CHAPTER I
INTRODUCTION

1.1 Background

More than one hundred years, *Hevea Brasilliensis* or para rubber has been the important biopolymer for Thailand and the world. It is one of top ten exports for Thailand (Table 1.1). The price of raw rubber such as rubber smoke sheet (RSS), block rubber (BR) and concentrated latex 60% (CL) are much lower than the rubber products such as tire, footwear, gloves etc in terms of Baht/g (The Customs Department, 2000). Recently Thai government has the policy to promote the value added products from raw rubber, such as gloves and condom in stead of concentrated latex 60% and medical rubber products such as catheter in stead of block rubber (Sombuntanon, 2000).

Natural rubber (NR) of high quality that can be used in the industry is from para rubber tree, *Hevea Brasilliensis*, a tree originating from the Amazonian area (Archer, 1965), (Table 1.2). The latex producing rubber trees grow to the height of 15-20 m and require 2,000-2,500 mm/y of rainfall. They trive at altitudes up to 300 m (Edgar, 1958). Phraya Rachadanupadit Mahidsorn Pakdee (Ko Simbee) had brought rubber seeds to plant in Trang Province in 1901. Rubber trees can be propagated either by seed germination method or by grafting bud. In normal practice, the trees are propagated by grafting buds from a single mother tree (St.Cyr, 1984). There are less than one hundred clones that are commercially grown for latex production. Since 1997 the clones recommended to plant in Thailand by the Rubber Research Institute of Thailand are: BPM24, RRIM600, GT1, PR255, Songkla 36, PB 255, PB 256, RRIC 110 and RRIT 251. These clones are of high priorities for the South and East Regions. Clones BPM 24, RRIM 600, GT 1 and PR 255 are the first clones of choice for the East and Northeast Regions (The Rubber Research Institute of Thailand, 1998).

As of June 2000, the world produced 6,790,000 tons of natural rubber and used 5,860,000 tons while, Thailand produced 2,240,000 tons and used within countries 200,000 tons (Table 1.3). Thailand exported natural rubber 1,084,115 tons, which included rubber smoke sheet (RSS) 524,244 tons, block rubber (BR) 391,762 tons, concentrated latex (CL) 163,201 tons and the other forms of rubber 4,908 tons (Table 1.4). Table 1.5 shows that Thailand exported natural rubber to many countries in the world such as Japan 241,086 tons, USA 204,600 tons, Malaysia 121,790 tons, China 180,550 tons, South Korea 74,057 tons, Singapore 47,525 tons and the other countries 214,507 tons (The Customs Department, 2000).

Table 1.1 Top ten exports of Thailand

Value: Million Baht

Item	1997	1998	1999	2000 (Jan-Oct)
1. Computer and accessories	220,302.7	320,525.6	304,982.2	270,700.1
2. Electronic circuit	75,837.7	93,833.1	111,767.4	141,864.2
3. Clothes	97,135.9	123,133.0	110,356.5	102,925.9
4. Vehicle and components	48,419.6	68,348.4	91,954.1	98,560.8
5. Plastic seed	23,980.2	40,786.3	46,025.8	61,547.8
6. Television and radio components	43,578.8	58,058.2	47,233.4	60,439.9
7. Seafood can	49,309.3	67,952.1	65,956.6	57,745.0
8. Jewelry	55,622.3	57,350.5	59,820.9	53,089.6
9. Rice	65,093.4	86,803.1	73,812.1	50,043.8
10. Natural rubber	57,450.0	55,406.5	43,941.7	48,839.6
Total (10 items)	736,729.9	972,196.8	955,850.5	945,756.7
Others	1,069,952.1	1,275,892.7	1,258,398.2	1,301,908.9
Total value	1,806,682.0	2,248,089.4	2,214,248.7	2,247,665.6

Source: The Customs Department, 2000.

Table 1.2 Taxonomy of rubber tree

Family	Euphorbiaceae
Genus	Hevea
Species	brasiliensis

Table 1.3 Production of Natural Rubber

	Year1997/1998	Year 1998/1999	Year 1999/2000
Production in the world (million tons)	6.43	6.59	6.79
Consumption of world	6.46	6.67	5.86
Production in Thailand (million tons)	2.17	2.16	2.24
Production/rai (ton/rai)	227	225	229
Consumption in Thailand (million tons)	0.17	0.20	0.20

Source: The Customs Department, 2000.

