CHAPTER 2

EXPERIMENTAL

The study in this thesis consists of two parts. The first part investigates the reactions between ENR and palm oil-based alkyds; while, the second part studies the reactions between ENR and palm oil-based mcl-PHA. In this chapter, all raw materials and reagents were presented in Section 2.1. For ENR, alkyd and mcl-PHA solutions, the preparation methods were explained in Sections 2.2, 2.3 and 2.4, respectively. The procedures used to prepare and study the reactions in the ENR/Alkyd and ENR/PHA blends were discussed in Sections 2.5 and 2.6. Then, the methods to determine the properties of the reacted polymers, such as the hydroxyl number, acid number, intrinsic viscosity and swelling of the reacted ENR in toluene were described in Sections 2.7 - 2.10. The following Sections 2.11 - 2.13 describe the analytical procedures in DSC, FTIR and NMR analyses.

2.1 Materials

Epoxidised natural rubber (ENR), grade ‘‘Epoxyprene 50’’, with 50 % epoxidation level, was supplied by Malaysian Rubber Board. Its batch number: EX5/80-04 with technical specification is shown in Table 2.1.

Palm kernel oil (PKO) is extracted from the nut of the oil palm (*Elaeis guineensis Jacq.*) fruit. The refined, bleached and deodorized PKO used in this research was supplied by Cognis Oleochemicals (M) Sdn. Bhd. It was utilized without further purification. The same batch of oil was used throughout the research in order to minimize the possibility of compositional variations in oil properties such as iodine values and minor impurities present therein. Table 2.2 lists the main characteristics and fatty acid composition of PKO.

Medium-chain-length polyhydroxyalkanoates (mcl-PHAs) was synthesized by Tan *et al.* from the Biotechnology Laboratory at the Institute of Advanced Studies, University of Malaya[181]. This mcl-PHA was produced by *Pseudomonas putida* PGA1 cultured on oleic acids ($C_{18:1}$). It was a copolymer consisting mainly of n-alkanoate monomers ranging from C_6 to C_{14}, with C_8 as the predominant component. Details of this mcl-PHA have been reported elsewhere[181]. The result of the yield of mcl-PHA is given in Table 2.3. Table 2.4 lists the monomer compositions of the mcl-PHA and indicates that the predominant monomer in the mcl-PHA is 3-hydroxyoctanoate.

Glycerol (>99.5 % pure) was supplied by Cognis Oleochemicals (M) Sdn. Bhd. Phthalic anhydride (PA), was purchased from P. T. Petrowidada, Indonesia. Maleic anhydride (MA), lithium hydroxide, toluene, chloroform, ethanol, methanol, potassium hydrogenphthalate, potassium hydroxide were purchased from Merck, and they were used as received.

Table 2.1

Technical specifications of ENR 50

Property	Mean	Method
Epoxidation level, %	50.0	^{1}H-NMR, UPB/P/019
Mooney viscosity* MLI +4 @ 100ºC)	72.0	ASTM D1646-68

* Test is carried out on samples homogenised by six passes through cold roll at room temperature with the rolls set of 1.66 mm[179].

Table 2.2

Characteristics and compositions of palm kernel oil[180]

Property		Palm Kernel Oil
Saponification value	-	246.0
Iodine value	-	18.1
Free fatty acid, % by weight	-	-
$C_{6:0}$	-	0.3
$C_{8:0}$	Caprylic acid	4.2
$C_{10:0}$	Capric acid	3.7
$C_{12:0}$	Lauric acid	48.7
$C_{14:0}$	Myristic acid	15.6
$C_{16:0}$	Palmitic acid	7.5
$C_{18:0}$	Stearic acid	1.8
$C_{18:1}$	Oleic acid	14.8
$C_{18:2}$	Linoleic acid	2.6
Others	-	0.1

Table 2.3

Properties of mcl-PHA derived from oleic acid*[181]

Property	Mean
Cell dry weight, g/ℓ	2.5
PHA content, % cell dry weight	19
PHA yield, g/ℓ	0.5

* Where the substrate concentration is 0.5 % w/v.

