



## CHAPTER III

### MATERIALS AND EXPERIMENTAL METHODS

#### 3.1 Materials

##### 3.1.1 Fresh field latex

Fresh latex from 4 clones of rubber, RRIM 600, PB 28/59, PB 5/51 and GT 1 were obtained from the rubber plantation in Rayong province. After removal of dirt by pouring the latex through muslin cloth, 15 ml of 25 % ammonia solution and 70 ml of 10 % Triton X-100 solution were added in 5 litre of fresh latex respectively as preservative and anticoagulant. The stabilized rubber lattices were kept in ice-box during delivery.

##### 3.1.2 Protease enzyme (papain)

Papain ( code number P3375, specified as crude extract ) was purchased from Sigma.

##### 3.1.3 Chemicals

Hydroxylamine hydrochloride and thiourea (thiocarbamide) were from BDH.

Anhydrous potassium sulfate, sodium metabisulfite, copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and phosphorus pentoxide were from M&B.

Selenium powder, sulfuric acid AR (Sp gr 1.84) were from Merck.

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

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

All Chemical ingredients in compound rubber formulation (Hisil 233S, ZnO active, 22 CP 46, wax, stearic acid, diethylene glycol, Shellflex, sulfur, MBTS, MBT and TMTM) were kindly provided by Banpan Research Laboratory Co. Ltd.

### 3.2 Apparatus

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

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

Oven model UL-80, Memmert, Germany.

pH Analyzer model 1054A and pH/ORP sensor model 399, Resemount Analytical Inc, U.S.A..

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 Resrarch Institute.

Durometer (Shore A) model 7206 (Zwick, Germany), Hydraulic press model TEE120 (Dahtyan hydraulic machine industrial Co.,Ltd., Taiwan), Instron testing machine model 1101 (U.S.A.), Micrometer model SM-114 (Teclock Co., Japan), Mooney viscometer model SMV 201 (Shimadzu, Japan), Rheometer model EK-100H (EEKONER IND Co.,Ltd, Taiwan), Two-roll mill model LRM 200 (Lab. Tech. Engineering Co., Ltd.) were kindly provided by Banpan Research Laboratory Co., Ltd..

#### 3.2.1 Reactor set

The reactor used in this research consists of vessel, impeller, water bath and pH analyzer (Figure 3.1).

The stainless steel cylindrical vessel is 41 cm inside diameter with two stainless steel baffles of 3 cm wide. The liquid in vessel is agitated by an impeller which is connected with a motor. The rotating speed of the motor can be adjusted from 30 to 200 rpm. The impeller is a two-flat blade paddle and made of stainless steel. The lower blade is 28 cm in length, 3 cm width and located close to the vessel bottom, the other is 12 cm in length, 3 cm width and 11.4 cm high from the first paddle. The liquid in the vessel is heated by hot water from water bath and pumped into a jacket around the vessel.



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a.



b.



Figure 3.1 A reactor set for deproteinization of rubber latex

a. Reactor set, b. Vessel

### 3.3 Experimental methods

#### 3.3.1 Determination of dry rubber content (DRC)

Five cm<sup>3</sup> of latex specimen was pipetted into a petridish and coagulated with 5 % acetic acid in ethanol. After complete coagulation, the coagulated rubber was washed with ethanol, creped through a two-roll mill, dried in a microwave oven at medium low power level for 15 minutes and weighed. The % DRC was calculated as follow.

$$\% \text{ DRC} = \frac{W}{\rho V} \times 100$$

where

W = weight of the dry rubber (g)

V = volume of the latex taken (cm<sup>3</sup>)

$\rho$  = density of the latex (g/cm<sup>3</sup>)

#### 3.3.2 Preparation of enzyme solution and chemical solution

Papain was weighed in p.h.r. unit (part per hundred of rubber) and dissolved in 200 ml of distilled water.

