

## CHAPTER III

### EXPERIMENTAL

#### 3.1 Materials

##### 3.1.1 Raw Materials

1. Concentrated latex (~60% DRC): N.Y. Rubber Co.,Ltd.
2. Field latex (~30% DRC): N.Y. Rubber Co.,Ltd.

##### 3.1.2 Chemicals

	Chemicals	Grade	Supplier
1.	Orthoboric acid	Analytical	Bang Trading 1993
2.	Potassium hydroxide	Analytical	Bang Trading 1993
3.	Sodium hydroxide	Analytical	Bang Trading 1993
4.	Phosphotungstic acid	Analytical	Bang Trading 1993
5.	Potassium hydrogen phthalate	Analytical	Bang Trading 1993
6.	Copper(II) sulfate pentahydrate	Analytical	Bang Trading 1993
7.	Sulfuric acid	Analytical	Bang Trading 1993
8.	Chloroform	Analytical	Bang Trading 1993
9.	D-chloroform	Analytical	Aldrich
10.	Sodium chloride	Analytical	Bang Trading 1993
11.	Toluene	Commercial	Bang Trading 1993
12.	Methanol	Commercial	Bang Trading 1993
13.	Tri-sodium citrate	Lab grade	APS Ajax Finechem
14.	Formic acid	Analytical	APS Ajax Finechem
15.	Potassium sulfate	Analytical	APS Ajax Finechem
16.	Calcium nitrate	Analytical	APS Ajax Finechem
17.	Sodium dodecyl sulfate	Analytical	APS Ajax Finechem
18.	Deoxycholic acid sodium salt	Biochemical grade	Fluka
19.	Selenium	Analytical	Fluka

20. Triton X-100	Analytical	MERCK
21. BDH	Analytical	MERCK
22. Trichloroacetic acid	Analytical	MERCK
23. Folin-Cioaltea's reagent	Analytical	Carlo Erba

### 3.2 Equipment and Instruments

- 1.) 500-cm<sup>3</sup> reactor flask
- 2.) Mechanical stirrer motor : HEIDON 1200G
- 3.) Magnetic stirrer hotplate : Ika Werker RW20, Germany
- 4.) General glassware and equipment
- 5.) Centrifuge : Beckman model J2-21, USA
- 6.) Total nitrogen analyzer : Gerhalt, Germany
- 7.) FTIR spectrophotometer : Nicolet (Impact 410), England
- 8.) Gel-permeation chromatograph
  - Column : styrene-divinylbenzene copolymers (exclusion limits of  $2.0 \times 10^7$  and  $5.0 \times 10^4$ )
  - Detector : refractive index detector (TOSOH LS-8000)
- 9.) Tensile tester : LLOYD (LR 5K), England

### 3.3 Purification of Natural Rubber Latex by Saponification

#### 3.3.1 Enzymatic Deproteinization

200 cm<sup>3</sup> of 30% DRC High Ammoniated-latex in 250 cm<sup>3</sup> flask reactor was incubated with 0.02% w/v proteolytic enzyme (Kao Kp-3937) and 1% SDS at room temperature for 24 h.. The reacted latex was diluted to 10% DRC followed by washed by double centrifugation and coagulated with methanol and dried under reduced pressure. Figure 3.1 shows the procedure of enzymatic deproteinization.

### 3.3.2 Saponification Deproteinization

Field latex and High Ammonia was diluted to 10% or 30% DRC and incubated with NaOH(1-5% w/v) in the presence of surfactant at 70°C for 3 h. The resulting latex was treated in two ways as shown in Figure 3.2.