Table 2.4

Monomer compositions of the mcl-PHA derived from oleic acid[181]

Relative amount of monomers in purified mcl-PHA/ % w/w						
C_6	C_8	C_{10}	$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$
10.4	83.2	3.4	0.9	0.2	2.0	0.5

2.2 Preparation of ENR solution

ENR was mechanically masticated using a laboratory two rolls mill to reduce the molecular weight and increase its solubility in toluene. About 10 g of the sample was cut and milled at a nip setting of 4 mm for 46 passes. The milled sheet was folded thrice before being put into the mill again. The rubber was then dissolved in toluene, shook on an orbital shaker overnight and subsequently the total solid content of the rubber solution was checked. About 2-3 g of the solution was weighed and dried in the oven at 110°C for an hour before cooled down in a desiccators. The end product was a dilute liquid containing 12 % w/w of ENR.

2.3 Preparation of PKO alkyd solutions

PKO alkyd A1 of oil length 50 was synthesized by solvent cook in a toluene medium. It was synthesized from PKO, glycerol, PA and lithium hydroxide. Later, alkyd A1 was incorporated with MA to produce the MA-modified alkyds A2, A3 and A4.

2.3.1 Preparation of PKO alkyd A1

A1 was formulated according to Patton's gel point calculation[182] and the composition of materials used for making alkyd A1 is given in Table 2.5.

Table 2.5

Formulation of alkyd A1

Material	Weight /g	Remark
PKO	242	PKO reacted with first portion of glycerol at stage 1
Glycerol	115	Divided into first portion 63 g, second portion 52 g
PA	148	PA and second portion of glycerol were added at stage 2

The alkyd A1 was synthesized in two stages. In the first stage, PKO and first part of glycerol (approximately 55 % of total glycerol used) and 0.15 % w/w of lithium hydroxide were placed into the reaction flask equipped with a stirrer through the central neck, and a Dean and Stark decanter on a side-neck, as shown in Figure 2.1. The mixture was heated to 225-230°C under alcoholysis to convert the triglycerides of PKO to monoglycerides. To check the completion of alcoholysis, one part of the reaction mixture from the cook was added to four parts of ethanol. When a clear mixing solution was formed, it indicated that alcoholysis had completed.

