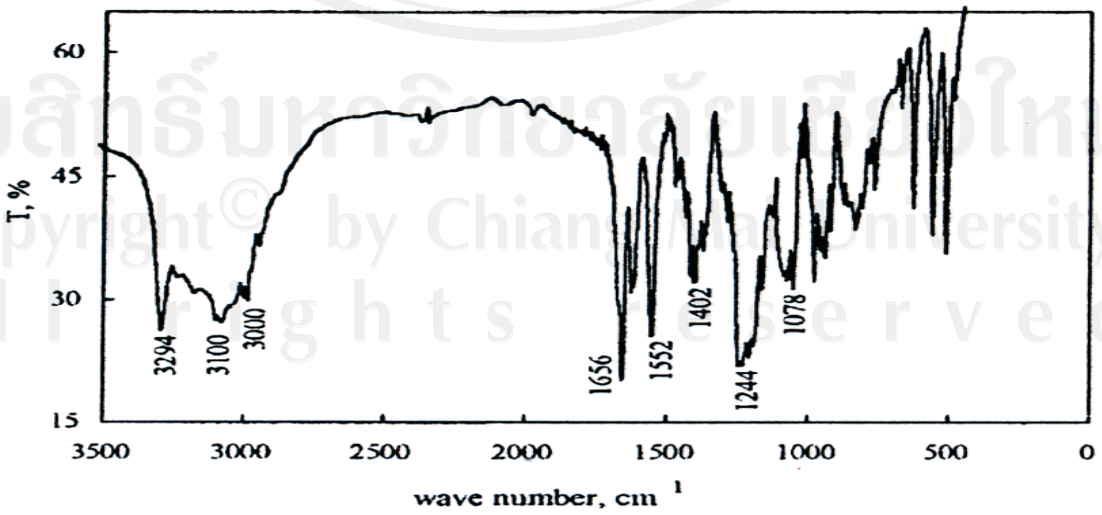
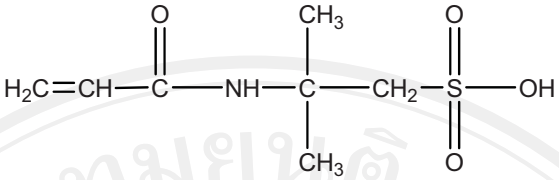


Figure 3.3: Reference infrared spectrum of AMPS [56]

Table 3.3 : Infrared absorption peak assignments for AMPS monomer precursor



Vibrational Assignment	Functional Group	Wavenumber (cm^{-1})	Peak Intensity*
S=O stretching	$-SO_3$	1373	s
		1245	
C=C stretching	CH_2=CH	1553	s
C=C bending	CH_2=CH	980	s
C=O stretching	-CONH-	1668	s
N-H stretching	-NH-	~3300	m
		3238	
N-H bending	-NH-	1613	s
C-H stretching	$-CH_2-$, $-CH_3$	~3000-2800	s-m
C-H bending	$-CH_2-$, $-CH_3$	~1450-1375	s, v
S-O stretching	$-SO_3$	650	s
C-N stretching	-C-N-	1127	s
O-H stretching	-O-H	~3300-3100	b

* s = strong, m = medium, v = variable, b = broad

The 400 MHz ^{1}H-NMR spectrum of the commercial AMPS acid is shown in Figure 3.4 below together with a reference spectrum for comparison in Figure 3.5. The proton assignments for the various peaks in the spectrum are given in Table 3.4.

