CHAPTER III

MATERIALS AND EXPERIMENTAL METHODS

Materials

1. Fresh field latex

Fresh field latex from 3 different clones of rubber. RRIM 600, PB 5/51 and GT 1 were bought from the rubber plantation in Rayong Province.

2. Protease enzymes

- Alcalase T, dust-free granulate form of Alcalase, was obtained from NOVO.
- Papain product code number P3375. specified as crude extract. was purchased from Sigma.

3. Chemicals

Sodium metabisulfite, anhydrous potassium sulfate. copper sulfate pentahydrate ($CuSO_4.5H_2O$) and phosphorus pentaoxide were from M&B.

Selenium powder, sulfuric acid AR (S.G. 1.84) were from Merck.

Ammonia solution C.G. 25%, RG was from Riedel-de Haen

Triton X-100 (Analyzed reagent) was from Packard Instrument Company Inc.

Hydroxylamine hydrochloride was from BDH.

All chemical ingredients in compound rubber formulation namely Hisil 233S, ZnO active, 22 cp46, Wax, stearic acid, diethylene glycol, Shellfex, sulfur, MBTS, MBT and TMTM were kindly provided by Banpan Research Laboratory Co.Ltd.

Apparatus

Autoclave model HA-30 , Hirayama Manufacturing Co.,Japan.

Oven model UL-80 , Memmert, Germany.

Microwave oven model TRX-2500 BS, Turbo international Co. Ltd, Japan.

pH meter model PHM83 autocal, Radiometer, Denmark.

Reactor set and Two-roll mill, Pan Dynamics Co. Ltd, Thailand.

Other apparatus and materials for raw rubber testing were kindly provided by the Rubber Quality Testing Unit at the Rubber Research Institute.

Mooney viscometer model SMV-201 (Shimadzu. Japan), Durometer (Shore A) model 7206 (Zwick, Germany), Hydraulic press model TEE 120 (Dahtyan hydraulic machine industrial Co.Ltd., Taiwan), Micrometer model SM-114 (Teclock Co., Japan), Rheometer model EK-100 H (EEKONER IND, Co.Ltd, Taiwan), Instron testing machine model 1101 (U.S.A) and Two-roll mill model LRM 200 (Lab.Tech.Engineering Co.Ltd) were kindly provided by Banpan Research Laboratory Co.Ltd.

1. Reactor set

The reactor set used in this research is consisted of a vessel having external jackets of 41 cm inside diameter and two baffles of 3 cm wide. (Figure 3.1 and 3.2) The stainless steel impeller is a two-flat blade paddle which is mounted 4 cm from the tank bottom and have 28 cm in length and 3 cm width. The reactants added to the vessel can be stirred with agitation speed from 30 to 200 rpm. Temperature of the reactor can be controlled by pumping hot water into a jacket around the vessel ,and pH of added reactants can be monitored by a pH analyzer.

Figure 3.1 Reactor set for deproteinization of field latex

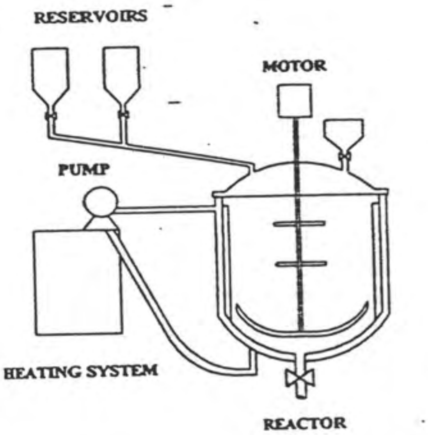


Figure 3.2 Diagram of reactor set used for deproteinization

Methods

1. Determination of dry rubber content (DRC)

A specimen of latex 5 ml was pipetted into a petridish and coagulated with 5% acetic acid in 95% ethanol. After complete coagulation, the coagulated rubber was removed, washed with ethanol and sheeted out by a two-roll mill. The coagulated rubber was then dried in a microwave oven at medium low power level for 20 minutes. Weigh the dried coagulum and calculate DRC content.