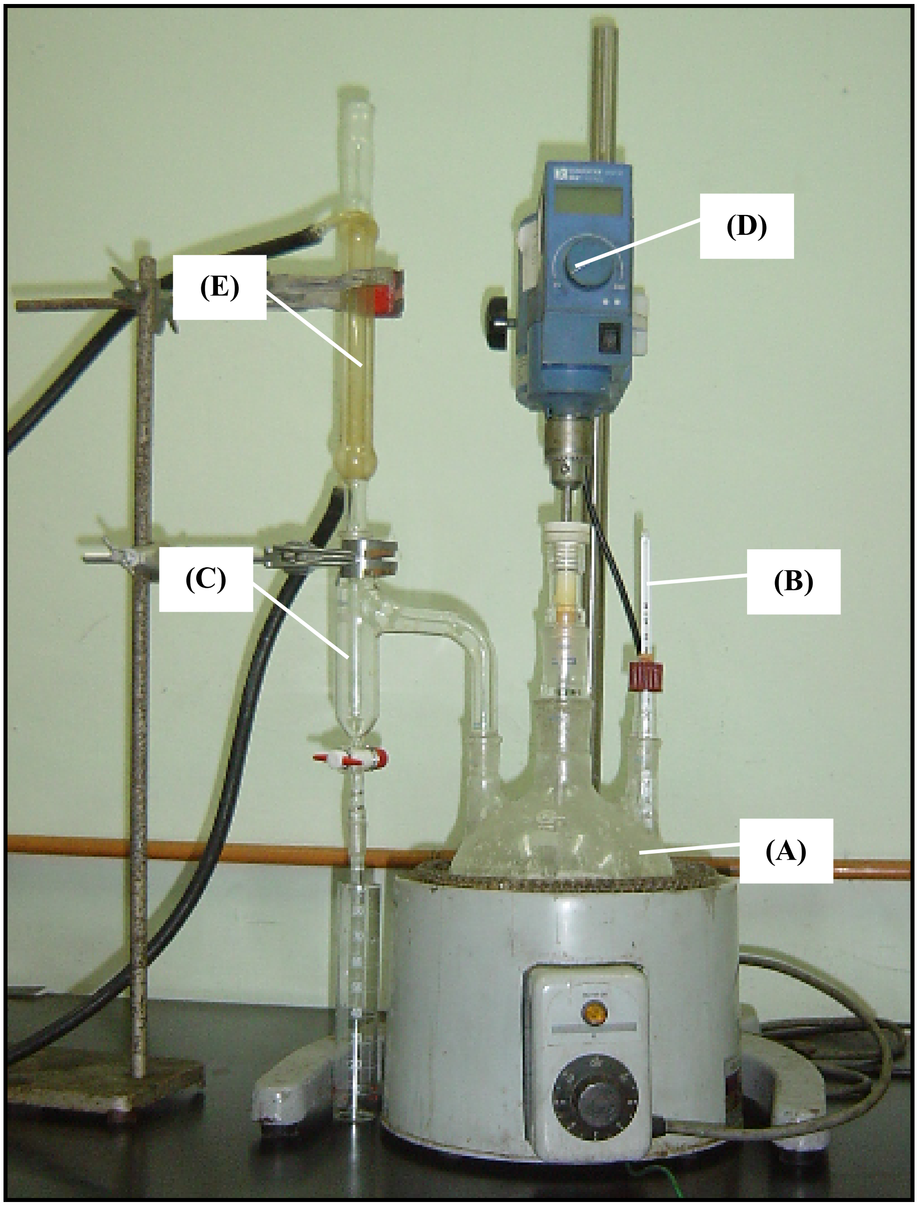

Figure 2.1: The set-up of an alkyd cook: 2-litre reaction flask (A); Thermometer (B); Dean and Stark decanter (C); Stirrer motor (D) and Condenser (E).

Next, the mixture was allowed to cool down to 180°C and the remaining glycerol, together with requisite amounts of PA and toluene was added. The time of addition was taken as the beginning of the esterification reaction. The mixture was heated and the reflux was maintained at 210-220°C, and the water generated was collected at the decanter arm and measured. During the reaction, the reflux mixture was sampled periodically to check the acid value, until it fell below 2 mg KOH g^{-1}. The total solid content of the alkyd solution was determined. About 2-3 g of the solution was weighed and dried in the oven at 110°C for an hour before cooled down in a desiccators. The product was a very viscous liquid containing 87.9 % of alkyd by weight.

2.3.2 Preparation of alkyd A2

Alkyd A2 was prepared by reacting 100 parts of A1 with 2.5 parts of MA in the same reaction set up as shown in Figure 2.1. The temperature was maintained around 120-130°C and the reaction was monitored by taking sample periodically to check the acid value. When the acid number was around 7 mg KOH g^{-1}, the reaction was stopped. A2 solution was a very viscous liquid containing 89.4 % of alkyd by weight.

2.3.3 Preparation of alkyd A3

Alkyd A3 was prepared using the same reaction set up as shown in Figure 2.1 by reacting 100 parts of A1 with 2.5 parts of MA. The reaction was maintained at a temperature range of 185-195°C and the reaction was stopped when water of reaction has stopped evolving. The acid value was checked periodically throughout the reaction. The product was a very viscous liquid containing 92.0 % of alkyd by weight, with an acid number fell below 2 mg KOH g^{-1}.

2.3.4 Preparation of alkyd A4

Alkyd A4 was prepared by reacting 100 parts of A1 with 11.4 parts of MA in the same reaction set up as shown in Figure 2.1. The preparation conditions were similar to alkyd A2 and it produced a viscous liquid containing 68.0 % of alkyd by weight and the acid value fell below 30 mg KOH g^{-1}.

2.4 Preparation of mcl-PHA solution

The mcl-PHA solution was prepared in two stages. The first stage involved cultivating the mcl-PHA, which was planned and conducted by Tan *et al.* in the Biotechnology Laboratory at the Institute of Advanced Studies, University of Malaya[181]. Thereafter, our lab continued the second stage in extracting the mcl-PHA and preparing of mcl-PHA solution.