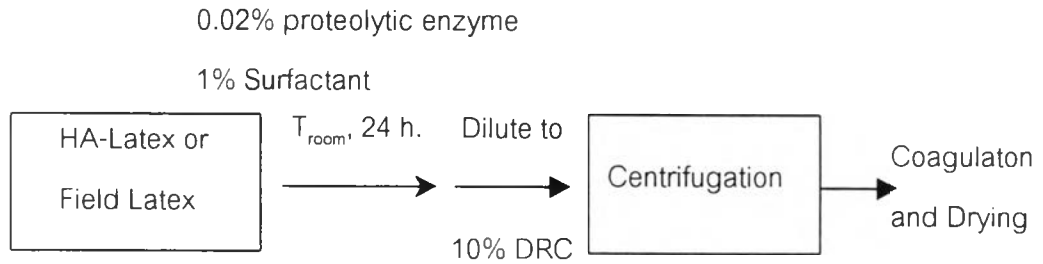


Figure 3.1 Enzymatic deproteinization of natural rubber latex

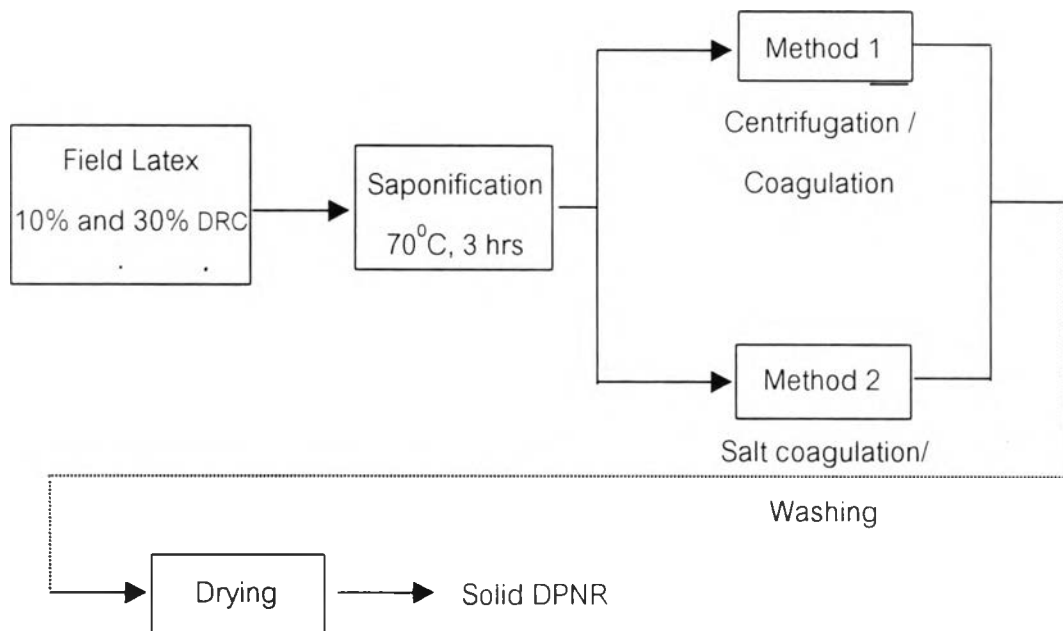


Figure 3.2 Saponification and washing method of natural rubber latex

### 3.3.2.1 Saponification of Latex and Washing by Centrifugation Followed by Coagulation

#### 1. Stability of latex during saponification and washing steps.

Two types of surfactant were studied. Sodium dodecyl sulfate (SDS) or Triton X-100, 1%, w/v was added during saponification. Stability of latex in saponification and washing step was observed by the coagulation of latex.

#### 2. Effect of number of centrifugation on washing efficiency.

Single and double centrifugation at 10,000 rpm, 30 min (for each) was applied in the washing step after saponification. Nitrogen content of saponified rubber was determined by modified RRIM (Rubber Research Institute of Malaysia) method.

#### 3. Effect of surfactant on saponification

10% DRC High-ammoniated latex was carried out with / with out Sodium hydroxide (5% w/v) in the presence of 0.1 and 0.2% w/v of Triton x-200. After that decomposed protein was washed by double centrifugation at 10,000 rpm for 30 min. Nitrogen content was determined by modified RRIM method.

#### 4. Effect of surfactant concentration on saponification and washing efficiency.

Saponification of 10% DRC High-ammoniated latex was carried out with 5% w/v NaOH in the presence of 0.1-0.4%w/v Triton X-100. The washing of saponified latex was carried out in the presence of Triton X-100 at first and second centrifugation as shown in Figure 4.2. Stability of latex was observed. Nitrogen content was determined by modified RRIM method.

#### 5. Effect of NaOH concentration on saponification efficiency.

Saponification of 10% DRC field latex was carried out with 1-5%, w/v NaOH in the presence of 0.2%, w/v Triton X-100. Decomposed protein in saponified

latex was washed by double centrifugation without Triton X-100. Cream of saponified latex was then coagulated by methanol and dried under reduced pressure.

Nitrogen and ash content were determined to observe these effects.

### 3.3.2.2 Saponification of Latex and Coagulation Followed by Washing

Saponification of 30% DRC field latex was carried out with 5% w/v NaOH in the presence of 0.1%, w/v Triton X-100. Saponified latex was diluted to 10% DRC, then the salt were added to coagulate at 50°C and high speed stirring. Crumb obtained from saponified latex was washed with water then dried under reduced pressure. Nitrogen and ash content of deproteinized natural rubber were determined.