Calculation

calculated % DRC as follows :

$$\% \text{ DRC} = \frac{w}{v} \times 100$$

where : w = weight of dry rubber (g)

v = volume of latex sample used (ml)

2. Preparation of fresh latex for deproteinization

Fresh field latex collected from rubber tree was poured through a sieve or muslin cloth to remove dirts and some contaminants. A 15 ml aliquot of 25% ammonia solution and 70 ml of 10% Triton X-100 (0.14g/100ml) were added in 5 liter of fresh latex as preservative and anticoagulant. Fresh latex was stirred and filled in the container. During transportation, the container was stored in an ice-box at 4°C.

3. Preparation of enzyme solution and chemical solution

Alcalase was weighed in p.h.r unit (part per hundred of rubber) and dissolved in 250 ml of distilled water.

Papain was weighed in p.h.r unit (part per hundred of rubber) and dissolved in 250 ml of distilled water.

Hydroxylamine hydrochloride and sodium metabisulfite were weighed in p.h.r unit and dissolved in 250 ml of distilled water.

4. Optimization of CV-DPNR production by using Papain

Deproteinization of fresh latex (modified from Visessanguan, 1992 and Koosakul, 1993)

Sodium metabisulfite and hydroxylamine hydrochloride were added into fresh field latex (DRC 30-40 %) until the final concentration of 0.05 p.h.r and 0.15 p.h.r in 25% DRC of latex were obtained to prevent enzymatic discoloration and storage hardening respectively. To stabilize fresh latex and prevent coagulation, 10% solution of Triton X-100 was added to the final concentration of 1.15 p.h.r. and then diluted latex with distilled water to 25% DRC. Before Papain treatment, the enzyme solution and latex were heated up to 50°C. Fresh latex was treated with enzyme solution in the stirred tank with agitation speed (50 r.p.m). Optimal conditions of deproteinization by Papain is in pH 7.6 $\pm$ 0.1, 50°C and 60 minutes in the reactor shown in Figure 3.1. Fresh latex was collected and coagulated with steam in an autoclave for 30 minutes at 121°C under pressure of 15 lb/in^2. The coagulum was pressed through a two-roll mill, washed with water and dried at 60 °C in an air circulating oven. Nitrogen content was determined by Semi-micro Kjeldahl method according to RRIM standard method (1970) as summarized in Figure 3.3.

Clones of rubber used in this experiment are RRIM 600 , PB 5/51 and GT 1

To investigate the effect of rubber storage on Mooney viscosity at various time, field latex was prepared at pH range 7-8 and treated with enzyme concentration 0.3 p.h.r at 50°C in a reactor with agitation speed (50 r.p.m). Latex sample was collected and determined for nitrogen content. Dried rubber was kept at room temperature and determined for Mooney viscosity at a month by period.