2.4.1 Cultivation of mcl-PHA

The mcl-PHA was first produced by *Pseudomonas putida* PGA1 using oleic acid ($C_{18:1}$) as carbon substrates[181]. Production of mcl-PHA was conducted using orbital shaking incubator set at 240 rpm with constant temperature of 30°C. Initially, *P. putida* cells were grown in E medium[183] supplemented with 0.1 % microelement solution[108] to produce the biomass. The cells were harvested after 20 hours of centrifugation. They were washed and then transferred to the nitrogen-limiting E2 medium[108] containing a specified carbon substrate at a concentration of 0.5 % w/w. Finally, cells were harvested after 48 hours of centrifugation, washed and lyophilized.

2.4.2 Extraction of mcl-PHA

To extract the mcl-PHA from the stock solution produced by Tan *et al*, the lyophilized cells (0.5 g) were first suspended in 100 mL of chloroform, then refluxed for 4 hours before filtered through a sintered glass filter (porosity no. 3). The residue cells that remained on the sintered glass filter were then extracted again in chloroform by repeating the same procedure for another two times. The filtrate was concentrated by evaporation using a Buchi Rotavapor R-114. The product was dissolved in toluene to produce a sticky liquid containing 41 % mcl-PHA by weight.

2.5 Reaction of ENR and alkyd

The reactions of ENR and alkyd resins were performed at ambient temperature of 27-29°C. ENR and alkyd solutions were mixed in conical flasks, flushed with nitrogen for 5 minutes before being closed with stoppers. The flasks were agitated on an orbital shaker.

Visual observation showed that the solutions could blend homogeneously and the viscosity increased with reaction time. At the end of the specified time, the mixtures were poured into 500 mL of methanol to precipitate the rubber, which was isolated by filtration. Any excess unreacted alkyds were washed off with methanol; while the reacted rubber was then dried in a vacuum oven at 50°C. ENR/A1, ENR/A2, ENR/A3 and ENR/A4 denote the reaction product of ENR with alkyds A1, A2, A3 and A4 respectively.