#### 1. Creaming and coagulation by sodium chloride (NaCl)

Sodium chloride or brine sodium was used as creaming agent or coagulant at concentration of 5, 10, 15% w/v. Coagulation process was carried out at 50°C and high speed mechanical stirring (~800 rpm). Cream or crumb of saponified latex was washed with water and dried under reduced pressure.

#### 2. Coagulation by calcium nitrate (Ca(NO<sub>3</sub>)<sub>2</sub>)

Calcium nitrate of 0.25, 0.5 and 0.1%, w/v in the solution concentration of 0.10, 0.25, 0.50 and 1%, w/v were used as coagulant. Coagulation process was carried out at 50°C and stirring speed of 800 rpm. Crumb of saponified latex was washed with water and dried under reduced pressure.

#### 2. Coagulation by neutralize with sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) in the presence of sodium stearate.

Neutralization or coagulation process was carried out with 5% w/v sulfuric acid. Volume of H<sub>2</sub>SO<sub>4</sub> was varied to 15 and 20 ml to obtain the latex at pH 8 and 4, respectively. Suspending agent or crumbing agent, 0.1 and 1% sodium stearate was used to obtain the fine crumb.

### 3.4 Determination of Rubber Properties

#### 3.4.1 Total Solids Content

Total solids concentrated latex and field latex were determined according to ASTM D 1076. Latex was weighed  $2.5 \pm 0.5$  g of the sample to the nearest 1 mg in the covered weighed dish. The cover was removed and the latex was distributed over the bottom of the approximately  $32 \text{ cm}^2$  ( $5 \text{ in}^2$ ) dish. This might be facilitated by carefully adding approximately  $1 \text{ cm}^3$  of distilled water to latex and gently swirling the dish. With the dish on covered, the specimen was dried in a vented air oven for 16 h at  $70 \pm 2^\circ\text{C}$  or 2h at  $100 \pm 2^\circ\text{C}$ . The cover was balanced and cooled in a desiccator to room temperature, and weighed. The drying was repeated and weighed until the mass was constant to 1 mg or less. Test was run in duplicate and checked within 0.15%. The average of the two determinations was taken as the result.

Calculation of percentage of total solids as follows:

$$\text{Total solids, \%} = [(C-A)/(B-A)] \times 100 \quad (3.1)$$

Where:

A = mass of the weighing dish, g

B = mass of the dish plus the original sample, g, and

C = mass of the dish plus the dried sample, g

#### 3.4.2 Dry Rubber content

Dry rubber content of concentrated and field latex was determined according to ASTM D 1076. Latex was weighed approximately 10 g to the nearest 1 mg into a porcelain evaporating dish. The distilled water was added until the total solid was approximately 25%. Sufficient acetic acid (2%) was added while the latex was stirred constantly over a 5-min period, to coagulate completely the latex.

The dish was placed on a steam bath and left undisturbed for 15 to 30 min. A clear serum was resulted, and 30 min was the maximum time allowed. A Coagulated latex particle was picked with the main body of the coagulum. The coagulated rubber was washed in running water and passed between rolls. This process was repeated 5 times and the sheet of coagulated rubber was reduced to a maximum thickness of 2 mm and dried at  $70 \pm 2^\circ\text{C}$  in a vented air oven. The coagulated rubber sheet was cooled in a desiccator to room temperature and weighed. Drying and weighing were repeat until the mass is constant to 1 mg or less

Calculation of the dry rubber content as follows:

$$\text{Dry rubber content, \%} = (\text{mass of dry coagulum}/\text{mass of sample}) \times 100 \quad (3.2)$$

#### 3.4.3 Determination of Low Nitrogen Content of Purified Natural Rubber by Modified Protein RRIM Method

Protein of natural rubber is normally related to the nitrogen content by the formula: Protein = 6.25 X nitrogen content. Nitrogen content of commercial natural rubber can be determined by the Kjeldahl method as described in RRIM SMR Bulletin No.7. However, it is rather difficult to determine accurately low levels of nitrogen in purified rubber ( $N < 0.03\%$ ). Therefore modified RRI method or RRIM method was applied.

0.2500 $\pm$ 0.01 g. of rubber sample was first digested in 10 ml of concentrated sulfuric acid at high temperature in the presence of 0.80 g of catalyst mixture (potassium sulfate: copper (II) sulfate pentahydrate: selenium = 15:2:1 in weight ratio). The sample was digested completely into ammonium hydrogen sulfate. The digest was then made to alkaline with 20 ml of distilled water and 10 ml of 40% NaOH. Liberated ammonia was subsequently steam distilled into 10 ml of 2% boric acid solution for 10 min and titrated with 0.005 N of sulfuric acid. Mixed indicator of methyl red and methylene blue was used. The end point of titration was observed by color change from green to purple.

