CHAPTER I

INTRODUCTION

1.1 Background

Hevea brasiliensis or Para rubber (family of Euphorbiaceae), a tree originating from the Amazonian area (Archer, 1965), is the most important and commercially exploited plant for natural rubber (NR) production. It was first planted in Thailand in 1901 by Praya Rachadanupadit Mahidsorn Prakdee (KO. Simbee) in Trang Province. At present, rubber plantations are distributed nearly all over the country. In 1998 Thailand produced 2,075,950 tons of natural rubber of which only 186,379 tons were used by the local rubber industry and the rest was exported (Figure 1.1, Thailand Rubber Statistic; 1999). The major exported rubber products in 1998 are rubber tires (37%) and rubber gloves (36%) which made up more than25, 000 million baht or 75% of exported products. (Figure 1.2)

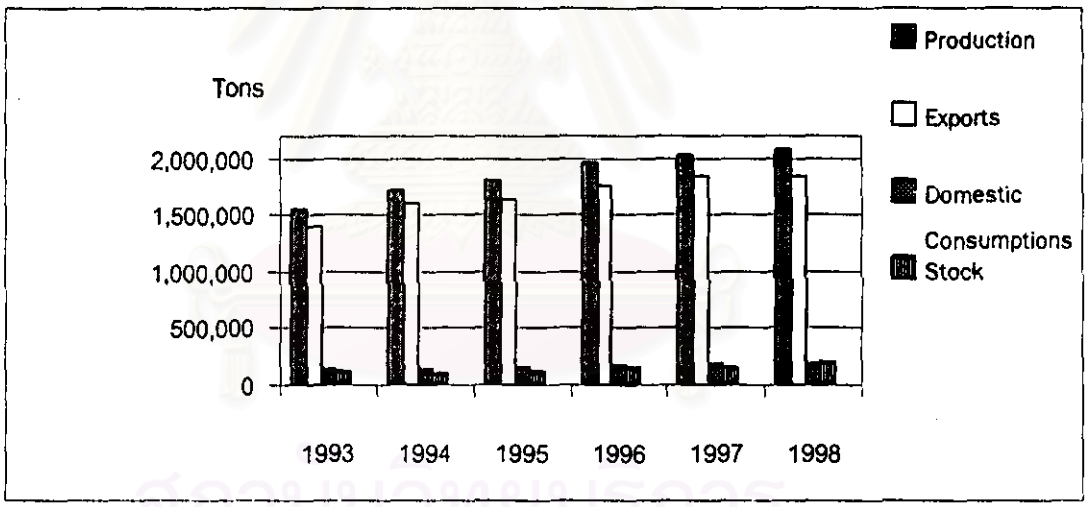


Figure 1.1 Natural rubber production of Thailand (Thailand Rubber Statistics, 1999)

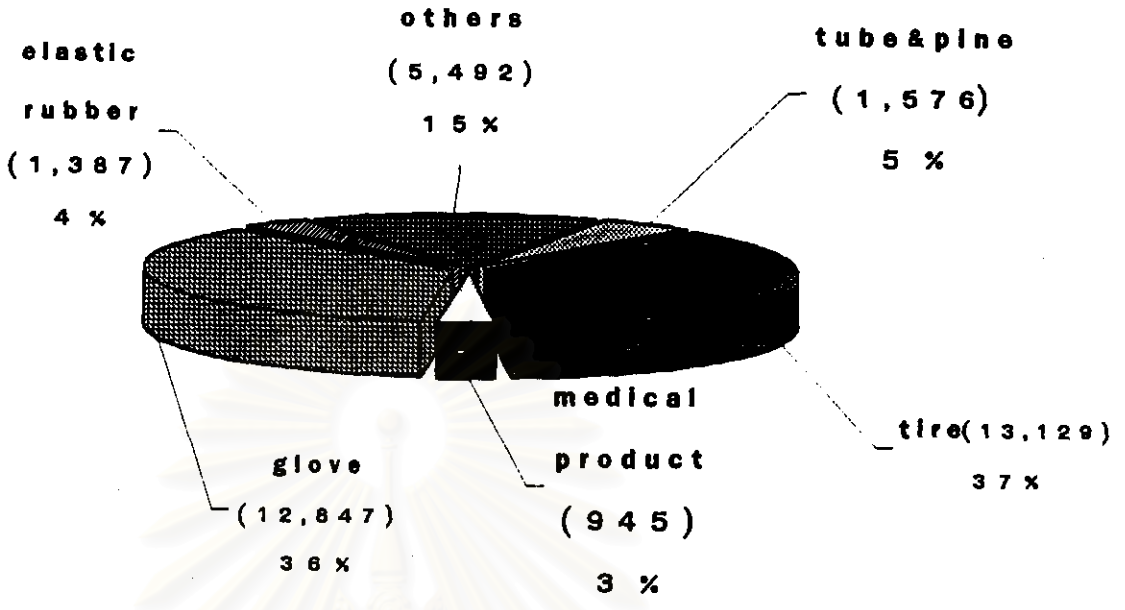


Figure 1.2 Values of exported rubber products (million Bath) of Thailand in 1998 (Thailand Rubber Statistics; 1999)

1.2 Natural rubber

Natural rubber (NR) is a unique elastomeric polymer produced from natural rubber latex (NRL). The latex is generally milky in appearance, and its function is believed to be protection of leaves and tissue of the tree against external infection (Archer et al., 1981). Fresh latex has the density of 0.975-0.980 g/ml with the pH of 6.5-7.0. This latex is obtained a rubber tree by process a known as tapping. Tapping involves diagonal incision into the bark of the tree, at two-or three-day intervals, to allow seepage of some of the liquid latex into a collection cup placed at the base of the incision. Usually about 300-400 ml latex can be obtained are obtained from each treeing at a single tapping.

Latex consists of rubber particles, which are spherical droplets of hydrocarbon enclosed in a fine phospholipoprotein envelope (Jacob et al, 1992) suspended in water (Figure 1.3). The average particle size is between 0.10 μm and 1.0 μm. The particle size distribution is very broad.

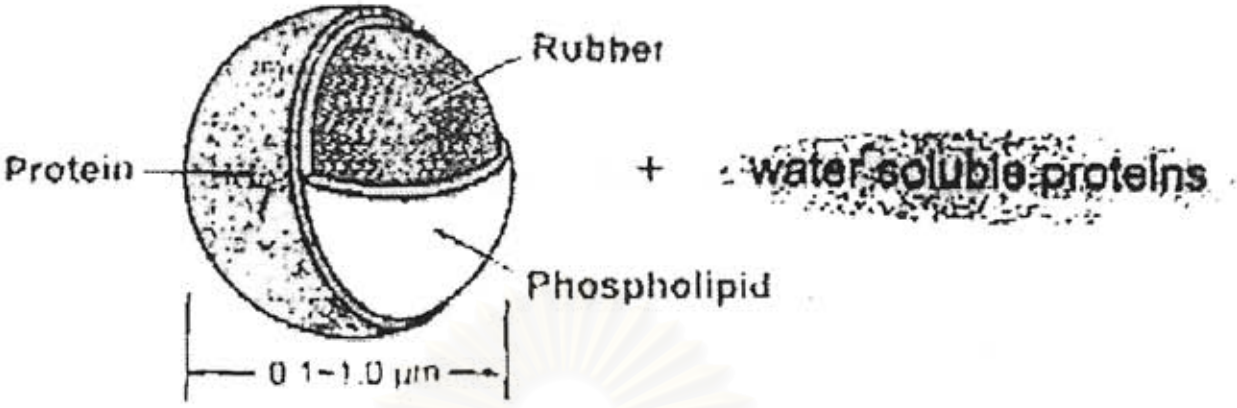
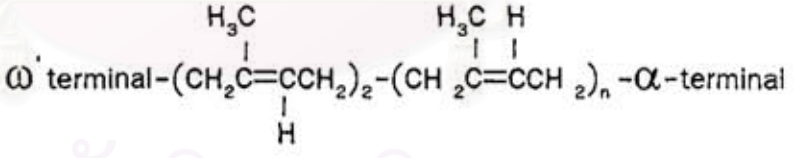