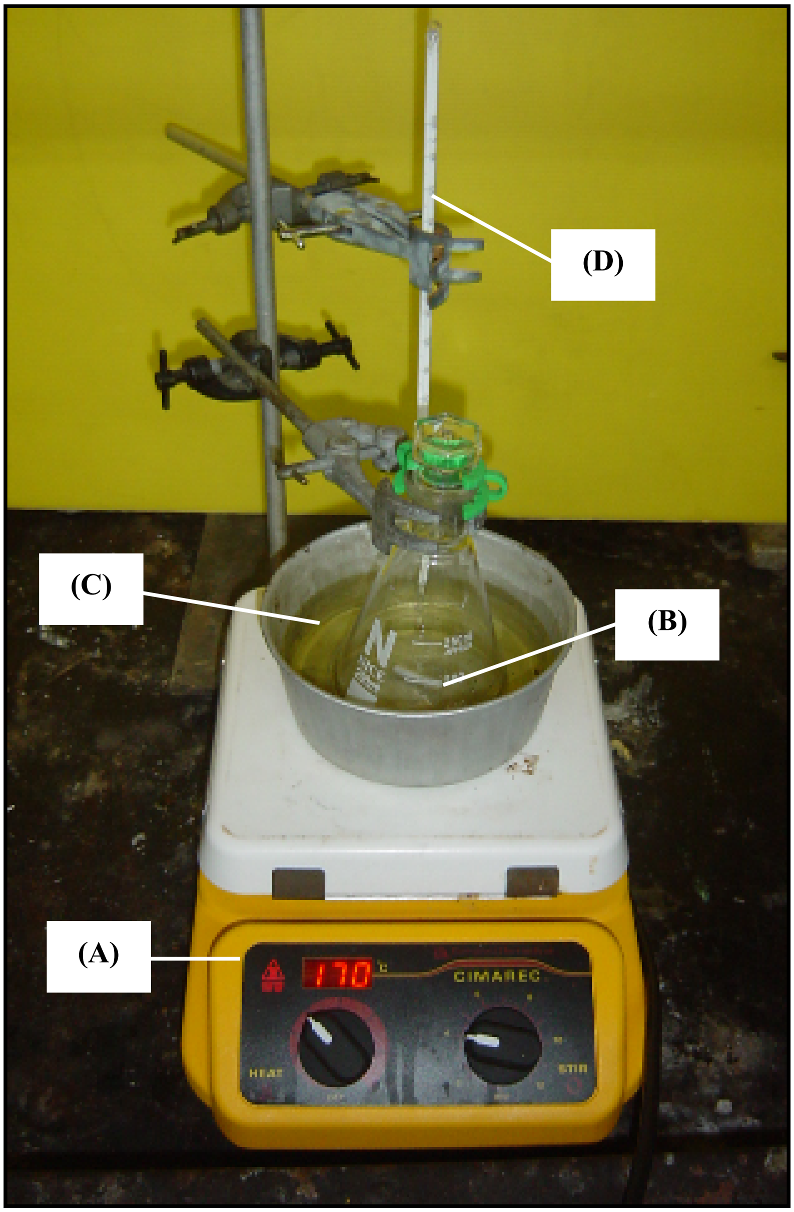

Figure 2.2: The reactions of ENR/mcl-PHA in an oil bath set at 170°C: Hot plate and stirrer (A); Magnetic stirrer (B); Oil bath (C) and Thermometer (D).

2.6 Reaction of ENR and mcl-PHA

ENR and mcl-PHA solutions were mixed in conical flasks, flushed with nitrogen for 5 minutes before being closed with stoppers. Then, the reaction of ENR and mcl-PHA was performed at two temperatures, i.e. ambient temperature of 27-29°C and elevated temperature of 170°C.

For the reaction investigated under ambient temperature, the mixture in the flask was agitated on an orbital shaker and allowed to react for the specified time. The mixture was then poured into 500 mL of methanol to precipitate the reacted ENR, which was isolated by filtration and dried in a vacuum oven overnight.

For the reaction investigated at elevated temperature, the mixture in the flask was put into an oil bath set at 170°C and stirred with a magnetic stirrer as shown in Figure 2.2. After being reacted for the specified reaction time, the mixture was then poured into 500 mL of methanol to precipitate the reacted rubber, which was isolated by filtration and the rubber dried as before. The reaction product of ENR with mcl-PHA was denoted as ENR/PHA.

2.7 Determination of hydroxyl number

The hydroxyl number was determined by a procedure adapted from ASTM D4274-94, test method B. Under this test, the hydroxyl groups in the sample was first esterified with phthalic anhydride (PA) reagent and the remaining reagent was then neutralized with the standard potassium hydroxide (KOH) solution. The hydroxyl number was calculated from the difference in titration of the blank and sample solutions. Overall, this test was carried out in two steps, where the first step was to prepare test reagents and standardize the standard KOH solution; whereas, the second step involved sample and blank titration.

2.7.1 Preparation of test reagents

The four reagents required for this test are redistilled pyridine, PA reagent, KOH solution and phenolphthalein indicator solution. These reagents are prepared as follows:

(a) Redistilled pyridine was prepared by distilling pyridine from PA. The fraction with boiling point around 114-115°C was collected and stored in a brown glass bottle.

(b) PA reagent was prepared by dissolving PA of about 113 g with 700 mL of redistilled pyridine in a brown bottle. The reagent was shaken vigorously on an orbital shaker until dissolved. It was left to stand overnight at ambient temperature before use. The product should be colourless and must be discarded if it developed a colour.

(c) Phenolphthalein indicator solution (1 % w/v) was prepared by dissolving 0.1 g of phenolphthalein in 10 mL of redistilled pyridine.

(d) KOH solution is the standard solution with concentration of 0.5 M. It was prepared by dissolving 5 g of KOH in 250 mL of distilled water.

The KOH solution has to be standardized before being used to titrate with the samples. About 10 g of potassium hydrogenphthalate ($KHC_8H_4O_4$) was dried in an oven at 110°C for 2 hours before cooled down in a desiccator. Then, about 4-5 g of the dried potassium hydrogenphthalate was weighed accurately in a 500 mL conical flask. About 200 mL of distilled water was added into the flask, flushed with nitrogen for 5 minutes before being closed with a stopper. The mixture was swirled gently until dissolved and 5 drops of phenolphthalein indicator was added into the flask. The mixture was then titrated with 0.5 M of KOH solution in a burette until the first appearance of a permanent pink colour. The molarity of KOH solution was determined as follows:

$$\text{Molarity of KOH (M)} = \frac{W_K}{0.2042 \times V_{KOH}} \qquad [2.1]$$

Where, W_K is the weight of potassium hydrogenphthalate and V_{KOH} is the volume of KOH solution.