Hydroxylamine hydrochloride ( 0.15 p.h.r. ) and sodium metabisulfite ( 0.05 p.h.r. ) were taken and dissolved in 200 ml of distilled water.

#### 3.3.3 Determination of optimum condition of CV-DPNR

The stabilized fresh latex was adjusted with mixture solution of sodium metabisulfite (0.05 p.h.r.) and hydroxylamine hydrochloride (0.15 p.h.r.). Sodium metabisulfite and hydroxylamine hydrochloride was added to improve light color and viscosity stabilized respectively. The treated fresh latex was once again adjusted with 10 % Triton X-100

solution. The pH was adjusted to  $7.6 \pm 0.1$  with 25 % ammonia solution. Then papain solution was added and diluted latex with distilled water to 25 % DRC. Before treatment, enzyme solution and latex were adjusted to the same temperature. Enzymolysis was carried out in the stirred tank by adding enzyme solution with 10 % solution of Triton X-100 added. Latex sample was collected at various time and coagulated. The coagulum was passed through the two-roll mill, washed with water, and dried at  $60\text{ }^{\circ}\text{C}$  in an air circulating oven (Figure 3.2). Nitrogen content was determined by Semimicro Kjeldahl method according to RRIM

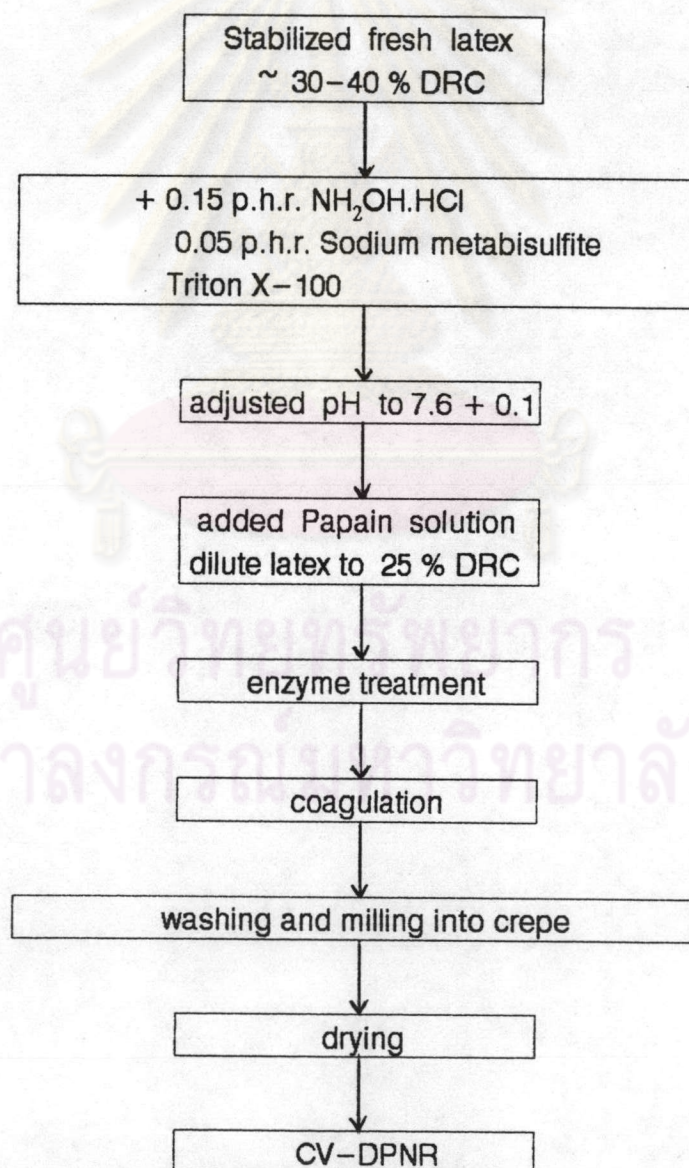


Figure 3.2 Scheme of CV-DPNR production

Clone of rubber in this experiment is RRIM 600. From previous study, papain was effective in pH range 7-8 (Visessanguan, 1992).