Figure 1.3 Presumed structure of solid rubber particle

NMR spectroscopy (Tanaka, 1984) revealed the chemical structure of natural rubber as the polyisoprene with the building unit of isoprene (C_5H_8) in majority of cis-unit and approximately 2-3 trans-units per polymer chain as shown in Figure 1.4 and Figure 1.5



ω' and α: unidentified terminal group

Figure 1.4 The chemical structure of natural rubber

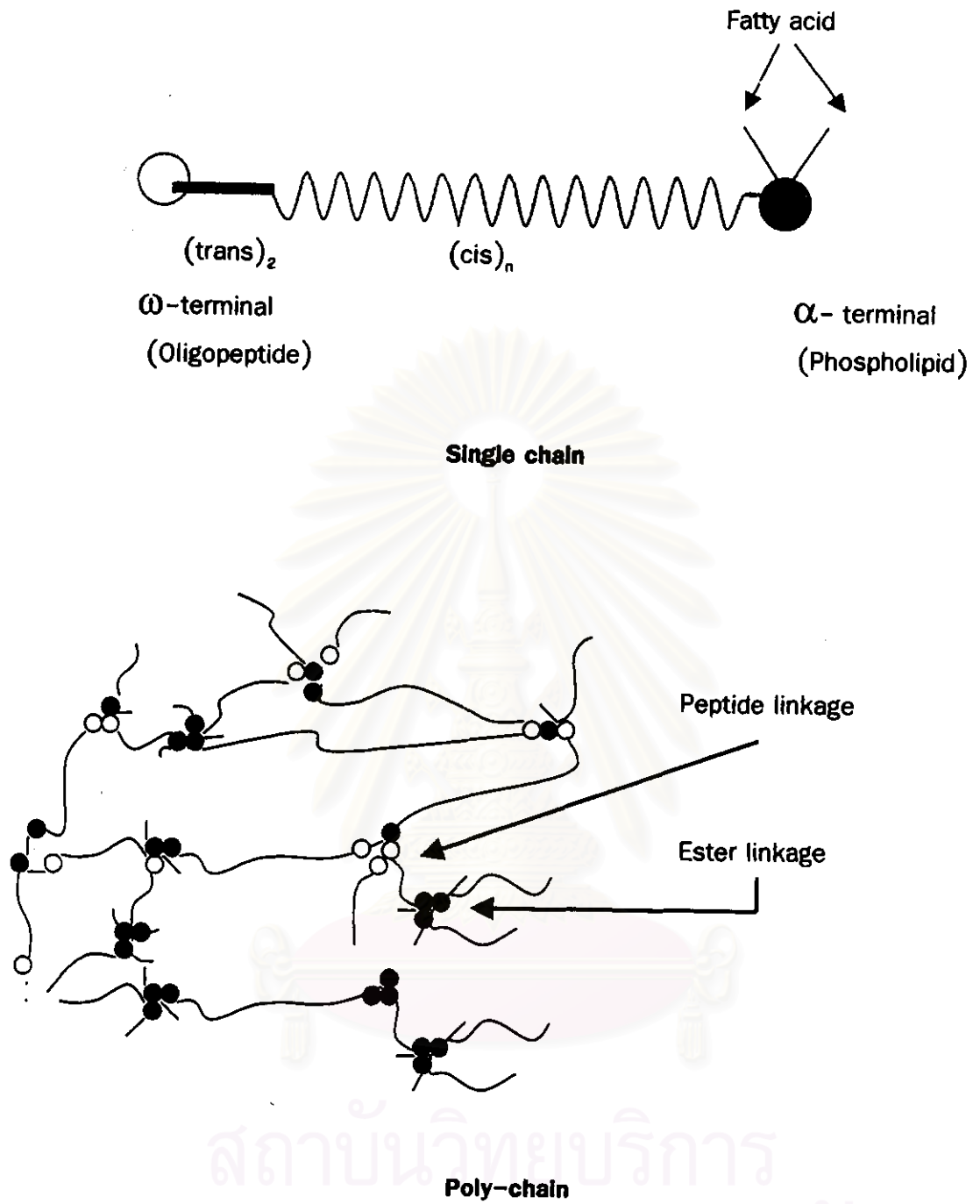


Figure 1.5 Presumed structure of natural rubber

When the latex is coagulated by 2% formic acid and dried, it contains the dry rubber content (DRC) about 25-45 % w/v depending on the season of tapping, clonal and other factors. The latex contains the total solids contents higher

than the dry rubber content of about 2-5 % (w/w). The difference between TSC and DRC is non-rubber portion, which made up mainly of 2-3% protein and phospholipid, 1% fatty acids, 0.4% carbohydrates, and 0.3% inorganic salts (Table 1.1).

Table 1.1 Composition of acid coagulated NR

Rubber hydrocarbons	93.7 %
Neutral lipids	2.4%
Glycolipids,Phospholipids	1.0%
Proteins	2.1%
Carbohydrates	0.4%
Inorganic constituents	0.2%
Others	0.1%

Centrifugation of latex consists of four major components: rubber particles, lutoids, Frey Wyssling particles, and cytosol. (Figure 1.6)

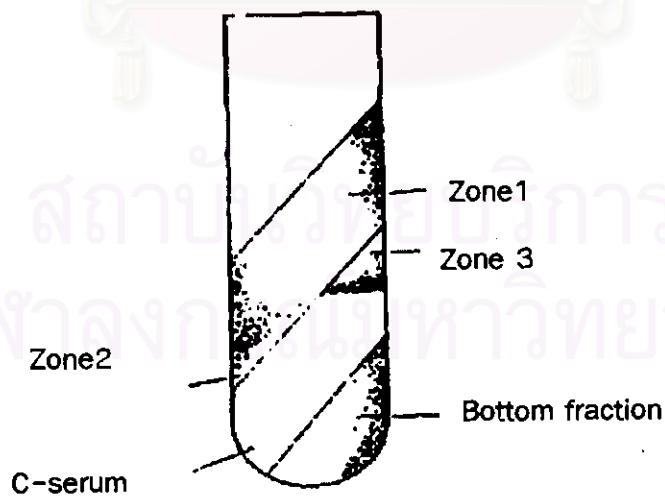


Figure 1.6 High-speed centrifugation of natural rubber latex, Moir, 1959

Rubber particle-Two proteins important in cis1, 4- polyisoprene synthesis were identified and sequenced in 1989 (Light et al., 1989). The first, cis-prenyl transferase (38 kD) is a hydrophobic membrane-bound enzyme, which catalyzes the addition of isoprene units, resulting in a polysioprene chain several thousand isoprene units in length. The second, rubber elongation factor, is a 14.6 kD stabilizing cofactor necessary for efficient function of cis-prenyll transferase (Dennis et al., 1989).

Bottom fraction or Lutoids are small vacuoles that comprise 10% to 20% of latex volume and are important for latex coagulation. Hevein (5 kD) and prohevein (20 kD) are major lutoid body protein (Archer, 1960). Hevein makes up 70% of proteins and has considerable structural homology with many plant agglutinins (lectins) such as those found in wheat, barley, rice, and potatoes. Hevamines (29 kD) are lysozymes that demonstrate homology with lysozyme in other plants such as ficus and papaw (Archer, 1976).