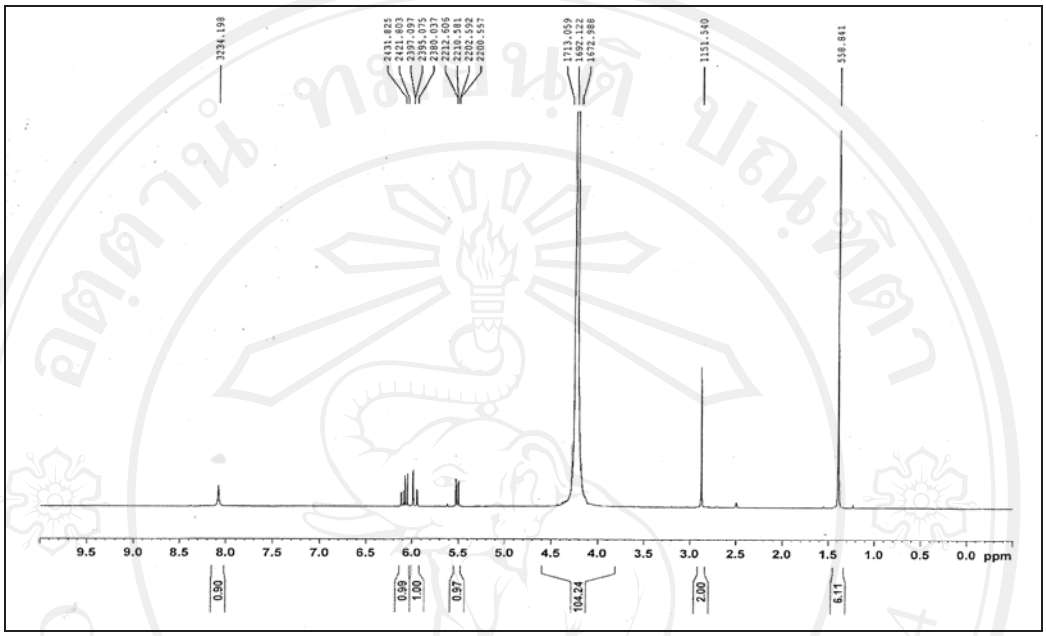


Figure 3.4 : 400 MHz ^{1}H-NMR spectrum of the commercial AMPS acid monomer precursor in d_6-DMSO as solvent at room temperature

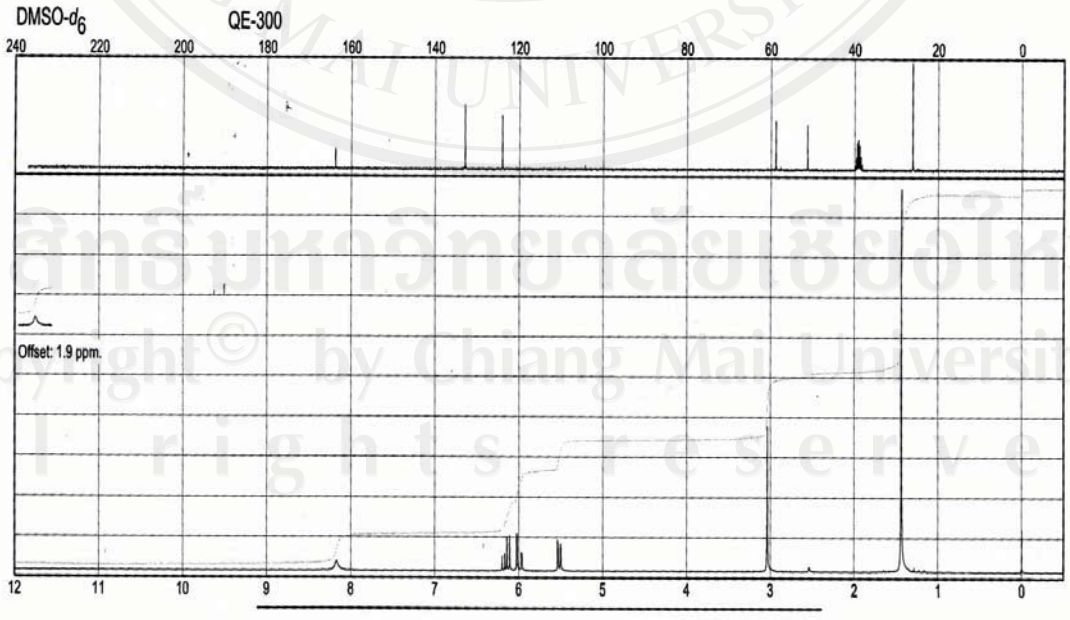
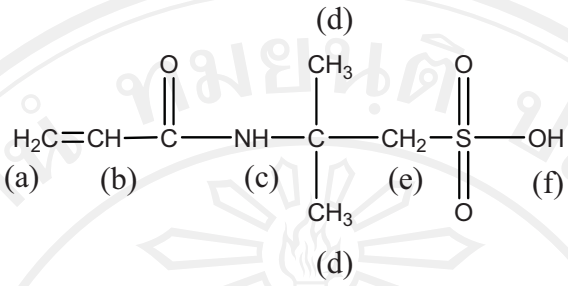