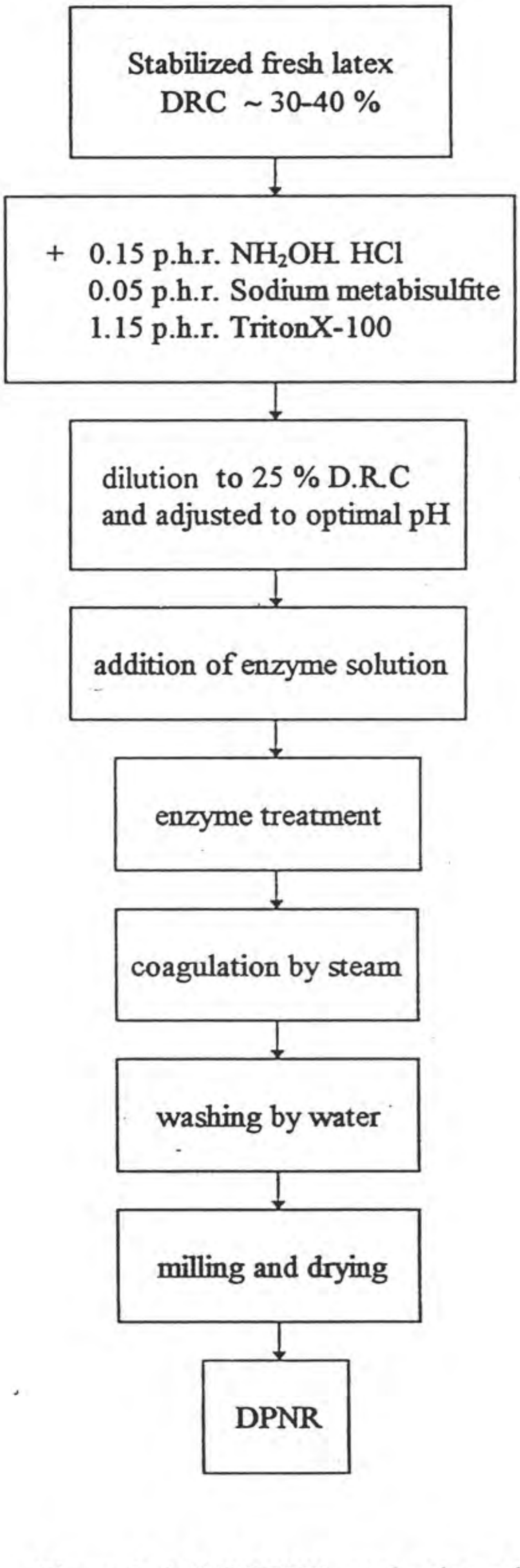


Figure 3. 3 CV-DPNR production scheme

To study the effect of mixing on Mooney viscosity of rubber, latex of rubber clone (RRIM 600) was treated by enzyme solution in a stirred-tank. DPNR production by both enzymes follows the previous works (Visessanguan, 1992 ; Koosakul, 1993). Optimum condition for deproteinization by Papain is at pH 7.6, 0.3 p.h.r enzyme concentration, 60 minutes and temperature at 50°C. For Alcalase, optimum condition is at pH 8.5, 0.4 p.h.r enzyme concentration, 6 hours and temperature at 60°C. Agitation speed of reactor was varied (45, 50, 55, 65 and 75 rpm). Latex sample (250 ml) was collected every hour and determined for nitrogen content. Dried rubber was stored at room temperature and determined for Mooney viscosity every month.

5. Optimization of CV-DPNR production by using Alcalase

To stabilize latex and prolong coagulation during Alcalase treatment, 10% solution of Triton X-100 was added at various concentration. Field latex was prepared in the optimal pH range 8-9 and treated with enzyme 0.4 p.h.r and agitation speed 60 rpm. After deproteinization, latex sample was collected and determined for % nitrogen content.

By varying time from 1 to 7 hr. at the optimal condition. RRIM 600 rubber was treated by Alcalase 0.4 p.h.r at temperature 60°C and pH range 8-9. After enzyme treatment , latex sample was collected and coagulated with steam. The optimal time was selected from the maximum percent reduction in nitrogen content.

CV-DPNR production

Field latex from three different clones of rubber PB5/51, RRIM 600 and GT1 were deproteinized at optimum condition and properties compared. DPNR was produced by using fresh field latex and both enzymes. Fresh latex was treated by adding 0.05 p.h.r sodium metabisulfite, 0.15 p.h.r hydroxylamine hydrochloride and 1.15 p.h.r Triton X-100 and diluted to 25% DRC. The stabilized latex was adjusted to optimal pH by adding 25% ammonia solution.

After deproteinization, the treated latex was coagulated in aluminium tray by steam in an autoclave under pressure 15 lb/in^2 at 121°C for 30 minutes. Control rubber of each latex source was prepared by steam coagulation without enzyme treatment.

Testing of the properties of CV-DPNR

Raw rubber testing (RRIM, 1970)

1. Preparation of homogenization testpiece

Before testing, Raw rubber sample was homogenized by a cold two-roll mill. Rubber sample was passed six times through a two-roll mill with the gap setting of 1.65 mm. between the rolls at room temperature. After each pass the rubber was rolled into a cylinder shape and then introduce endwise for the next pass. The homogenized rubber was cut and divided into approximate weight portions for each test. Raw rubber properties of DPNR and its control rubber were determined according to RRIM specifications including dirt, ash, nitrogen, volatile matter, plasticity (P_0), plasticity retention index (PRI), color index, Mooney viscosity, molecular weight averages and molecular weight distribution.