Frey Wyssling particles comprise 2% to 3% of latex volume; their biologic role has not been clearly defined. The remaining cytosol forms 40% to 50% of latex volume and contains soluble carbohydrate, organic acids, amino acids, nucleotides, and proteins important in isoprene synthesis.

C-serum has about 48% of the total protein. About fifty different enzymes are distributed in this phase. Data from two-dimensional gel electrophoresis show the presence of about 30 components (Kekwick, 1993).

1.3 Molecular characteristics of natural rubber

1.3.1 Molecular weight (MW) and molecular weight distribution (MWD)

The MW and MWD of NR vary according to various factors such as clonal origin, environmental conditions of the rubber tree, methods of rubber isolation and treatment of the rubber sample before measurement. Subramanian, 1980 has categorized the rubber clones according to their molecular weight distribution into 3 types as shown in Figure 1.7. The molecular weigh distribution of NR is normally in the region of 10^5-10^7 with a high molecular weight peak centered around 10^6 and low molecular weight weak around 10^5. The weight average molecular weight $\overline{Mw}$ and number average $\overline{Mn}$ are 1.6-2.3x10^6 and 2.2-5.2x10^5 respectively; the molecular weight polydispersity expressed by $\overline{Mw}/\overline{Mn}$

is extremely wide ranging from 2.8-10. The proportion of high molecular weight fraction correlate with the Mooney viscosity of rubber.

The broad molecular weight distribution of Hevea rubber is presumed to be associated with branching and crosslinking reactions through some special functional groups.

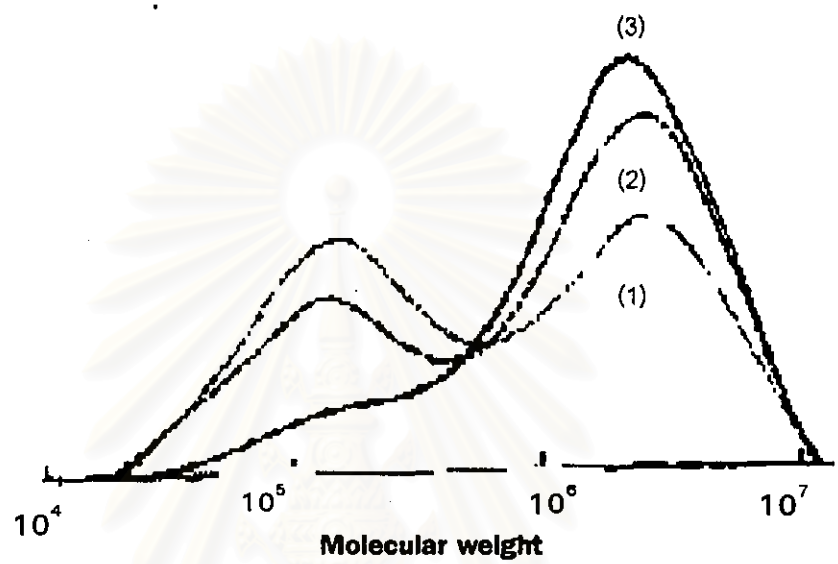


Figure 1.7 Types of molecular weight distribution curves of natural rubber (Subramaium, 1980)

Type 1 (.....) Distinctly bimodal distribution where the peak height in the low molecular weight region is nearly equal or slightly less than the peak height at the high molecular weight region.

Type 2 (---) Distinctly bimodal distribution where the height of the low molecular weight peak is only half or less than the height of the high molecular weight peak.

Type 3(___) Skewed unimodal distribution with " shoulder" or a plateau in the molecular weight region.

1.3.2 Gel phase

Heavea rubber contains 5-50% gel phase, which is insoluble in rubber solvent such as toluene, depending on the clonal origin of the rubber, processing conditions and the period and temperature of storage (Allen et al., 1963). Insoluble phase consists of microgel and macrogel. The gel phase contains a larger proportion of nitrogenous impurities than the sol phase; e.g. the nitrogen of the gel is 2.57%, while that of the sol is 0.05%. These facts may well be accounted for by the formation of a protein-containing network between the crosslinked small particles on coagulation as illustrated in Figure1.8. This is further supported by the observation that gel content of rubber isolated from high ammonia preserved field latex decreased drastically after purification (Tanaka et al., 1992).

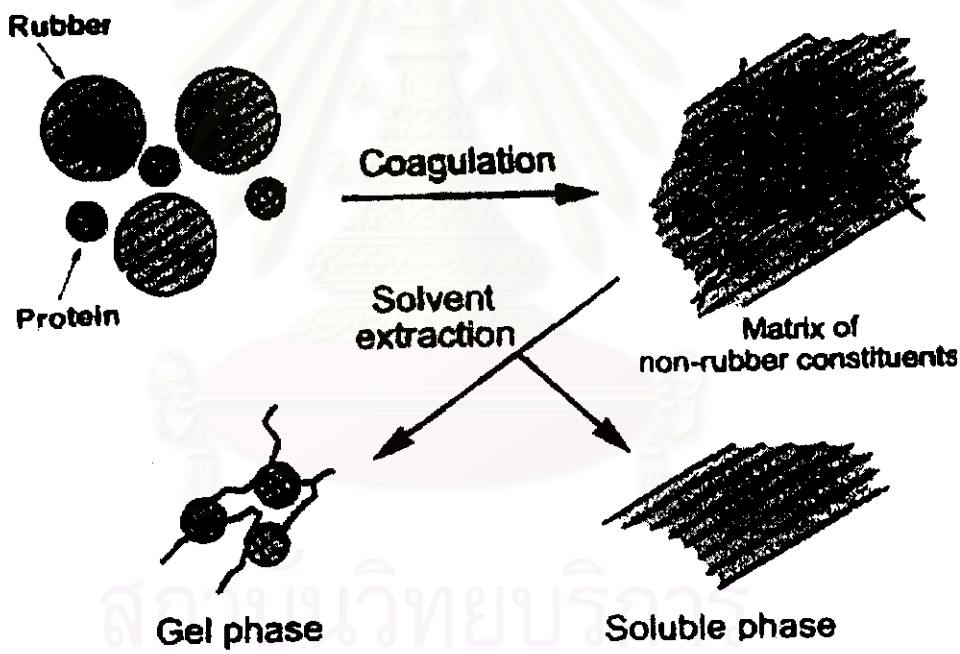


Figure 1.8 Schematic representation of gel phase in latex and in rubber (Allen et al., 1963)

1.3.3 Acetone extract or non-rubber content

Subramaian (1975) has reported that total non-rubber content of rubber from different Hevea clones in Malaysia varies between 2-5% of total rubber content and broadly correlate with the Mooney viscosity and Wallace plasticity.

1.3.4 Structural characteristics of NR by FT-IR

Proteins in NR have been thought to be physically held by the polymer. Although they have been assumed to be responsible for the branching and crosslinking of NR (Tanaka, 1989). In these crosslinked species the carbonyl groups can be detected by FT-IR spectroscope, due to the high absorptivity of carbonyl; band around 1,700-1,750 cm^{-1} carboxyl, ester carbonyl and aldehyde carbonyl groups are distinguishable from one another.

The presence of protein is evidenced by the characteristic bands of N-H stretching at 3,280 cm^{-1}, -(NH)C=O at 1,650 cm^{-1} and N-H bending at1,540 cm^{-1} as indicated in Figure 1.9 (Eng., et al. 1993.)