Figure 3.5 : Reference ^{1}H-NMR spectrum of AMPS in d_6-DMSO as solvent [57]

Table 3.4 : ^{1}H-NMR peak assignments for AMPS monomer precursor

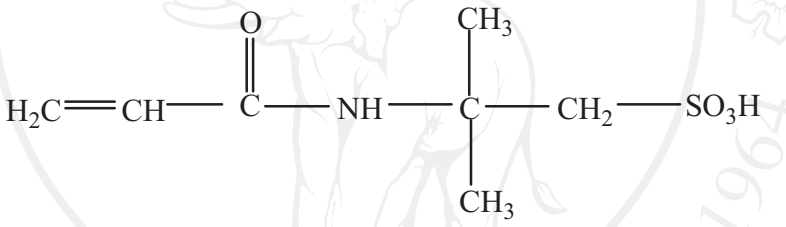


Proton Assignment	Peak Multiplicity	Chemical Shift *, δ (ppm)
a	doublet	5.5
b	triplet	6.1
c	singlet	8.1
d	singlet	1.4
e	singlet	6.0
f	singlet	2.9

* given as the peak center

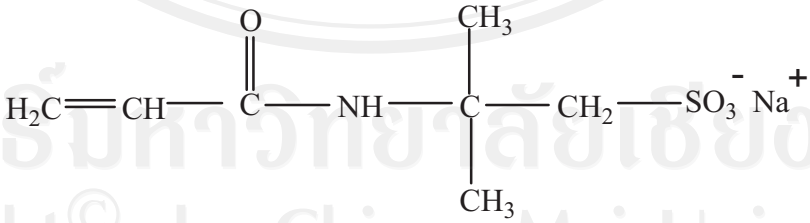
3.2.2 Preparation of 2-Acrylamido-2-Methylpropane Sulfonic Acid Sodium Salt (Na-AMPS)

The commercial AMPS acid was converted into its sodium salt (Na-AMPS) by neutralization with sodium hydroxide (NaOH) in aqueous solution. 40 g AMPS acid, in the form of solid white flakes, was dissolved in about 60 ml distilled water. The acidic solution (pH ≈ 1) was then neutralized to pH 7.0 by dropwise addition of 1 M aqueous NaOH with magnetic stirring while cooling in an ice bath, as shown in Figures 3.6 and 3.7. The resultant Na-AMPS solution was then made up to 100 ml with distilled water to give a concentration of 40% w/v before being transferred to a tightly sealed container and stored in a refrigerator at 4°C until required for use in polymerisation.



AMPS acid

neutralization with NaOH



Na-AMPS

The main reason for converting the AMPS acid into Na-AMPS, both of which can polymerise equally well, was so that the hydrogel obtained from polymerisation would be pH neutral rather than acidic. This has obvious importance if the hydrogel is to be used in a biomedical application such as a wound dressing in which it comes into direct contact with regenerating cellular tissue.