To stabilize latex and prolong coagulation during papain treatment, 10 % solution of Triton X-100 was added at various concentration and treated with 0.3 p.h.r. papain concentration at pH  $7.6 \pm 0.1$ , 50 °C, and agitation speed 37 rpm. The treated latex was coagulated with steam by autoclaving 10 minutes at 121 °C under pressure 15 lb/in<sup>2</sup>. The coagulum was passed through a two-roll mill, washed, dried and the nitrogen content determined. The optimum concentration of Triton X-100 was determined when coagulation under steam and for maximum nitrogen reduction.

To study the effect of temperature on deproteinization, fresh latex was prepared in pH  $7.6 \pm 0.1$ , using a stirred tank with fix agitation speed (37 rpm) and treated with 0.3 p.h.r. papain concentration at various temperature (30, 35, 40, 45, 50 and 55 °C). At fixed time interval latex samples were collected and coagulated in a microwave oven. The coagulated samples were passed through the two-roll mill, washed and dried. Their nitrogen content were later determined.

To study the effect of agitation speed on deproteinization, A small reactor with agitation speed of 57 rpm and a large reactor which agitation speeds were varied (37, 50, 60, 80 and 100 rpm) were used at 0.3 p.h.r. enzyme concentration and optimum temperature. Latex sample was collected and determined for nitrogen content.

Several papain concentrations were used (0, 0.2, 0.25, 0.3 p.h.r.) at optimum temperature and agitation speed in order to study the effect of papain concentration on deproteinization, The samples

were taken at intervals for nitrogen content determination.

The optimum condition (Triton X-100 concentration, temperature, agitation speed and papain concentration) is chosen when reaction of nitrogen is highest and the nitrogen content must be lower than the standard DPNR specification (%N < 0.12). The shortest time used for deproteinization is also selected.

### 3.4 CV-DPNR production

Three different clones of rubber PB 28/59, PB 5/51 and GT 1 were also deproteinized at this optimum condition and their properties compared.

For each clone of rubber deproteinization was carried out 4 times to check repeatability. The latex was treated by adding 0.15 p.h.r. hydroxylamine hydrochloride, 0.05 p.h.r. sodium metabisulfite, Triton X-100 and pH adjusted in the agitated tank and then papain added. After deproteinization, the treated latex was coagulated in aluminium tray by steaming under pressure 15 lb/in<sup>2</sup> in an autoclaving at 121 °C for 10 minutes. The coagulum was passed through a two-roll mill, washed and dried at 60 °C in hot air oven. Control rubber of each latex source was prepared by steam coagulation without papain treatment.

### 3.5 Testing of the properties of DPNR

#### 3.5.1 Raw rubber testing

The raw rubber properties test procedures for CV-DPNR and its control were based on RRIM specification (for ash content, dirt content, nitrogen content, volatile matter, initial plasticity ( $P_0$ );



plasticity retention index (PRI) and color index). Before testing, rubber samples were homogenized. For Mooney viscosity, molecular weight average and molecular weight distribution by GPC, the test procedures were carried out according to ASTM (See Appendix 2).

### 3.5.2 Vulcanized rubber testing

As most as rubber products are in the form of vulcanizates, vulcanization test is of vital importance to the quality and performance of their products. Before vulcanization test, the rubber was mixed with chemicals to form compound. The compound was determined cure characteristics (scorch time, cure time, cure rate and torque rise) by running on Rheometer at 155 °C for 8 minuts.

The vulcanized samples were prepared by compression moulding at 155 °C for its optimum cure time plus 3.5 minutes indicated by Rheometer graph. The vulcanized rubbers were left for 24 hours, after that they were cut into test pieces for physical properties measuring. The physical properties tests such as hardness (Shore A), 300 % modulus, elongation at break, tensile strength, tear strength and specific gravity were tested according to ASTM (See Appendix 2).

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