1.1 Determination of nitrogen content

Weigh accurately about 0.2-0.3 g of the homogenised rubber into a micro-Kjeldahl tube and add about 0.65 g of catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: SeO = 30 : 4 : 1) and 7-8 ml of concentrated sulfuric acid. The mixture was heated gently in the digestion unit until the solution becomes clear green or colorless with no yellow tint after cooling. The digestion requires about 2-3 h. Cool the digest and transfer to the distillation apparatus followed by three washing with distilled water. Add 10 ml of boric acid solution to the receiving flask, add 2-3 drops of methyl red - bromecresol green indicator and place the receiver so that the end of the condenser dips below the surface of the boric acid solution. Add about 10 ml of 40 % NaOH solution to the digestion flask and pass steam from the generating flask through the distillation apparatus until the volume of distillate in the receiving flask is about 125 ml. This requires about 4 min. Lower the receiver until the condenser tip is well above the solution and continue distilling for 1 min. The total volume should be about 150 ml. Wash the end of the condenser with distilled water. Titrate the distillate with standardized 0.01 M H_2SO_4. The color changes from green to a light violet at the end point. Blank can be prepared by using all the reagents but omitting the sample.

Calculation

calculated the nitrogen content as follows :

$$\text{Nitrogen (\%)} = \frac{(V_1 - V_2) \times 2.8 \times M}{W}$$

where: V_1 = volume of H_2SO_4 required for titration of the contents of the receiving flask (ml)

V_2 = volume of H_2SO_4 required for titration of the blank test (ml)

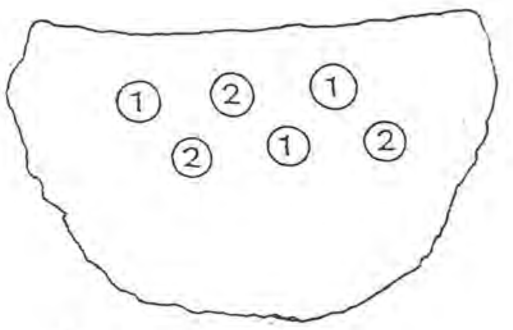
M = Molarity of the H_2SO_4

W = weight of rubber sample used (g)

1.2 Determination of plasticity retention index (PRI)

The plasticity retention index is a measure of the resistance of raw natural rubber to thermal oxidation. A high value of the index denotes high resistance to thermal oxidation.

A test portion of 20 $\pm$ 5 g of the homogenized piece was passed twice between a two-roll mill with nip setting adjusted to the final sheet thickness 1.6-1.8 mm, then immediately doubled and pressed lightly together by hand. Cut six test pellets from the double sheet with the Wallace punch as illustrated in Figure 3.4 . The test pellets are divided into two sets, one set each for plasticity determination before and after ageing in an oven at 140°C for 30 minute. The test pellet was sandwiched between two pieces of cigarette paper and pressed between the two parallel plate to a fixed thickness of 1 mm with compressive force of 10 ± 0.1 kg_f for 15 seconds. The thickness at the end of this period is taken as the measure of rapid plasticity.



Punched pellet for

1. initial plasticity determination
2. aged plasticity determination

Figure 3.4 Six test pellets from the double sheet with the Wallace punch

Calculation

calculated the PRI by using the median plasticity value of three unaged test pellets and the median plasticity value of the three aged test pellets as follows :

$$PRI = \frac{P_{30}}{P_0} \times 100$$

Where P_0 = the median plasticity value of three unaged test pellets.

P_{30} = the median plasticity value of three aged test pellets.

1.3 Determination of color index (ASTM D3157 . 1988)

A test piece of 15 ± 5 g from homogenized rubber was prepared by the same procedure as PRI test. Cut two test pellets from the doubled sheet with the punch. The two test pellets shall be laminated together by lightly pressing with the fingers. The two test pellets were pressed in the mold between two sheets of polyester or cellulose film using mold covers at not less than 3.5 mN/m^2 pressure of the cavity areas of the mold for 5 min at 150 ± 3° C. The color of test pellet was determined as Lovibond index by matching as closely as possible with the appropriate color standard over lighting box.