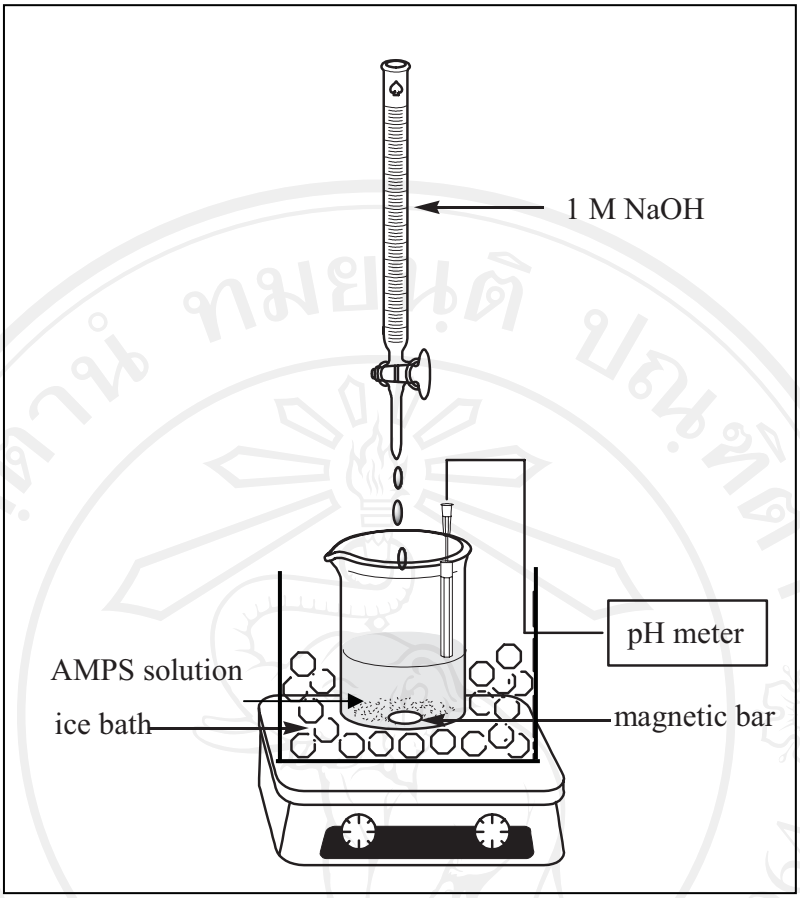


Figure 3.6: Schematic arrangement of the apparatus used in the preparation of Na-AMPS solution

Figure 3.7: Photograph of the apparatus used in the preparation of Na-AMPS solution

3.2.3 *N*-Vinyl Pyrrolidone (NVP)

The commercial NVP as supplied was purified by vacuum distillation with the constant boiling fraction at 72-75 °C / 3 mm Hg being collected (cf., lit. b.pt. 68°C / 2 mm Hg [58]). The Fourier-transform infrared (FT-IR) spectrum of the purified NVP monomer is shown in Figure 3.8 below and can be compared with the reference spectrum in Figure 3.9. The various peaks in the spectrum are assigned to their corresponding bond vibrations in Table 3.5.