2.7.2 Sample and blank titration

The sample used for titration was first dried in an oven at 110°C for 2 hours. This was to remove excess water in the sample which might disrupt the esterification reagent during titration. The amount of sample needed for the test was calculated as follows:

$$\text{Sample size} = \frac{561}{\text{Estimated hydroxyl number}} \qquad [2.2]$$

The calculated sample was dissolved in 25 mL of PA reagent into a conical flask closed with stopper. Blank solutions were also prepared by pipetting 25 mL of PA reagent into conical flasks closed with a stopper. Both the sample and blank solutions were clamped as close as possible in a water bath and allowed to heat for 2 hours at 98-100°C. The flasks were then removed from the bath and allowed to cool to ambient temperature. Next, 50 mL of redistilled pyridine and 0.5 mL of phenolphatalein indicator were added to each sample and blank solutions. The solutions were titrated with KOH solution in a burette, until the first appearance of a permanent pink colour.

In order to obtain the results close to the estimated hydroxyl number, this test must fulfil two requirements. In the blank titration, it was important to note that 25 mL of PA reagent should consume 95-100 mL of 0.5 M standard solution. It was also crucial that the net titration (blank minus sample) was between 18-22 mL. If the titration results did not meet these requirements, then the titration process have to be repeated by adjusting the sample size accordingly. The hydroxyl number of the sample was calculated using the equation [2.3],

$$\text{Hydroxyl number} = \frac{56.1 \times M \times (V_b - V)}{W} \qquad [2.3]$$

where, V and V_b are the volumes of KOH solution to titrate sample and blank, respectively, W is the mass of sample and M refers to the molarity of the KOH solution.

Sample and blank titration were carried out in duplicates and the hydroxyl number was reported by averaging the results.

2.8 Determination of acid number

The acid number was determined by a procedure adapted from ASTM D 1980-87. Under this test, the free acids in the sample were neutralized with the standard potassium hydroxide (KOH) solution. The acid number was determined from the amount of KOH (in milligrams) required to neutralize the free acids in 1 g of sample. This test was carried out in two steps. The first step was to prepare test reagents and standardize the ethanolic KOH solution. The second step involved sample and blank titration.

2.8.1 Preparation of test reagents

There are two reagents required for this test, which are KOH solution and phenolphthalein indicator solution. These reagents were prepared as follows:

(a) Phenolphthalein indicator solution (1 % w/v) was prepared by dissolving 0.1 g of phenolphthalein in 10 mL of ethanol.

(b) KOH solution is the standard solution with concentration of 0.05 M. It was prepared by dissolving 0.7 g of KOH in 250 mL of ethanol.

The KOH solution has to be standardized before titrating the samples. About 2 g of potassium hydrogenphthalate ($KHC_8H_4O_4$) was dried in an oven at 110°C for 2 hours before cooled down in a desiccators. Subsequently, about 0.5 g of the dried potassium hydrogenphthalate was dissolved in 50 mL of distilled water in a conical flask. The mixture was swirled gently until dissolved. Later, 5 drops of phenolphthalein indicator was added into the flask and the mixture was then titrated with 0.05 M of ethanolic KOH solution in a burette until the first appearance of a permanent pink colour. The molarity of KOH solution was determined as follows:

$$\text{Molarity of KOH (M)} = \frac{W_K}{0.2042 \times V_{KOH}} \qquad [2.4]$$

where, W_K is the weight of potassium hydrogenphthalate and V_{KOH} is the volume of KOH solution.