1.4 Determination of volatile matter

Cut about 25-30 g of the homogenized rubber and wrap it airtight in a polyethylene bag. The test portion was cut 10 g from homogenized rubber and weighed to nearest 0.1 mg. Pass the test portion through the cold mill with a nip setting at 0.5 ± 0.05 mm. Place test portions on aluminium tray. Tray was heated in an oven at 100°C ± 3°C for 4 h. After heating, open the oven door, remove a tray and close the oven door immediately. Each test portion was kept in a polyethylene bag and hung on the rack. Allow the test portions to cool for half an hour in an air-conditioned room.

Calculation

calculated as follows :

$$\text{volatile matter (\%)} = \frac{A - B}{A} \times 100$$

Where A = weight of test portion before drying (g)

B = weight of test portion after drying (g)

1.5 Determination of dirt content

Weigh about 25-30 g of the homogenized rubber and pass twice through a cold rolls of a laboratory mill with nip set at 0.33 mm. Immediately weigh a test portion of 10 g to the nearest 0.1 g. Cut into small strips and placed in a 500 ml conical flask containing 250 ml of mineral turpentine and 1 ml of rubber peptizing agent. Place flask with its content on the infra-red lamps and heat with occasional agitation to assist dissolution which is completed in 1 h - 2 h. After dissolution completed , the hot solution in flask was filtered through a previously weighed. clean and dry sieve. Wash twice the flask with 30-50 ml of hot mineral turpentine and filter through the sieve. Repeat washing with a hot mineral turpentine around the sieve. The sieve was dried in an oven at 90-100°C for about 1 h, cooled in a dessicator and weighed to the nearest 0.1 mg.

Calculation

calculated as follows :

$$\text{Dirt content (\%)} = \frac{B - A}{C} \times 100$$

Where A = weight of clean dry sieve (g)
B = weight of the sieve plus dirt (g)
C = weight of the test portion (g)

1.6 Determination of ash content

Weigh accurately 5-10 g portion of the homogenized rubber. Wrap in ashless filter paper and place in a crucible which has been previously ignited and weighed. Introduce the crucible into a muffle furnace controlled at a temperature of 550 ± 20° C until free form carbon (2-4 h). When ashing was complete, allow the crucible to cool in a dessicator and the weigh it the nearest 0.1 mg.

Calculation

calculated as follows :

$$\text{Ash content (\%)} = \frac{A - B}{W} \times 100$$

where A = weight of crucible plus ash (g)

B = weight of empty crucible (g)

W = weight of rubber sample (g)

1.7 Determination of Mooney viscosity (ASTM D1646,1988)

Before testing, temperature of test should be 100 ± 1° C. Place the rotor in the die cavity to heat up to test temperature. Remove the hot rotor from die cavity and place the preheated rotor through the center of one portion. Weigh 150 ± 5 g of rubber sample and passed ten times through a two-roll mill having a roll temperature of 70 ± 5 °C and having a gap setting of 2.5 ± 0.1 mm. Divide about 25 g of the homogenized rubber into two equal portions. One half was placed in the lower die cavity and the rotor was placed followed by another half on top of the rotor. Close the die immediately. Before starting the motor, preheat the specimen for 1 min. Set the running time for 4 minutes. The dial gauge reading were recorded at 1 minute. The viscosity of rubber sample was taken as the dial gauge reading at the end of running time. The viscosity was reported as Mooney unit, ML(1 − 4) 100° C; the rotor size (L for large), the number of minutes for warming up in the machine (1 min), the number of minutes at actual test (4 min.) and temperature (100°C).