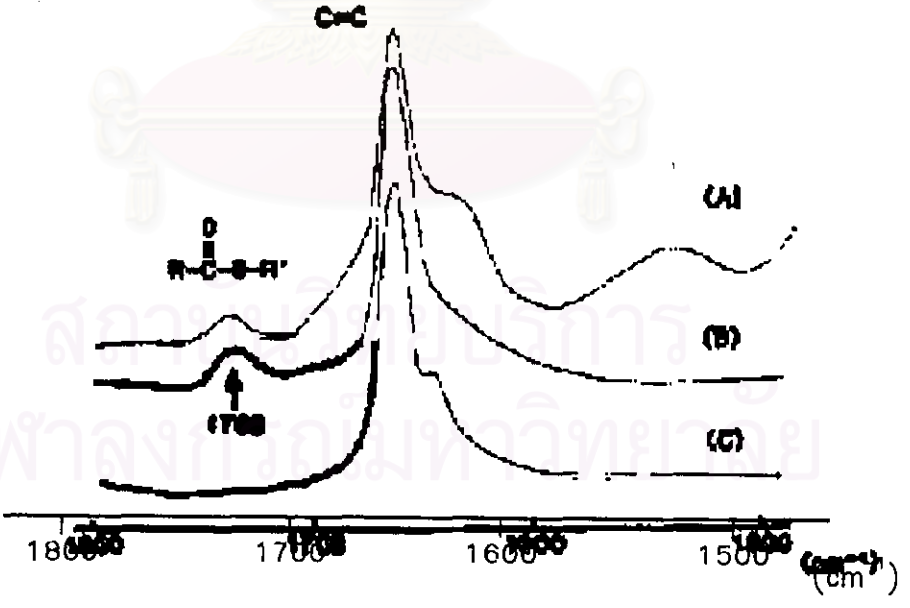


Figure 1.9 Infrared spectra of natural rubber (A) and synthetic cis-1,4 polyisoprene (B) (Tanaka, 1989)

1.4 **Commercial natural rubber**– Commercial natural rubber can be classified into two major groups, solid NR and latex concentrate (Kajornchaiyakul, 1986). Solid NR has been produced from fresh field latex and skim latex. Solid NR produced from skim latex can be divided into skim block and skim crepe. Solid NR produced from fresh field latex can be divided into four groups, ribbed smoked sheet (RSS), air dried sheet (ADS), crepe rubber and block rubber or standard Thai rubber (STR), depending on its derived process (Figure 1.10). In each group of solid rubber, the small number indicates for less impurities or better grade; such as STR 5 has much less impurities than STR 20, especially STR 5L the letter " L" indicates for "light color grade. ", usually require bleaching.

1.5 **Natural rubber products** (NRP)– Natural rubber products from latex have been used widely for over a hundred years. This is attributed to the superior-processing behavior and high physical strength of rubber. There are several products made from latex which can divided in to two groups. The first is NRP made from concentrated latex such as gloves, condom, tip catheters, endotracheal tubing latex balloon, baby bottle nipples, and dental cofferdams. The second is NRP made from solid rubber such as tire, shoes, adhesives, elastic rubber, and medical products (Figure 1.11).

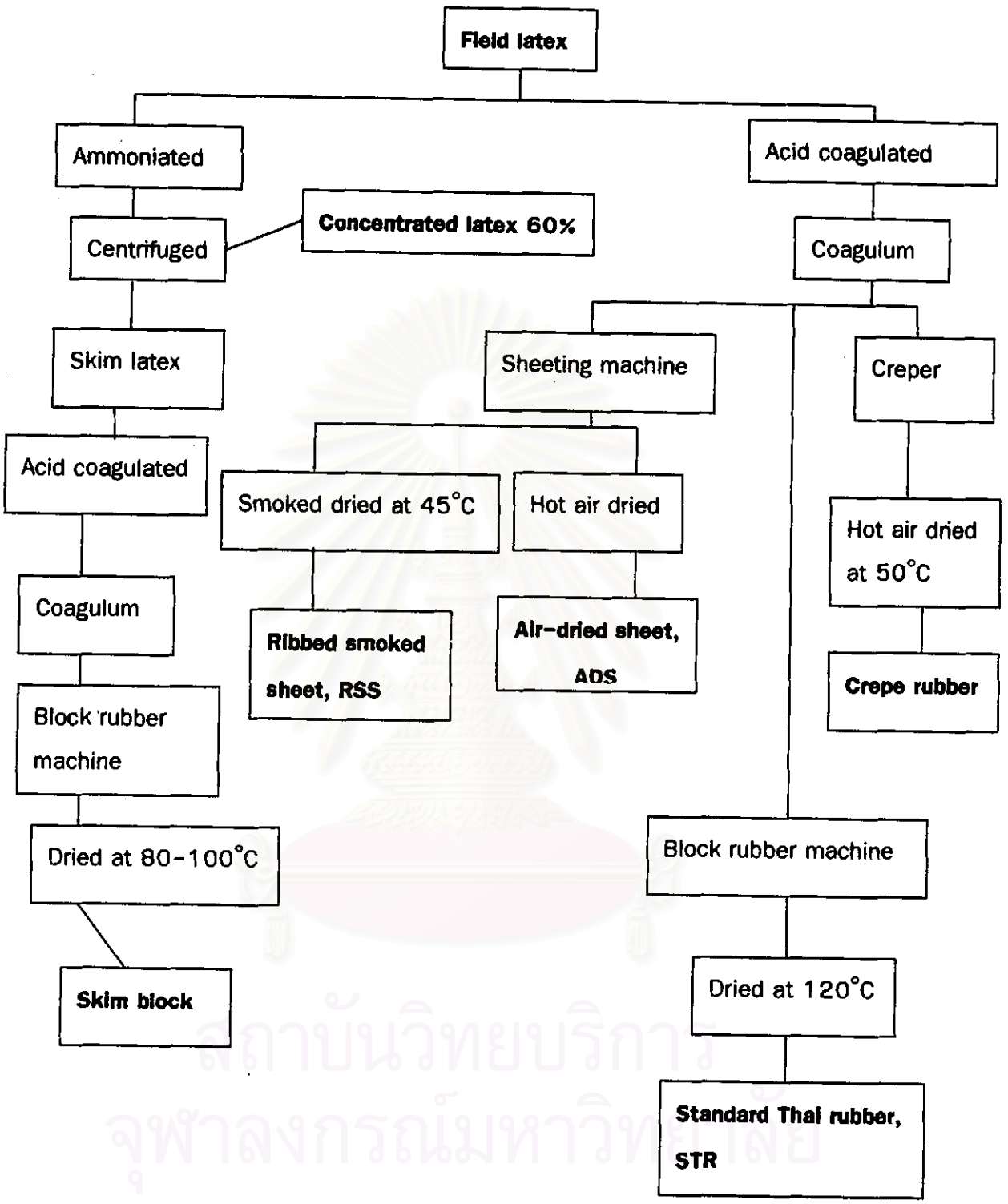


Figure 1.10 Natural rubber production process in Thailand (From Para Rubber Bulletin, Thailand 7, 1986)