Table 1.4 Exports of Natural Rubber

The kind of rubber	Jan-Jun 1999		Jan-Jun 2000	
	Quantity (ton)	Value (million Baht)	Quantity (ton)	Value (million Baht)
Total	881,493	18,977.57	1,084,115	29,699.78
RSS	465,979	10,828.70	524,244	13,724.31
BR	234,800	4,903.00	391,762	9,829.65
CL	176,945	3,179.53	163,201	6,051.25
Other	3,769	66.34	4,908	94.57

RSS: Rubber smokes sheet, BL: Block rubber, CL: Concentrated latex 60%

Source: The Customs Department, 2000.

Table 1.5 Exports of Natural Rubber to Foreign Countries

Countries	Jan-Jun 1999		Jan-Jun 2000	
	Quantity (ton)	Value (million Baht)	Quantity (ton)	Value (million Baht)
Total	881,493	18,977.57	1,084,115	29,699.80
Japan	240,398	5,802.85	241,086	6,027.00
USA	114,202	2,565.30	204,600	5,322.02
Malaysia	92,840	1,710.78	121,790	2,496.70
China	86,696	1,824.88	180,550	5,307.40
South Korea	77,302	1,380.91	74,057	1,773.77
Singapore	30,360	671.23	47,525	1,093.08
Other	239,695	5,021.60	214,507	7,679.84

Source: The Customs Department, 2000.

1.2 Natural rubber latex

Natural rubber latex (NRL) is a milky white or slightly yellowish opaque liquid, which flows from the plant after wounding. It is believed that the latex is for protection of leaves and tissues of the tree against external infection (Archer et al., 1981). The fresh natural rubber latex is collected from the tree by a process call tapping, which has been described as a controlled wounding of the tree (St.Cyr, 1984). Tapping involves diagonal incision into the bark of the tree. Tapping is usually done for two or three consecutive days and followed by one day rest, the latex about 300-400 ml exudes onto the surface of the cut and flow down the cut into a collection cup at a single tapping. Coagulation of latex occurs naturally. In order to prevent coagulation, a small amount of preservative, usually ammonia, sodium sulfite, formaldehyde, or boric acid, is added to the latex.

1.2.1 NRL composition separated by acid coagulation

Hevea latex contains, in addition to the rubber hydrocarbon, a large number of non-rubber constituents present in relatively small amounts. The latex has the density of 0.975-0.980 g/ml with the pH 6.5-7.0. NRL contains the dry rubber content (DRC) about 25-45% (w/v) depending on the season of tapping, clonal and other factors. The dry rubber content is usually about 2-5% (w/w) less than the total solids content (TSC). The difference between TSC and DRC is non-rubber portion, which made up mainly of 2-3% protein and phospholipid, 2-3% neutral lipid, 0.4% carbohydrates, and 0.3% inorganic salts (Table 1.6).

Table 1.6 Composition of acid coagulated natural rubber

Composition	Per cent (%)
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

Source: Archer et al., 1981.

1.2.2 NRL composition separated by ultracentrifugation

Fresh latex can be separated into four major zones by ultracentrifugation:

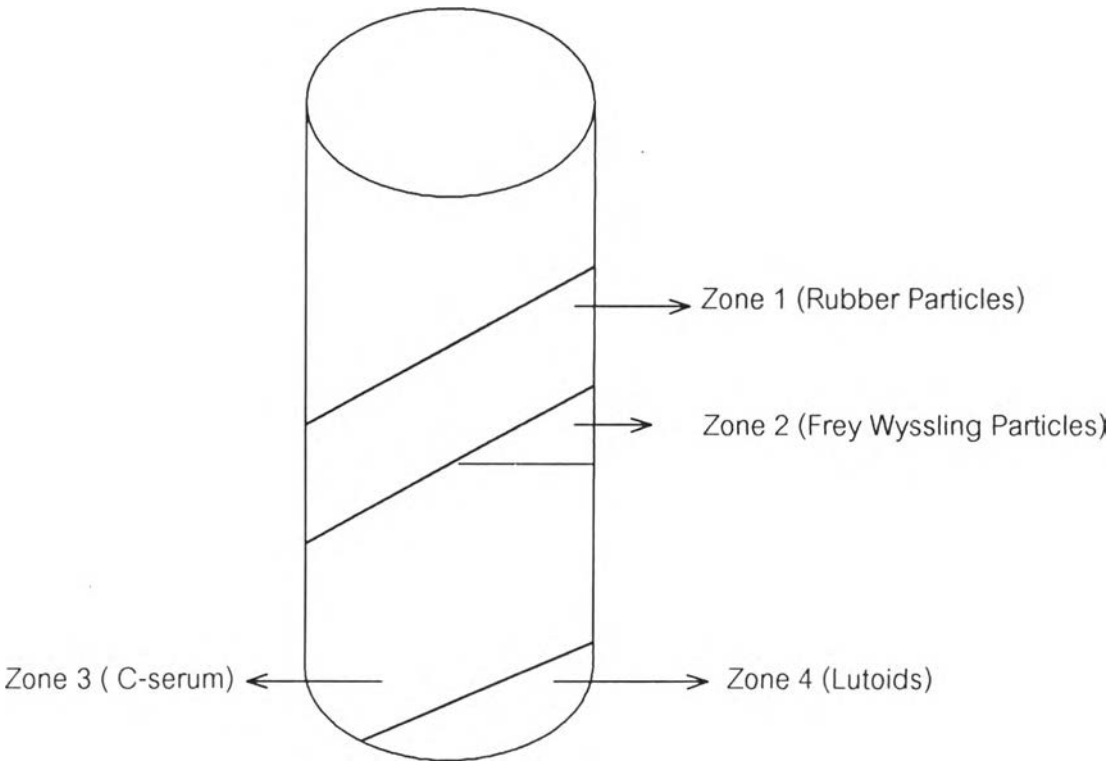


Figure 1.1 High-speed centrifugation of natural rubber latex (Moir, 1959)

1.2.2.1 *Zone 1 (Rubber Particles)*

The uppermost layer constitutes 25-45% of the volume of latex. The rubber particles are usually spherical droplets of hydrocarbon enclosed in a fine phospholipoprotein envelope (Jacob et al., 1992) suspended in water (Figure 1.2). The average particles size were reported between 0.1 μm and 1.0 μm. with a diameter ranging from about 0.02 μm to 3 μm. The particles in fresh latex are protected and stabilized by negatively charged complex film containing proteins and lipids (Smith, 1953 and Fong,

1992). Two proteins important in cis 1,4-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 polyisoprene 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).

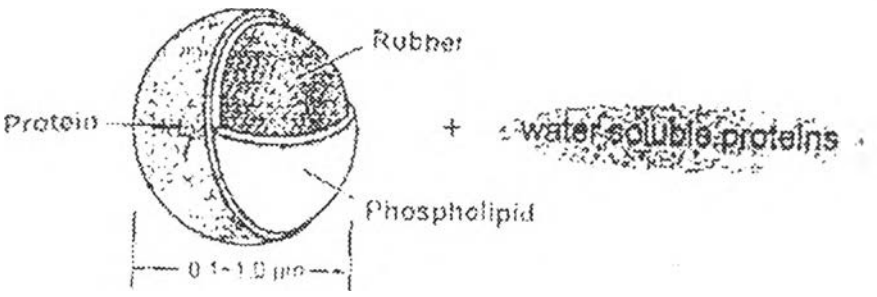


Figure 1.2 Presumed structure of solid rubber particle (Jacob et al., 1992)

1.2.2.2 *Zone 2 (Frey Wyssling Particles)*

A small yellow layer below the rubber zone constitutes 2-3% of the volume of latex. The particles are larger spherical and have slightly higher density than rubber particles. The bright yellow color is due to the presence of carotenoid pigments. Their biological role has not been clearly defined.