1.8 Determination of molecular weight averages (Mw) and molecular weight distribution (MWD) by gel permeation chromatography (ASTM D3536, 1980)

Weigh accurately 0.01 g of rubber sample into a 20 ml vial with screw cap containing 10 ml of tetrahydrofuran (THF). After dissolution was complete, the solution was filtered through a membrane filter with pore sizes 5 μm before injection (100 μl). Analysis was performed on a Waters 510, with series of 4 Ultra styragel columns having nominal exclusion limits of 10^3, 10^4, 10^5 and 10^6 °A at a controlled temperature of 35°C and equipped with differential refractometer, using THF as eluters at the flow rate of 1 $ml.min^{-1}$. The Mw, Mn and polydispersity (Mw/Mn) were analyzed by the MAXIMA software for GPC by comparing with the standard calibration graph of Polystyrene Standard MW ranging from 8.5×10^3 - 1.06×10^7.

Weight average molecular weight (Mw) is related to the weight of the individual molecule as represented by the equation:

$$Mw = \Sigma(M_i.W_i)\ \Sigma W_i$$

where M = MW of rubber in fraction i

w = Weight of the rubber in fraction i

and i = fraction number (from o to x)

Number average molecular weight (Mn) is dependent on the number of molecules having each discrete Mw and is represented by the equation.

$$Mn = \Sigma W_i / \Sigma N_i$$

where N = the number of molecules in fraction i

The Mw and Mn values may be used to provide an index for the range of molecular weight distribution as follows:

$$Mw/Mn = d$$

where d = polydispersity value

2. <u>Testing of the rubber vulcanizates</u> (ASTM D412, 1989)

2.1 <u>Preparation of rubber compounding</u>

The compounding formulation shown in Table 3.1 was chosen to prepare rubber vulcanizate for comparison of the cure characteristic and physical properties of DPNR from each clones and its control rubber

Table 3.1 The compounding formulation chosen for assessing the cure characteristics of natural rubber

Ingredients	**Gram**
Natural rubber	100.0
Hisil 233S	45.0
Zinc oxide active	3.0
22 cp 46	0.3
Wax	1.0
Stearic acid	1.2
DEG	3.5
Shelflex	1.5
Sulfur	2.0
MBTS + MBT + TMTM	1.08

where

Hisil 233S (precipitated silica) is reinforcing filler.

Zinc oxide active and stearic acid are the activators of vulcanization.

MBTS (2-2 di-benzothiazyl disulfide) , MBT (2-mercaptobenzothiazole) and TMTM (tetramethyl thiuram monosulfide) are accelerators of vulcanization.

22 cp 46 (2,2 methylene bis-4-methyl-6-p-butyl phenol) and wax are antioxidants.

DEG (diethylene glycol) is a depressor of surface active absorption.

Shelflex is the processing oil.

Sulfur is the crosslinking agent.

The raw rubber (300 g) was pressed through a smooth two-roll mill and then mixed with the vulcanizing ingredients. The homogenized rubber was added with the mixture of Hisil 233S, DEG, Shelflex and Stearic acid followed by mixing with the mixture of wax, 22 cp46 and ZnO active. The compound was left at room temperature until cooled and added the mixture of MBTS, MBT and TMTM. Finally, sulfur was mixed with the compound. The compound was sheet out and left at room temperature for 24 hours before determination of vulcanization characteristics.

2.2 Determination of cure characteristic

The cure characteristic of the compound rubber was determined by using a Rheometer Model EK-100H (EEKONER, Taiwan) for 8 minutes at 155°C. The rubber sample was loaded on the top of the oscillating disc and the die immediately closed. The samples were then cured in a heated press in their respective mold for time to reach optimum cure. From the Rheometer curve recorded (Figure 3.5), all the necessary readings was measured and reported.

The cure parameters were recorded as scorch time, cure time, cure rate, minimum and maximum modulus. Scorch time, T_s was the minimum time for processing the compound rubber before the rubber was changed from plastic form to vulcanized state. Cure rate, T_{90}-T_S means the cure time required to give product to its optimum cure state.