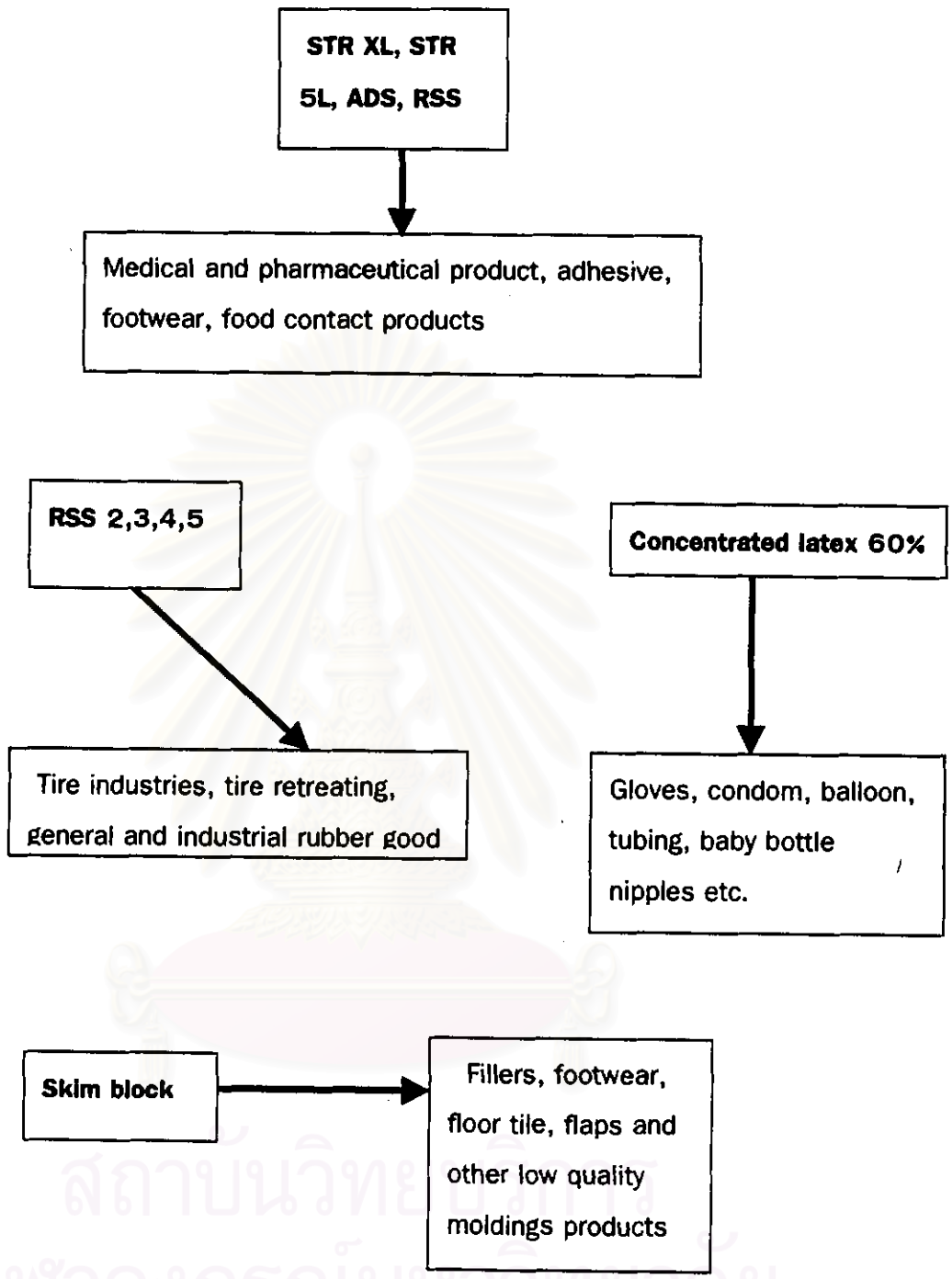


Figure 1.11 Natural rubber products in Thailand (From Para Rubber Bulletin, Thailand 7, 1986)

1.6 **Latex allergy**

Two types of allergy caused by natural rubber products (NRL) are known: type I, immediate type and type IV delayed type hypersensitivity.

1.6.1 Type I immediate allergic reactions

This type occurs within an hour of exposure to NRP. The contact urticaria syndrome includes localized urticaria, angiedema, asthma, and anaphylaxis. Typical reactions occur as a result of IgE-mediated hypersensitivity to natural rubber proteins. The first case of an immediate reaction to NRL was reported in 1927 by Stern who described severe generalized urticaria caused by a rubber dental prosthesis. In 1979, Nutter reported the first glove related case of this type, contact urticaria.

Warshaw, 1998 received that type I, immediate allergic reaction cause 15 deaths related to latex gloves and barium enema catheter tips. The food and Drug Administration (FDA) in U.S.A has received more than 1,000 reports of describing severe systemic allergic reactions to natural rubber latex medical devices. The FDA thus published a bulletin identifying the risk of life-threatening anaphylaxis associated with NRL devices. (FDA. Med. Bull, 1991)

1.6.2 Type IV delayed type hypersensitivity (DTH)

This type occurs about 48 hours after exposure to NRP. The sensitizing agents that caused this delayed type contact dermatitis are used in the rubber vulcanization such as mercaptobenzothiazole, carbamate and p-phenyenediamine. The contact dermatitis syndrome may be occurring as a result of exposure to latex because some chemical such as 1,2-benziothiazolin-3-ene may be added to the raw latex. Ingredient of glove powder such as epichlorohydrin and sorbic acid may cause DTH.

Prevalence of latex sensitivity in the general population is probably less than 2 % (Table 1.2). Studies using RAST with serum samples from blood donors indicate higher rates of sensitization may be due to different test method or probably because health cares workers, known to be at risk for latex allergy, are more likely to donate blood.

Table 1.2 Latex sensitivity in atopic and general patients, health care workers and blood donor

Population	Sample size	Test *	% Positive	Author(s)
Consecutive allergy clinic patients	130	Scratch	0.8	Turjanmaa, 1987
Allergy clinic patients without risk factors	272	SPT	0.4	Moneret-Vautrin et al.,1993
Consecutive preoperative patients	800	SPT	0.13	Turjanmaa, 1994
Patients seen for annual check-up	365	SPT/RAST	2.3	Porri et l., 1995
Health care workers	224/405	SPT/Quest	15.2/ 12.3	Teeraratkul et al.1997
Blood donors	1000	RAST	6.5	Ownby et al. 1994
Blood donors	1436	RAST	7.9	Merrett et al. 1995
Blood donors	352	EAST	4.5	Harncharoen K.,1996

(Adapted from Warshaw et al., 1998)

* SPT: Skin prick test, RAST: Radioallergosorbent test, EAST: Enzyme allergosorbent test, Quest: Questionnaires

1.6.3 Latex Antigen identification

As summarized in Table 1.3 immunobloting studies show that IgE from sera of latex-allergic patients binds heterogeneously to many different proteins ranging from 4 to 200 kD. Identification of one or two major allergens in daunting task. Currently, there is no consensus on which proteins are most important. Some authors believe that proteins of 14.6 kD (rubber elongation factor, Hev b1), 20 kD, 22 and 23 kD and 27 kD are particularly important in spina bifida. Others believe that hevein (4.7 kD) and prohevein (20 kD) may be important antigens. Several other potential antigens have recently been identified with molecular weights of 10, 16, 18, 21, 23, 25, 30, 36, and 66 kD.