2.8.2 Sample and blank titration

About 2-3 g of sample was weighed into a conical flask, followed by addition 50 mL of a solvent mixture. This solvent mixture consists of 16.7 mL ethanol and 33.3 mL toluene at a mixing ratio of 1:2, then the mixture was swirled gently until dissolved. To prepare the blank solutions, 50 mL of solvent mixture was pipetted into conical flasks and 0.5 mL of phenolphatalein indicator was later added to each sample and blank solutions. The solutions were titrated with KOH solution in a burette until the first appearance of a permanent pink colour. The acid number was calculated as follows:

$$\text{Acid number} = \frac{56.1 \times \text{M} \times (\text{V - V}_b)}{\text{W}} \qquad [2.5]$$

where, V and V_b are the volumes of KOH solution to titrate sample and blank, respectively, W is the mass of sample and M refers to the molarity of KOH solution.

Sample and blank titrations were carried out in duplicates and the acid number was reported by averaging the results. The difference between the two determinations should be less than 10 % of the mean acid number.

2.9 Determination of intrinsic viscosity

Figure 2.3 shows an Ubbelohde viscometer which is used in determining the intrinsic viscosities, $[\eta]$ of a dilute sample solution. Following the procedure adapted from ASTM D 446-93, this test was carried out in three steps, where the first step was to measure the solvent flow time, t_0, the second step involved preparation of sample solution and finally the measurement of solution flow time, t_s. All measurements were conducted in a water bath maintained at 30.0 ± 0.1°C. The data collected can be used to find the viscosity average molecular weight, M_v, provided that the two Mark-Houwink constants, K and a, for a specific polymer with respect to the temperature and solvent used in the experiment, are known.

2.9.1 Determination of solvent flow time, t_0

10 mL of toluene was measured into the viscometer through capillary (A). It was then placed in a water bath maintained at 30.0 ± 0.1°C for at least 2 minutes to allow the system to attain thermal equivalent. After that, a rubber pump was placed at capillary (B); whereas capillary (C) was blocked with finger to allowed toluene to be sucked up to capillary (B), in order to fill the bulb between the mark XY and reach the bulb above X (Figure 2.3).

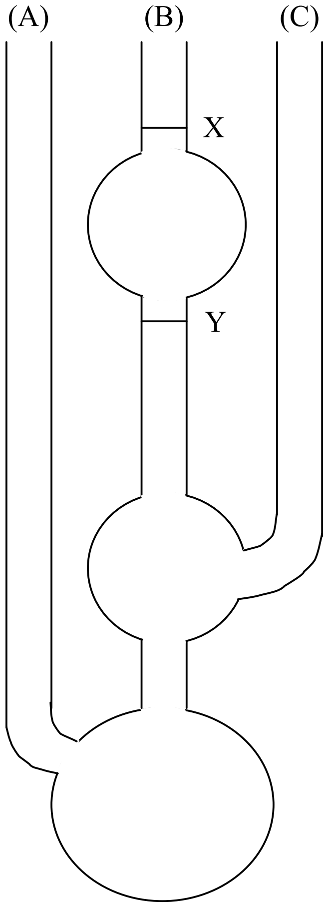

Figure 2.3: The Ubbelohde viscometer

After that, toluene was allowed to flow under gravity. A stop-watch was started when the upper meniscus reaches X and stopped when the meniscus reaches Y. The solvent flow time, t_0 is the time when the solvent past through the mark XY. This test was repeated five times and the average of the three most consistent readings was recorded as t_0.

2.9.2 Preparation of sample solution

A dilute sample solution was prepared by dissolving 0.25 g of the sample into a 25 mL volumetric flask. Toluene was added and adjusted to the mark of the flask. The mixture was swirled gently until dissolved.

2.9.3 Determination of solution flow time, t_s

10 mL of sample solution was pipetted into the viscometer through capillary (A) (Figure 2.3). The solution flow time, t_s was measured by following the same procedure as mentioned in Section 2.9.1. This test was repeated five times and the average of the three most consistent readings was taken as the t_s.

The concentration of the solution was reduced by adding 2-3 mL of toluene directly into the viscometer. Then, the viscometer was shook for a few minutes to ensure homogeneous mixing. It was then placed in a water bath, maintained at 30.0 ± 0.1°C for at least 2 minutes to allow the system to attain thermal equivalent before the next solution flow time was determined. This dilution process was repeated five times and the average of the three most consistent readings was calculated to be t_s.