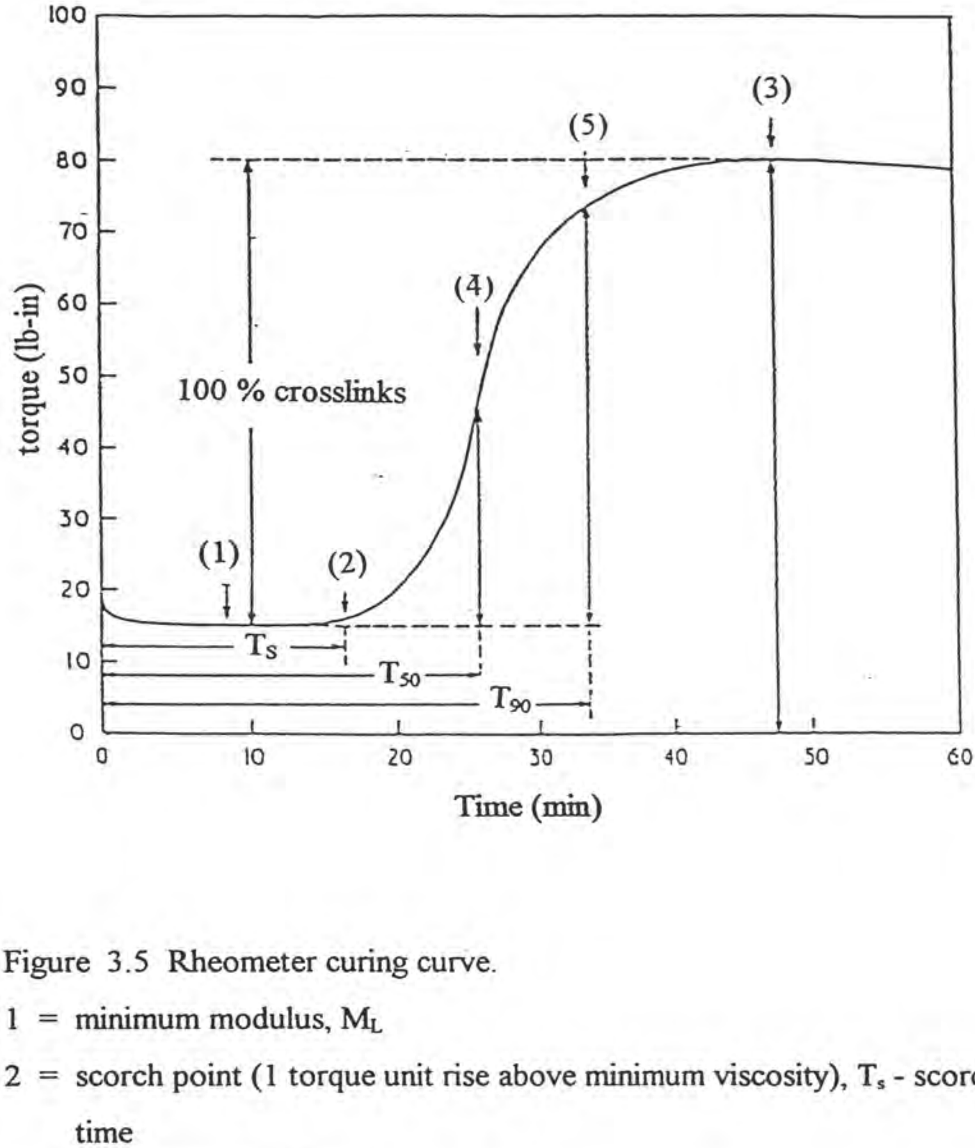


Figure 3.5 Rheometer curing curve.

1 = minimum modulus, M_L

2 = scorch point (1 torque unit rise above minimum viscosity), T_s - scorch time

3 = maximum modulus, M_H

4 = 50% crosslink , T_{50} - time to 50% crosslink

5 = 90% crosslink or optimum cure , T_{90} - time to optimum cure

6 = cure rate , T_{50} - T_s or T_{90} - T_s

2.3 Preparation of testpiece for physical property test.

The compound rubber was prepared by compression molding at 155°C for its optimum cure time indicated by Rheometer curing curve. The vulcanized rubbers were stored at room temperature for 24 hours before cutting into testpiece for measuring physical properties. The physical properties test of vulcanized rubber such as hardness (ShoreA), specific gravity, tensile strength, 300% modulus, %elongation at break and tear strength were tested according to ASTM .