Table 1.3 Identified latex antigens

Author(s) (year)	<5	10	15	20	25	30	35	40	45	50	>50
Turjanmaa et al1988	**2 5**					**30**					
Moralws et al.,1989		**10**		**24**			**35**				**100**
Turjanmaa and Reunala, 1989	3	10									
Turjanmaa et al1990		**10**									
Alenius et al.,1991	4		14	21							70
Alenius et al.,1992			**14**	**21**		**29**				**53**	
Chambeyron et al., 1992		**10**	15	18	20 25	**30**	35				60
Fuchs and Wahl,,1992						**28**					
Jaeger et al,1992			14			30			45		
Slater and Chhabra, 1992			**14**	**20**							
Tomazic et al., 1992	4			20 (AL)							200 (NAL)
Alenius et al.,1994			**14**	**20**	**27**						
Czuppon et al. 1993			**14.6**								**58** (tetramer of 14.6)
Alenius et al.,1993			**14**	**20**	**27**						
Alenius et al.,1994			14	20							200
Slater and Trybul, 1994			**14.3**		**26.7**				46		
Alenius et al., 1995			**14**	**20**	24	**30**	**36**		46		
Chiu et al. 1997			**14**	**18**	**23 25**						**66**
Eriksen et al. 1997			**14**		21		30	35		44	
Nieto et al. 1997		**11 12 13**				27	32				
Yeang and Ward 1997				22 23							

Adapted from Warshaw et al., 1998 AL-ammoniated latex, NAL-nonammoniated latex, Boldface type indicates major antigen

1.7 The development of deproteinized natural rubber (DPNR)

There are several attempts to produce a rubber product with a very low protein content. This rubber has been known as 'Deproteinized Natural rubber', DPNR or ' Low Nitrogen Natural Rubber, LNNR'. There are several methods that have been used for deproteinze natural rubber latex can be divided into 3 groups: 1) by centrifugation or washing of latex with surfactants 2) by enzymatic reaction and 3) by chemical reaction. These approaches may be combined such use of enzyme with surfactants.

In 1955, Firestone Plantations produced skim rubber by alkali treatment to brake down the protein in spontaneous coagulation of skim latex. The crumb was soaked in a solution of lime followed by sodium hydroxide solution. They were able to reduce the nitrogen content for about 35%.

In 1971, John produced solid rubber from latex with low protein by treating field latex with di-octyl sodium sulphosuccinate and an anionic surfactant at neutral pH. He obtained a solid rubber with about 30% less nitrogen than acid ordinary coagulated rubber.

In 1975, Yapa had prepared low nitrogen content and constant viscosity rubber. In this case, he started from field latex, which was diluted, to 1/2 with water and papain (0.05% on volume) was added then it was stabilized by chemical solution such as hydroxylamine hydrochloride, semicarbazide hydrochloride. The latex was allowed to stand for overnight in order to decompose latex protein by enzyme. He found that papain and hydroxylamide were suitable for the manufacture of low nitrogen CV-rubber and reduced nitrogen content to about 40%.

In 1978, Chang, Lau and Nambiar had a preparation of viscosity stabilized latex DPNR from clarified field latex. The clarified latex was added with 10% solution of sodium metabisulfite (0.05 p.h.r) and hydroxylamine neutral sulfate (0.15 p.h.r) followed by adding 10% solution of potassium naphthenate and 2.5% solution of the Alcalase or Superase. The reaction was carried out in a tank with a slow-speed stirrer for 24 hours. Then the treated latex was diluted to 3% total solids and coagulated with 2% mixture of parts by weight of phosphoric and sulfuric acid. The nitrogen content was 0.12 g%.

In 1977, John, Nadarajah and Chan had prepared DPNR by papain treatment and surface active agent, Nonidet P-40.

In 1977, Yapa had prepared solid DPNR and CV-DPNR by difference proteolytic enzymes. Field latex was diluted to 1:1 with water and papain was added (0.05% w/v). In the case of bacterial protease, Novo (BPN) or Superase, enzyme concentration of 0.1% w/v was used. Latex was coagulated on leaving overnight and the coagulum was granulated and soaked for 24 hours in a solution of 1% NaOH. Rubber was then removed from the alkali solution and washed in running water and soaked in fresh water overnight with several change of water. Next, the rubber was soaked in solution of 1-% oxalic acid overnight and washed with water. For CV-DPNR, hydroxylamine hydrochloride was added before enzyme treatment. Papain treatment was found to be better than Superase. Alkali treatment after enzyme treatment reduced protein better than enzyme treatment only.

In 1978, Yapa et al. prepared solid DPNR from skim latex. Skim latex was creamed with sodium alginate and ammonium oleate for 24 hours. The creamed latex was diluted 1:1 with water and mixed with fresh latex 1:1 and diluted with water 1:1 again. The mixed latex was coagulated with papain (0.08% w/v).

In 1980, Yapa et al. prepared latex DPNR from field latex by pineapple juice (PAJ) treatment. In this method, field latex was diluted with water (1:1) and added pineapple juice or bromelain. The rubber was left overnight for coagulation.

In 1992, Visessanguan prepared solid CV-DPNR from fresh latex and concentrated latex by enzyme treatment. Processing of DPNR from fresh latex was carried out as follows; latex was added with 0.9 p.h.r of Triton X-100 and then diluted to 25% DRC at pH 7-8 with water, ammonia solution and chemicals (hydroxylamine hydrochloride and sodium metabisulfite). The latex was treated with papain 0.3 p.h.r with shaking at 50 °C for 2 hours followed by coagulation with steam. The nitrogen reduction was 70-75 %. For concentrated latex 60%, ammonia was evaporated from latex and the latex was diluted to 25% DRC with water and chemicals. The latex was adjusted to pH 8-9 and treated with Alcalase 0.3 p.h.r. in shaker at 50 °C for 10 hours. The treated latex was diluted to 5% DRC and coagulated with 2% mixture of sulfuric and phosphoric acids. The coagulum was dipped in 2% thiourea solution. In this method, nitrogen can be reduced by about 70-75%.

In 1992, Eng, Tanaka and Gan had prepared solid purified natural rubber from concentrated latex. The latex was diluted to about 4.5% DRC and stabilized with 0.12 % (w/v) sodium naphtenate. After adjusting the pH to 9.2 with sodium dihydrogen phosphate, the latex was allowed to react with 0.04% (w/v) Alcalase 2.0T for 24 hours at 37 °C. The latex was then either centrifuged once or twice and coagulated by addition of 2% (v/v) phosphoric acid, and washed extensively with water. The rubber was dried under vacuum and extracted with acetone for 16 hours. It was then redissolved in toluene at 1% w/v and centrifuged, the clear solution was separated and the rubber precipitated into excess methanol. The nitrogen content was 0.05 %.

In 1993, Eng, Kawahara and Tanaka had prepared solid DPNR from commercial high ammonia latex. In this case, the latex was diluted to 6%-23% DRC and stabilized with 0.2%-1% sodium dodecyl sulphate. After adjusting the pH to 9.2 with sodium dihydrogen phosphate, the latex was allowed to react with 0.04% w/v Alcalase 2.0T for 24 hours at 37 °C. The treated latex was then either centrifuged once or twice and the rubber coagulated by acetone. The coagulated rubber was pressed and cut into small pieces, washed extensively with water and dried under vacuum. The nitrogen content of DPNR was 0.05 %.

In 1997, Nakade et al. had prepared latex highly deproteinized natural rubber (HDPNR) from commercial HA latex. The latex was diluted with water to 30% DRC and 0.02% (w/v) of a proteolytic enzyme (KP-3939, Kao. Co.) was mixed. The mixture was incubated for 24 hours at room temperature under slow stirring. The reacted mixture was washed once or more. The nitrogen content of HDPNR was 0.013 %.