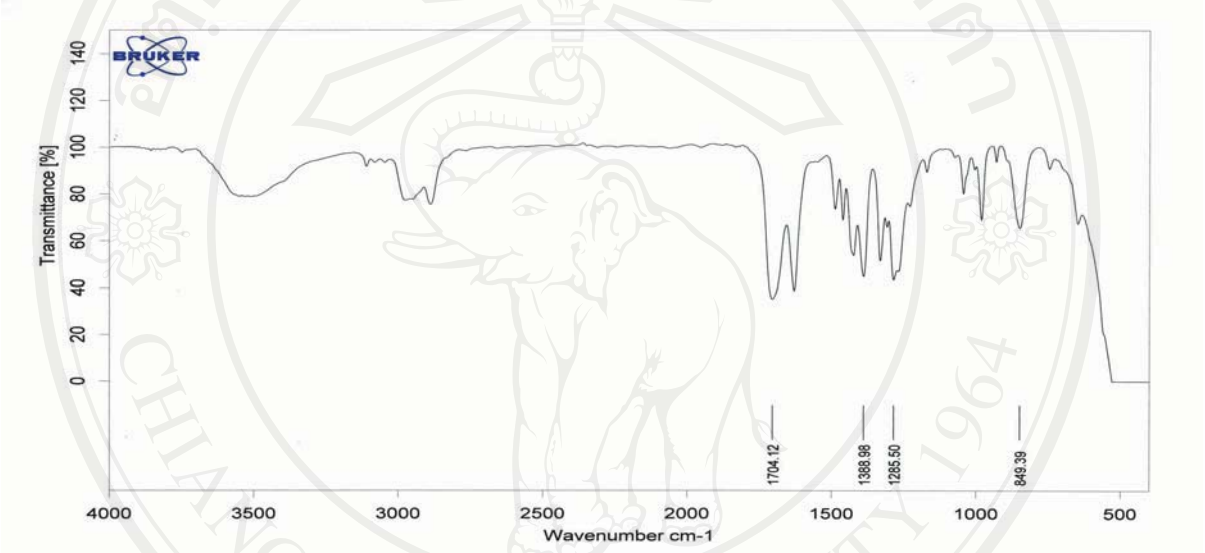


Figure 3.8 : Infrared spectrum of the commercial NVP monomer used in this work

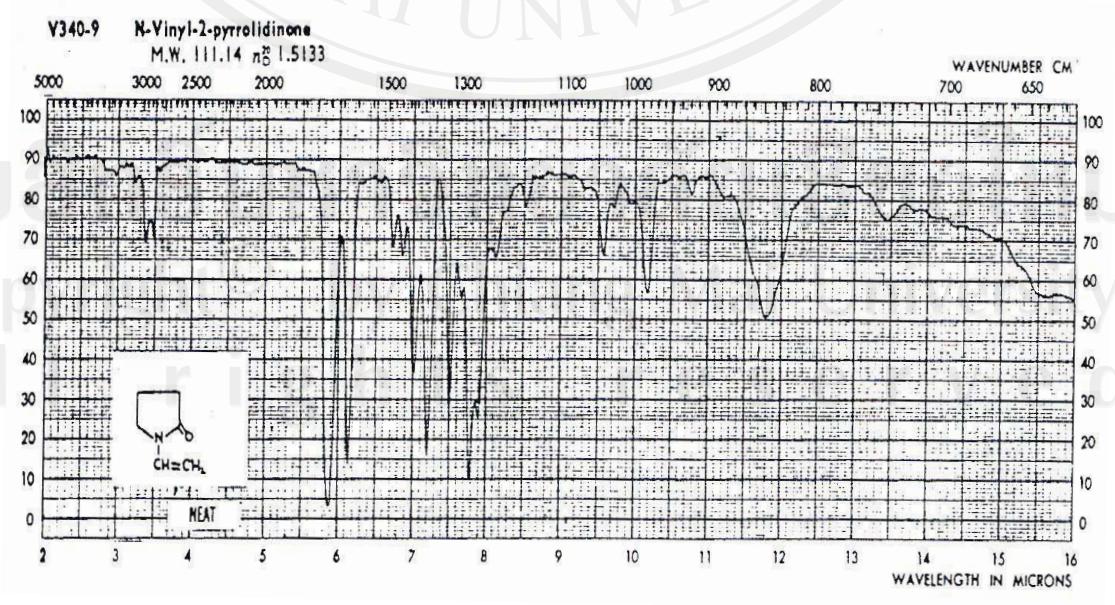
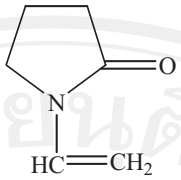


Figure 3.9 : Reference infrared spectrum of NVP monomer [59]

Table 3.5 : Infrared absorption peak assignments for NVP monomer



Vibrational Assignment	Functional Group	Wavenumber (cm^{-1})	Peak Intensity*
C=C bending	CH_2=CH	980	s
C=O stretching	-CO-N-	1704	s
C-H stretching	-CH_2-	~3000-2800	m, b
C-H bending	-CH_2-	~1400-1388	s, v
C-N stretching	-C-N-	1285	s

* s = strong, m = medium, v = variable, b = broad

The 400 MHz ^{1}H-NMR spectrum of the purified NVP is shown in Figure 3.10 below together with a reference spectrum for comparison in Figure 3.11. The proton assignments for the various peaks in the spectrum are given in Table 3.6.