2.4 Hardness test (ASTM D1415 , 1988)

The international hardness test was based on measurement of the penetration of a rigid ball into the rubber specimen under specified condition. Rubber vulcanizate was prepared as a flat and smooth sheet having thickness sufficient to fit the gap of type A durometer. The plunger of durometer was pressed with the minor force on the rubber specimen, the scale was pointed and read as the hardness in Shore A at room temperature. The median value of five different points distributed over the specimen was recorded.

2.5 Specific gravity test (ASTM D3184, 1989)

The specific gravity test was based on water replacement of testpiece. The weight of rubber vulcanized testpiece was weighed in the air and weighed again in the water. The specific gravity of rubber vulcanized was calculated as follows:

$$\text{Specific gravity} = \frac{\text{Weight of testpiece in air (g)}}{\text{Weight of testpiece in water (g)}}$$

2.6 Tensile strength, 300% modulus and % elongation at break (ASTM D412, 1987)

The five dumbbell testpieces (Figure 3.6) were cut out from the vulcanized rubber by punching with die using a single stroke of a press. A reference of length

2.0 cm. was marked and the thickness of the dumbbell testpiece measured along the reference length by a micrometer dial gauge.

The two ends of the dumbbell testpiece were clamped into the two grips of the testing machine. The testpiece was stretched at a constant rate of moving grips of 500 ± 50 mm/min. The force per cross-sectional area of testpiece required to stretch the sample to 300% of reference mark length and to breakage were automatically recorded and calculated as 300% modulus and tensile strength (stress at break) respectively. The maximum stretching of reference mark was recorded and calculated as follows:

$$\text{Tensile strength} = \frac{\text{Force at break (kg)}}{\text{cross-sectional area (cm}^2\text{)}}$$

$$\text{300\% modulus} = \frac{\text{Force at 6.0 cm (kg)}}{\text{cross-sectional area (cm}^2\text{)}}$$

$$\text{\% elongation at break} = (\text{length of reference mark at break} - 2) \times 50$$

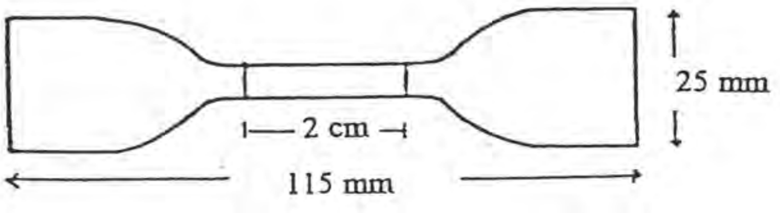


Figure 3.6 Shape of a dumbbell testpiece for tensile strength, 300%modulus and % elongation at break.

2.7 Tear strength test (ASTM D624 , 1989)

The five testpieces (Figure 3.7) were cut out from the rubber vulcanized by punching with die using a single stroke of a press. The thickness of the testpiece was measured by a micrometer dial gauge. The two end of the testpiece were clamped into the two grips of the testing machine. The highest force per thickness of the testpiece required to tear were automatically recorded and calculated as follow :

$$\text{Tear strength} = \frac{\text{Highest force (kg)}}{\text{Thickness of testpiece (cm)}}$$

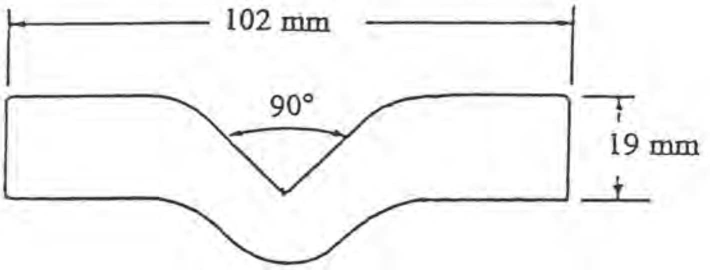


Figure 3.7 Shape of testpiece for tear strength test

2.8 Heat ageing tests

Heat ageing changes in bulk properties such as hardness, 300% modulus, tear strength, tensile strength, % elongation at break and 300% modulus and surface appearance. Rubber valcanized was stored in oven at 70°C for 7 days. After ageing, properties of rubber vulcanized are measured initially and then samples subjected to high temperature for specified period of time. Properties of samples was measured again.