In 1997, Tangpakdee and Tanaka had prepared latex DPNR from fresh field latex (FL-latex) by different treatment, i.e. enzymatic deproteinization, transesterification and saponificaiton. FL-latex was preserved in 1% w/v sodium dodecyl sulfate. In the case of enzymatic deproteinization, the reaction was carried out by treatment of 10%DRC latex with 0.04%w/v Alcalase 2.0T and 1%w/v Triton-X100 at 37 °C for 24 hours followed by centrifugation. The cream rubber was redispersed in1%w/v Triton-X100 to make 10% DRC and recentrifugation twice. The nitrogen content was bout 0.016 %. In case of transesterification, the reaction was carried out by treatment of 1%w/v solution of rubber in toluene, with freshly prepared 1 M $NaOCH_3$ under nitrogen atmosphere in the dark at room

temperature for 2.5 hours, followed by concentration with a rotary evaporation at 45 °C and precipitation in methanol. This method resulted in nitrogen content of 0.21%. Deproteinization by saponification was performed by reaction of 1%w/v of rubber in hexane/toluene (5:3 v/v) with 1.5 M KOH solution in 2-propanol/water (5:1v/v) in the presence of 0.1% w/v methanolic pyrogallol as an antioxidant. The reaction was refluxed at 70 °C for 2 hours under nitrogen atmosphere. The hot saponfied mixture was then filtrated and washed several times with hot distillated water then concentrated by evaporation and precipitated in methanol. The nitrogen content of saponified solid rubber was 0.011%.

In 1998, Tanaka had prepared latex DPNR from commercial high ammonia latex and fresh field latex by saponification. The lactics were diluted to about 30% DRC by water. Concentrated KOH or NaOH aqueous solution and 2-propanol were added to the latex to make 5% w/v and 10% v/v solution respectively. Saponification was carried out at 70 °C for 3 hours without stirring. The saponified rubber obtained has reduced the nitrogen content to 0.008 %.

In 1998, Rungvichaniwat et al. had prepared skim rubber by treatment of NaOH soaking in wet skim crumb and skim latex treated with NaOH techniques. The reaction of NaOH soaking in wet skim crumb was carried by soaking wet skim crumb in 3% NaOH at room temperature for 24 hours. Skim crumb rubber obtained has reduced the nitrogen content to 0.52 %. Skim latex treated with NaOH technique was treated skim latex with 5% of NaOH at room temperature for 24 hours, the treated skim was divided into 2 groups: 1) coagulated by sulfuric acid and followed by treated with 3% NaOH at room temperature for 24 hours. The skim rubber obtained has reduced the nitrogen to 0.52% 2) treated with 5% NaOH at room temperature for 24 hours then coagulated by sulfuric acid then treated with 3% NaOH at room temperature for 24 hours .The skim rubber obtained has reduced the nitrogen content to 0.37 %.

1.8 Deproteinization of rubber by saponificalton process

Saponification reaction is normally used for hydrolysis of esters bond by alkali solution, a carboxylate ion formed, in the presence of a cation, become a carboxylate salt which are soluble in water.

The reaction is as follows

R, = fatty acyl chain

R = alkyl chain

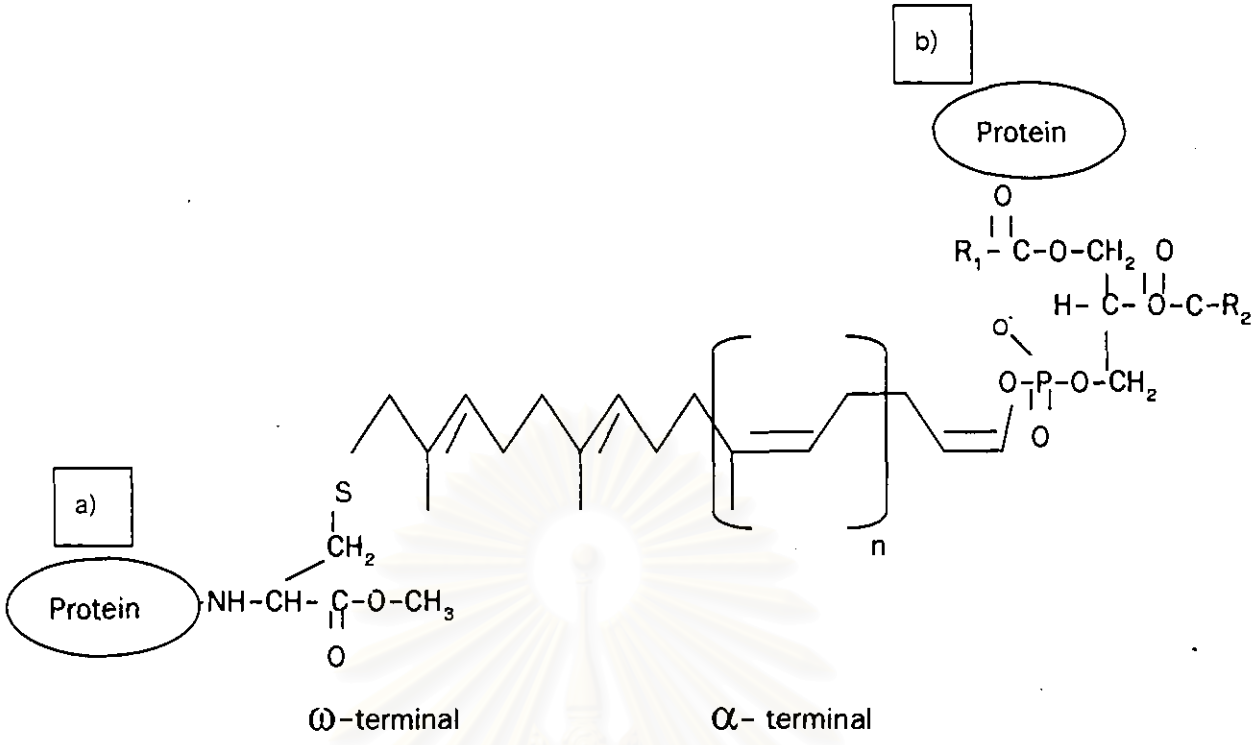
It is well known that rubber particles are envelope with phospholipids and proteins. Figure 1.12a and Figure1.12 b show the presumed structure of linkage between protein and NR that can be removed by saponification. The presumed structure of linkage between protein and NR are:

1. Proteins may covalently link to isoprene group in the ω-terminal of NR by S-cysteine methy ester, so called Prenylated Proteins

2. Proteins may associated with fatty acyl groups of phospholipids by hydrophobic interactions

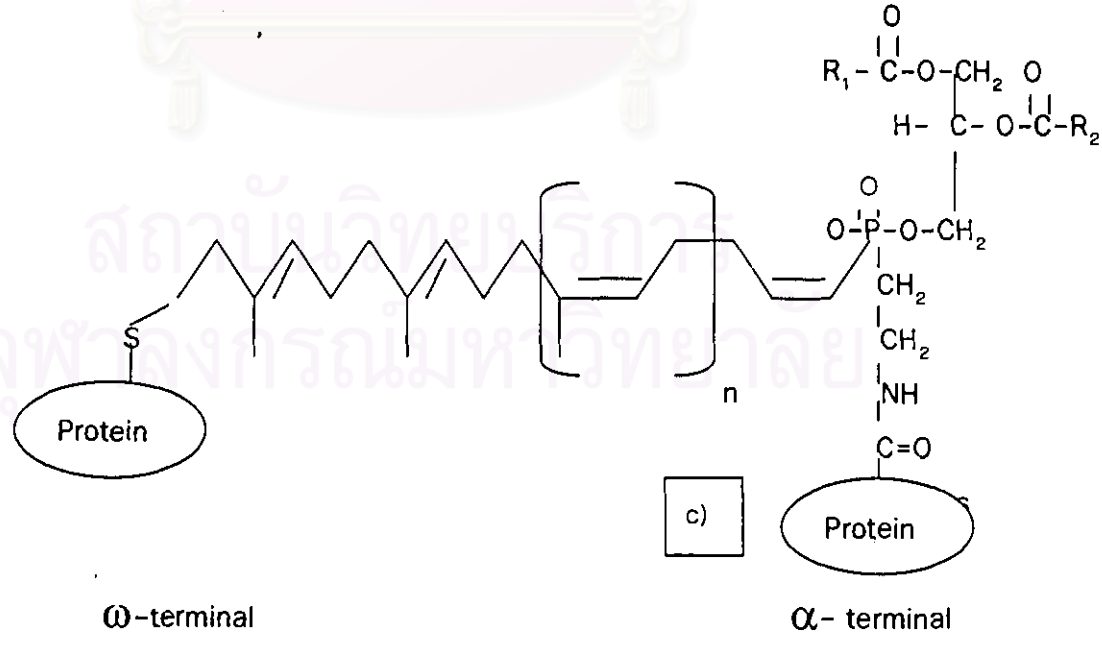
3. Proteins may covalently link to α-terminal of NR through phospholipids such as phospho ethanolamine