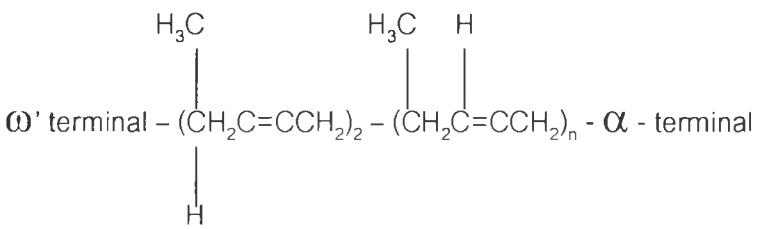
1.2.2.3 *Zone 3 (C-serum)*

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. Data from two-dimensional gel electrophoresis show the presence of about 30 protein bands (Kekwick, 1993).

1.2.2.4 *Zone 4 (Lutoids)*

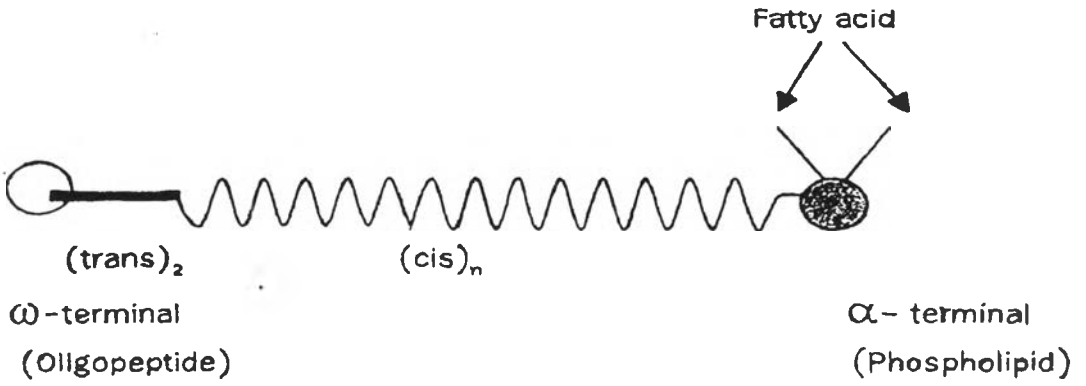
Lutoids or bottom fraction constitutes about 10-20% of the volume of latex. Lutoids, which are spherical membrane-bound bodies typically 2 μm to 5 μm in diameter and have negatively charged surface. It is important for latex coagulation. Inside the lutoid is an aqueous solution pH 5.5, called B-serum which contains dissolved substances such as acids, mineral salts, proteins, sugars and a polyphenol oxides while the pH of ambient serum is about 6.5-6.9 (Fong, 1992).

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 1.3 and Figure 1.4

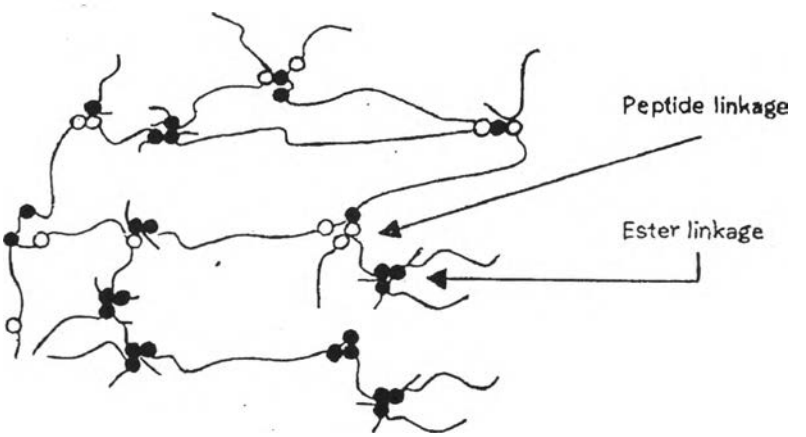


ω' and α : unidentified terminal group

Figure 1.3 The chemical structure of natural rubber (Tanaka, 1984)



Single chain



Poly-chain

Figure 1.4 Presumed structure of a single chain and poly-chain of natural rubber (Tanaka, 1984)