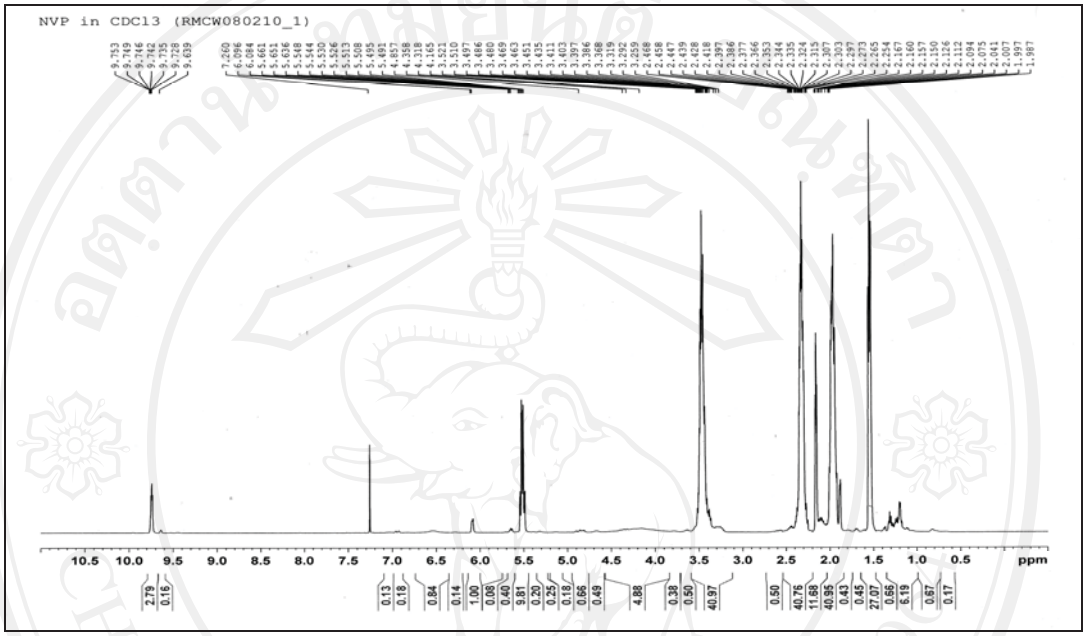


Figure 3.10 : 400 MHz ^{1}H-NMR spectrum of the purified NVP in $CDCl_3$ as solvent at room temperature

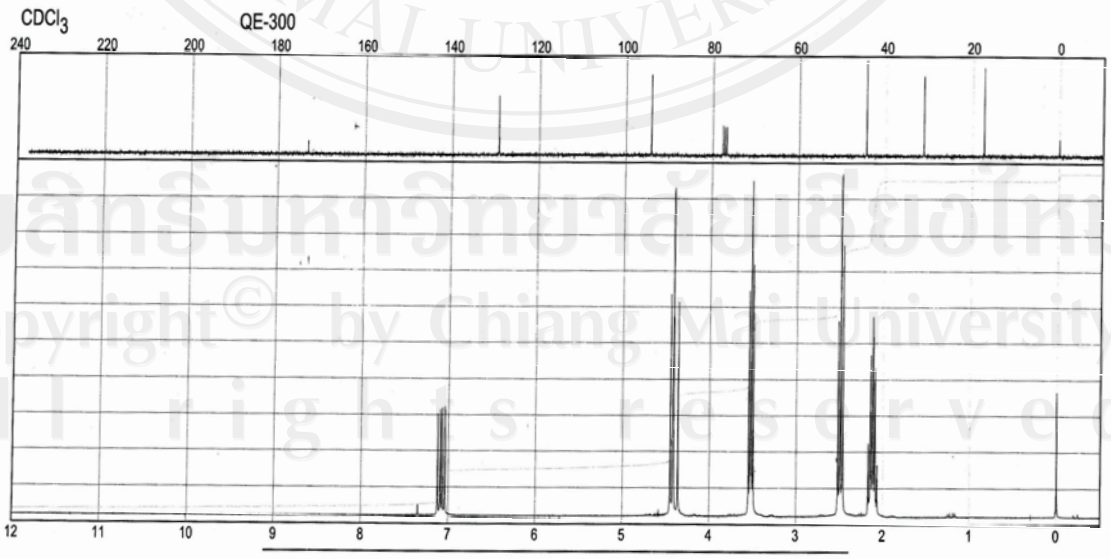
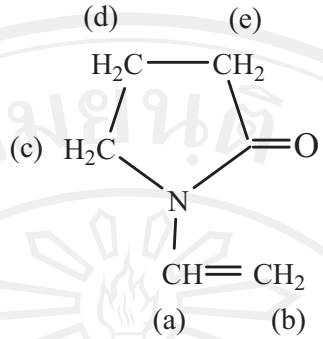