4. Proteins may covalently link to α-terminal of NR through glycosylphosphatidylinositol (GPI), so called GPI-proteins



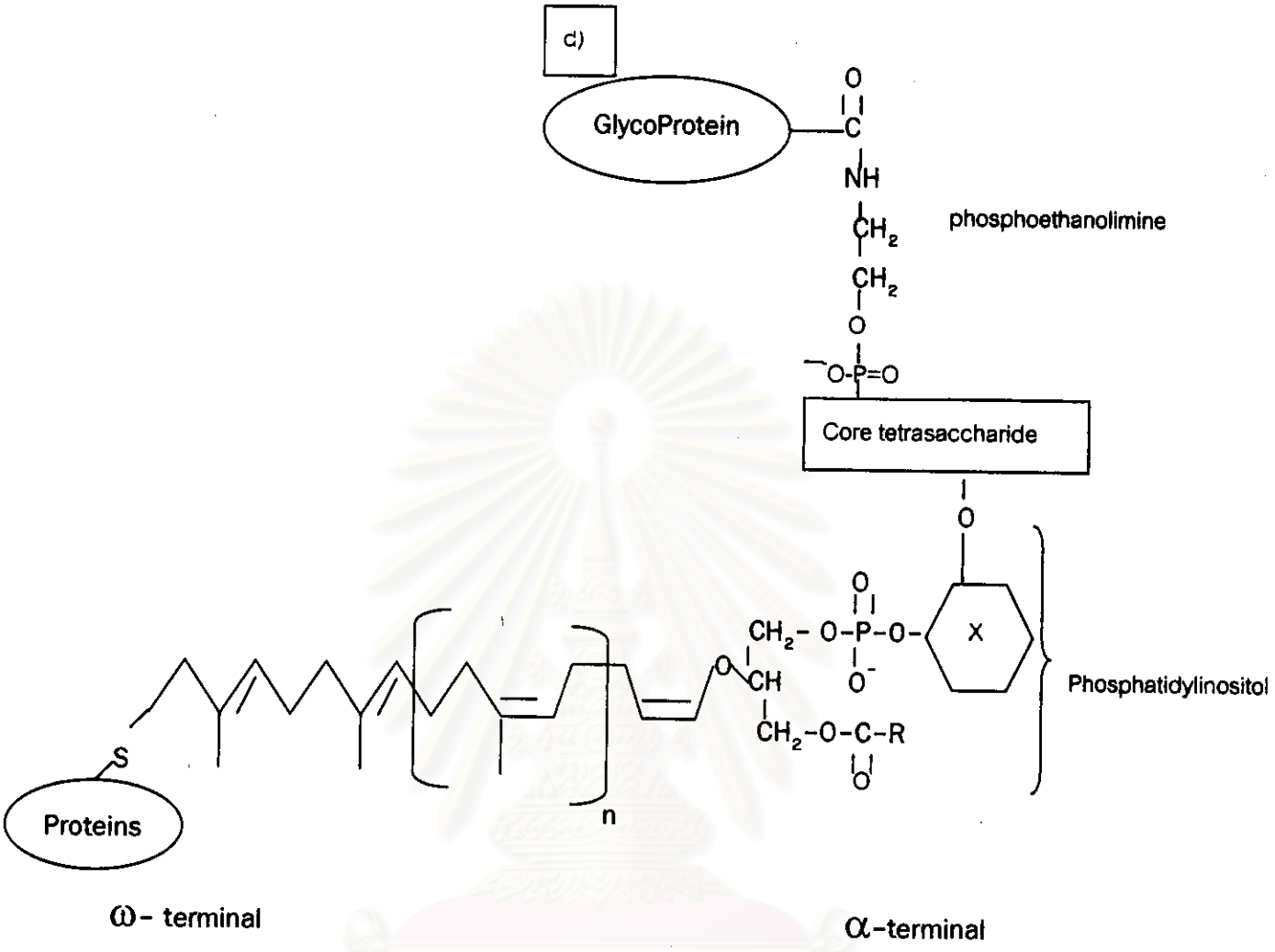
a) Proteins may covalently link to isoprene group in ω-terminal of NR by S-cystein methy ester so called prenylated proteins

b) Protein may associated with phospholipids by hydrophobic interactions



c) Proteins link to α-terminal through phosphoethanolamine

Figure 1.12 a) The presumed structure of linkage between protein and NR



d) Glycoprotein may covalently link to glycosylphosphatidylinositol (GPI), so called GPI-protein

Figure 1.12 b) The presumed structure of linkage between protein and NR

By saponfication the proteins that can be removed are:

1. non-covalently linked proteins that associate with fatty acyl groups of phospholipids of NR
2. proteins that covalently linked to ω-terminal of NR
3. lipoproteins that covalently linked to α-terminal of NR
4. glycoproteins that covalently linked to α-terminal of NR

The expected merit of saponification is low cost if the reaction is carried out without organic solvent, can be recoved or neutralized of alkali. Sapinfication can also remove of fatty acyl ester which link rubber.

1.9 The rationale and purposes of this study

It is well known that natural rubber is one of the most important export goods of Thailand. The rationale of this research is to promote the production and use of purified natural rubber for more value added products. It is hoped that, a better quality solid rubber products developed in this research should replace ribbed smoked sheet (RSS) in the near future. The disadvantage of RSS compared to other rubber is its inconsistency of purity, so that the price of rubber sheet produced by small holders are usually sold at the cheapest grade. The visual specification for the best quality of RSS1-3 is light and uniform color and low amount of dirt. The new requirement of the domestic and world market is highly qualified form of solid rubber for more value-added rubber goods such as allergen-free gloves and other medical rubber products. Thai domestic rubber industries should be supplied with low-allergen solid rubber and latex concentrate to meet with the world market. Certification on contact and type of potential NRL allergens should be available for those new grades of raw rubbers.

It is hoped that research and development of new solid rubber products will lead to the production of highly qualified form of more value-added rubber goods. A standard operation procedure for production of a new grade of solid rubber very low in latex protein allergens is the main goal of this research. These raw NR materials should be the first milestone rubbers to produce allergen free rubber products such as medical and pharmaceutical devices, adhesive, and food contact package. To reach this ultimate goal, deproteinization by saponification is one approach to

upgrade solid rubber of various degrees of nitrogen contents. representating protein impurities.

1. To optimize the conditions for latex deproteinization by saponification.
2. To deproteinize solid rubber by saponification, from both starting materials: crumb rubber obtained from ammoniated latex and skim latex, under the optimized latex deproteinization conditions.
3. To study physical properties of raw solid rubber and vulcanized rubber under these conditions.
4. To identify water extractable protein by SDS-PAGE in solid rubber obtained before and after saponiffication.
5. To study prevalence of latex allergy in general population, and compare for the allergenic response between control and saponified rubbers by skin prick (SPT) and enzymeallergosorbent test (EAST) or enzyme-linked immunosorbent test (ELISA).