Nitrogen content was calculated as follows:

$$\text{Nitrogen, \%} = [(V_1 - V_2) M \times 0.0140] / W \times 100 \quad (3.3)$$

where:

$V_1$  = Volume of sulfuric acid used in sample titration,  $\text{cm}^3$

$V_2$  = Volume of sulfuric acid used in blank titration,  $\text{cm}^3$

$M$  = concentration of sulfuric acid, M (N)

$W$  = weight of rubber sample, g

The measurement was done with an automatic Kjeldahl analyzer (TT215 Gerhalt, Germany).

#### 3.4.4 Gel Content Analysis

The gel content was determined by dissolve rubber sample in dried toluene, which was kept in activated molecular sieve 4A, to make a concentration of 0.2% (w/v) and kept in the dark without shaking or stirring for one week at room temperature. The solution was centrifuged at 5000 rpm for 40 min to separate gel fraction from sol fraction. The gel was washed with methanol and dried under reduced pressure at room temperature. The gel content and toluene-soluble fraction were calculated from the following expression:

$$\text{Gel content, \%} = \frac{\text{weight of gel}}{\text{weight of original rubber}} \times 100 \quad 3.4$$

$$\text{Toluene - soluble fraction, \%} = 100 - (\%, \text{ gel content } ) \quad 3.5$$

#### 3.4.5 Molecular Weight and Molecular Weight Distribution Determination by GPC

The molecular weight and molecular weight distribution of rubber was determined by gel permeation chromatography (GPC) using two 8 mm ID x 500 mm columns in series, packed with styrene-divinylbenzene copolymers, having exclusion limits of  $2.0 \times 10^7$  and  $5.0 \times 10^4$ . Measurements were made using THF as eluent with a flow rate of 0.5 ml/min. at  $35^\circ\text{C}$ , monitoring with a TOSOH LS-8000 with refractive index and low-angle laser-light-scattering (LALLS) detectors. Saponified rubber was dissolved to



make 0.01-0.02% (g/dl) solution in THF. The solution was filtered through a Millipore LS prefilter and 0.2 micron membrane filter before injected to column.

A calibration curve is obtained by plotting the molecular weight against the elution volume. Since narrow fraction natural weight *cis*-1,4 polyisoprene standards are not available, it is usual to use narrow fraction polystyrene standards instead. When tetrahydrofuran is used as the solvent the following equation can be used to convert the polystyrene molecular weights into natural rubber molecular weights of equivalent coil size:

$$\log M_{PI} = 0.185 + 0.950 \log M_{PS} \quad 3.6$$

#### 3.4.6 Ash Content Analysis

The ash content of field latex and saponified latex was determined according to RRIM B6 (Appendix A)

#### 3.4.7 FTIR Analysis of Saponified Rubber

Rubber sample was weighed approximately 0.05 g and dissolved in 5 ml of chloroform. Rubber solution was dropped to NaCl cell then the stream of nitrogen gas was passed to form a round transparent film of about 1 cm in diameter. The measurement was performed by Nicolet FTIR spectrometer Model Impact 410 at a resolution of  $2 \text{ cm}^{-1}$  with 200 scans.

##### 3.4.7.1 Determination of Nitrogen Content

The nitrogen content of saponified latex was determined by FTIR spectroscopy via the decrease of intensity of the infrared band at  $3280 \text{ cm}^{-1}$  and shifted to  $3316\text{-}3320 \text{ cm}^{-1}$ .

##### 3.4.7.2 Determination of Ester Content

Methyl stearate was used as a model compound for the FTIR analysis of ester groups in natural rubber. The absorbance of the carbonyl groups was measured

by using a calibration curve obtained by using a series of mixtures of methyl stearate and synthetic *cis*-1,4-polyisoprene (Carliflex-305)

The ester content of fatty acid groups was determined by FTIR. The height ratio of peaks at  $1738\text{ cm}^{-1}$  (C=O) to  $1664\text{ cm}^{-1}$  (C=C),  $(A_{1738}/A_{1664})$ , was plotted against the concentration of the added ester groups in the rubber. A mixture of synthetic *cis*-1, 4-polyisoprene and methyl stearate was used as standard. The height of peak at  $1738\text{ cm}^{-1}$  and  $1664\text{ cm}^{-1}$  was measured.

The ester content of purified or saponified rubber was then obtained by substituting the height of peaks at  $1738\text{ cm}^{-1}$  to  $1664\text{ cm}^{-1}$  into the following expression;

$$\text{Ester content (mmol/kg rubber)} = (A_{1778}/A_{1668}) \times \text{Gradient of calibration curve} \quad (3.7)$$