Figure 3.11 : Reference ^{1}H-NMR spectrum of NVP in $CDCl_3$ as solvent [60]

Table 3.6 : ^{1}H-NMR peak assignments for purified NVP



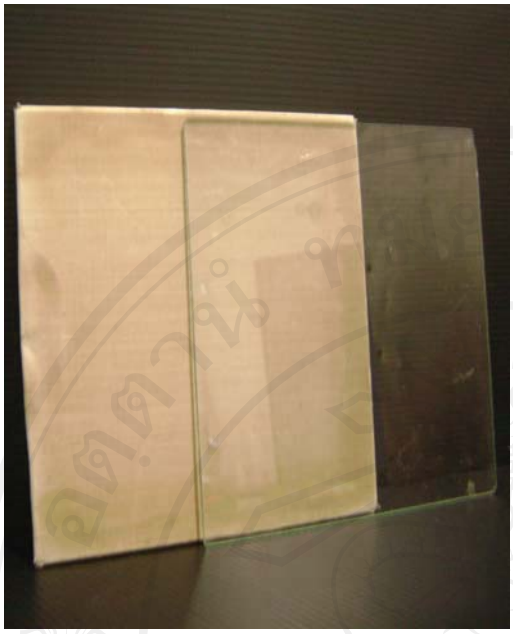
Proton Assignment	Peak Multiplicity	Chemical Shift *, δ (ppm)
a	triplet	5.5
b	doublet	3.5
c	triplet	2.4
d	multiplet	2.1
e	triplet	1.6

* given as the peak center

3.3 Mould Design and Construction

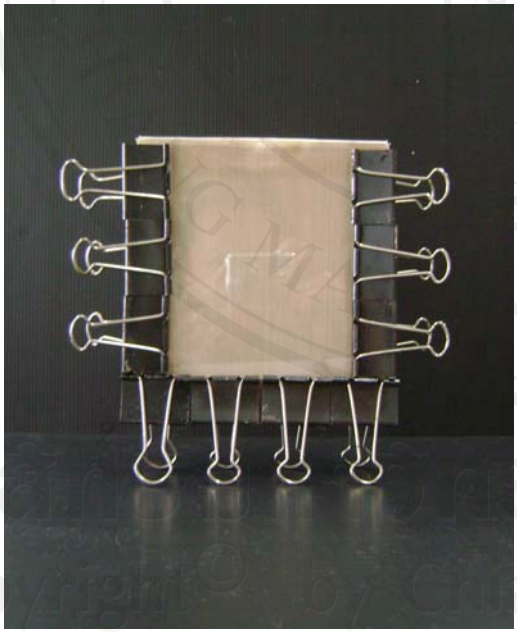
In this work, the aim was to synthesize hydrogels in the form of thin sheets in view of their intended application as wound coverings. This aim also included combining the hydrogel synthesis and sheet forming processes into a single operation for both efficiency and convenience. Consequently, it was necessary to design and construct a purpose-built mould inside which the Na-AMPS monomer could be polymerised in aqueous solution and in which the hydrated poly(Na-AMPS) hydrogel obtained would be simultaneously formed *in situ* as a thin sheet of the required thickness.