2.10 Swelling in toluene

The extent of swelling of a rubber sample by immersing in toluene was expressed as the percentage by weight of toluene that could be held by the swollen gel of the reacted ENR. This test comprised of two sections, where the first section was to determine the density of sample and the second section was to measure the amount of swelling of the sample in toluene. The data collected can be used for further calculations in determining the percentage of swell (% swell), crosslink number average molecular weight (M_c) and modulus of the swollen rubber (G).

2.10.1 Determination of density

The weight of density bottle was weighed and recorded as W_0. It was then calibrated with water and the weight was recorded as W_a. The volume of density bottle, V_1, was calculated as follows:

$$\text{Volume of density bottle, } V_1 = \frac{W_0}{\rho_w} \qquad [2.6]$$

where, W_0 is the weight of density bottle, ρ_w is the density of water that is 1 g/mL.

About 0.39-0.41 g of dried rubber sample was cut into small pieces and weighed in a beaker. Then, 10 mL of glycerol was pipetted into the beaker, followed by the addition of methanol and glycerol alternately until the sample was suspended in the middle of the solvent mixture. Hence, the amount of methanol and glycerol must be balanced, in which the rubber might float in the solvent mixture if glycerol was in excess, but sink if methanol was in excess. The solvent mixture was then transferred into the density bottle and weight was recorded as W_1. The density of rubber sample was calculated as follows:

$$\text{Density of rubber, } \rho = \frac{W_1}{V_1} \qquad [2.7]$$

where, W_1 is the weight of solvent mixture of methanol and glycerol and V_1 is the volume of density bottle.

2.10.2 Amount of swelling

About 0.39-0.41 g of dried rubber sample was cut into small pieces and weighed as W_2. The rubber pieces were then transferred into a 100 mL conical flask, together with 25 mL of toluene and subsequently closed with stopper. The flask was then kept in the dark at ambient temperature for 20 hours before the excess toluene was removed and the swollen sample was transferred to a wire mesh (50 mesh). The weights of the wire mesh alone and the wire mesh with the swollen sample were recorded as S and U, respectively. The amount of swelling was calculated as follows:

$$\%\,\text{Swell} = \frac{\text{swollen weight - original weight}}{\text{original weight}} \times 100 \qquad [2.8]$$

$$= \frac{(W_g - W_2)}{W_2} \times 100$$

where, W_g is the weight of the swollen rubber which is (U-S) and W_2 is the weight of the original dried rubber

2.11 Differential Scanning Calorimetry (DSC)

Glass transition temperature, T_g, of sample was measured with a Mettler Toledo DSC 822 thermal analyzer. The instrument was calibrated with high purity indium to ensure the accuracy of the calorimetric data. Sample was dried in an oven to remove the volatile solvent. About 4-8 mg of dried sample was weighed and encapsulated in an aluminium pan. A similar empty pan was used as a reference. Analytical grade nitrogen (99.9 %) was used as the purge gas throughout the experiment. The DSC measurement was carried out at the temperature range of -100°C to 100°C at a scan rate of 10ºC min^{-1}. To nullify the effect of thermal history, the sample was scanned twice and DSC thermograms recorded during the second heating cycle was used for the interpretation. The onset temperature was used as the T_g of the samples.

2.12 Fourier Transform Infrared analysis (FTIR)

Sample solution of 1 % w/v was prepared by dissolving about 0.01 g of sample into 1 mL of chloroform. A layer of polymer film was cast from the solution and then was dried in cool air using a hair dryer to eliminate the volatile solvent. Subsequently, these films were analyzed at ambient temperature using the Perkin Elmer FTIR 1600 Series FTIR spectrometer, with 16 scans from 4000 cm^{-1} to 600 cm^{-1} with the resolution of 2 cm^{-1}.

2.13 Nuclear Magnetic Resonance (NMR)

Samples were dissolved as approximately 4 % w/v solutions in deuterated chloroform, containing 99.9 % $CDCl_3$ and 0.1 % trimethylsilane (TMS) as internal standard. ^{1}H-NMR spectra were recorded using a JEOL JNM-LA 400 FTNMR System spectrometer at ambient temperature using the FTNMR spectrometer, with 8 scans at 400 MHz.