As shown in Figure 3.12, the mould was designed and constructed as a vertical sheet-forming mould consisting of two parallel plates (15 cm x 15 cm x 5 mm), one of which was made of glass and the other plastic (Perspex®). The glass plate was lined on its inside surface with an adhesive Teflon® sheet as a release liner to facilitate removal of the hydrogel sheet which would otherwise adhere to the glass. A Teflon-covered plastic spacer was placed in between the plates as a means of controlling the hydrogel sheet thickness.. The complete mould assembly was held together by strong clips along the bottom and both sides. The mould cavity had a volume of approximately 20 ml. The mould assembly and its component parts are shown in Figure 3.12.

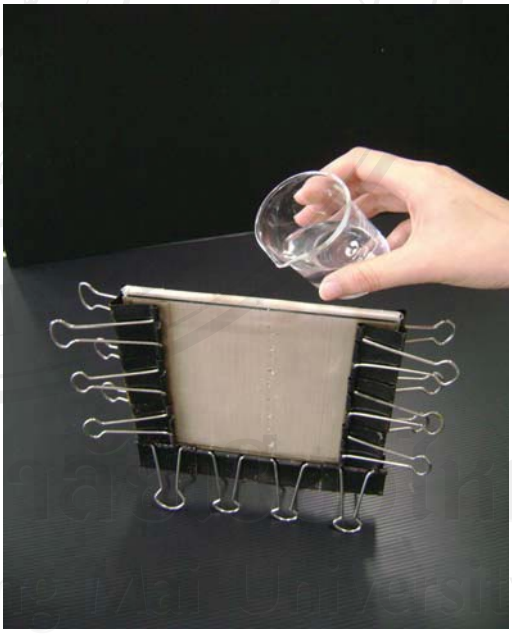


(a)
Parallel plastic (front) and
Teflon-covered glass (back) plates

(b)
Teflon® covered spacer



(c)
Mould assembly (side view)



(d)
Mould assembly (top view)

Figure 3.12 : Mould used for making hydrogel sheets showing (d) the Na-AMPS monomer solution being poured into the filling space at the top of the mould

3.4 Photopolymerisation Apparatus

Altogether, 3 different methods for polymerisation initiation were considered and compared, namely:

(1) Thermal initiation using potassium persulfate ($K_2S_2O_8$) as the free radical initiator at a concentration of 0.1% mol (relative to the monomer) and at a temperature of 60°C [61]

$$K_2S_2O_8 \rightarrow 2\,K^+ + S_2O_8^{2-}$$

$$S_2O_8^{2-} \rightarrow 2\,SO_4^{-}\bullet$$

(2) Redox initiation using potassium persulfate ($K_2S_2O_8$) as the free radical initiator at a concentration of 0.5% mol (relative to the monomer) and potassium metabisulfite ($K_2S_2O_5$) and ferrous sulfate ($FeSO_4$) as coinitiators (reductants) at room temperature [62].

$$S_2O_8^{2-} + Fe^{2+} \rightarrow SO_4^{2-} + SO_4^{-}\bullet + Fe^{3+}$$

$$S_2O_8^{2-} + S_2O_5^{2-} \rightarrow SO_4^{2-} + SO_4^{-}\bullet + S_2O_5^{-}\bullet$$

(3) Photoinitiation using a commercially available UV lamp at room temperature

The photoinitiation apparatus is shown in Figure 3.13 and consisted of an aluminium cabinet, adjustable laboratory jack (to support the mould) and a commercially available UV lamp (Philips Solarium Model MD1-15). The lamp consisted of 4 parallel 15 watt fluorescent tubes which emitted UV light in the wavelength range 100-400 nm.

(a)
Aluminium cabinet

(b)
UV Lamp

(c)
Mould supported vertically
on an adjustable lab jack inside the cabinet

(d)
UV lamp in position
against the cabinet during use

Figure 3.13 : Photopolymerisation apparatus used for making hydrogel sheets