1.3 Rubber particle stabilization

The rubber particle is stabilized because the surface of particle is surrounded by a layer of proteins absorbed which carry negative charge, and proteins are hydrophillic substances. Then there is an envelope of water molecules surrounding the rubber particle. These water molecules, acting as a sheath, prevent direct contact between particles. To increase the stability of latex, stabilizing agent such as ammonia solution and Triton X-100 is used. Ammonia used as a preservative is an alkali, not harmful, has no effect on rubber, no deposits and can easily be deammoniated. It increases the pH of latex and inhibits bacterial growth, sequesters some metallic ions and deactivates the carbohydrates, which act as enzymatic substrates forming fatty acid anions, resulting in the increasing of stability. Triton X-100 (Iso-octyl phenoxypolyethoxyethanol, Nonidet P-40) is nonionic detergent that has the capacity to solubilize proteins and also stabilize the colloidal state of rubber particles (John et al., 1977). Chemical destability agents are acids, metallic compounds, organic solvents etc. that destabilize the structure of water molecule and bring about the coagulation of latex.

1.4 Coagulation of latex using steam

Latex can be auto-coagulated and complete coagulation in about 48 hours by the action of bacterial and yeasts on indigenous substrates and produced acid (John, 1966a and 1966b). There is disadvantage to rubber process due to time-consuming. Coagulation with acid usually takes 2-4 hours for complete yield. But John and Sin (1974) found the coagulation by steam. They found that complete coagulation (clear serum separating from the coagulum) occurred by steam with 1 kg/cm^2 pressure for 10 minutes at a depth of latex up to 4 cm and range of 5-40%DRC.

1.5 Proteins and their effects

Among non-rubber constituents, proteins are major components. About 20% of total proteins in the latex are absorbed on the surface of rubber particles, and a similar proportion is associated with the bottom fraction (or B-serum). The remainders are dissolved in the aqueous phase of latex or C-serum (Archer, 1963). The proteins adsorbed on the rubber particles, together with adsorbed lipids, import colloidal stability to the latex and have isoelectric point (pI) ranging from pH 4.0 to pH 4.6 depending on the clone; pI of RRIM 600 is about 4.3. The variation of pI indicates that more than one protein is adsorbed on the rubber particle and the relative proportion of the adsorbed proteins is a clonal characteristic.

From various types of electrophoresis it has been shown that C-serum protein consist about 19 anionic and 5 cationic proteins (Tata and Edwin, 1970) of which α-globulin, isolated by Archer and Cockbain (1955), form the major protein. α-Globulin is the protein present in the highest concentration in fresh latex serum. Coagulation by heat and precipitation from the solution at approximately the same pH at which fresh latex is coagulated. It may be one of the proteins adsorbed on the surface of the rubber particles and thus partly responsible for the colloidal stability of the latex

The proteins in the bottom fraction consist of 8 anionic and 5 cationic proteins (Karunakaran et al., 1961) in which hevein (4.7 kD) and hevamine (29-30 kD) have been isolated (Archer, 1960; Archer, 1976). Approximately 20% of the dry matter in the bottom fraction of latex from mature tree is water-soluble protein, of which about 70% is hevein. Hevein contains an abnormally high amount of sulfur (about 5%) which is present as disulfide groups in cysteine. It is readily soluble in water and is not coagulated by heat. These properties indicate that sheet rubber should contain very little hevein.

However, there are many more kinds of protein distributed in different phase of *Hevea* latex. Proteins, by their polar and hydrophilic nature, are believed to affect the properties of rubber in many aspects from the raw rubber properties to the vulcanizate properties (Perera and Siriwardena, 1985). Tanaka (1984) had shown that newly formed network, containing a large proportion of nitrogen content, may occur by the formation of

protein with other particles resulting in the hardening phenomenon. Nadarajan and Karunaratue (1971) reported that polyphenol oxidase, is in charge of latex and rubber discoloration. Borgotrom (1968) also believed that enzyme polyphenol oxidase (PPO) is in charge of latex or natural rubber discoloration.

During processing, protein has been suggested to act as filler, having variable stiffening effect resulting in modulus variation (Metherell, 1980), poor dispersion of vulcanized curatives and finally leading to local over cross-linking and properties variation (Bloomfield, 1973) and absorb water affecting the degree of cross-linking in the vulcanizing system (Elliott et al., 1970). Moreover, it has been recently reported in United States that medical instruments with the use of natural rubber such as surgical gloves can give rise to allergic symptoms (EP 0 584 597 A1,1993).

1.6 Commercial natural rubber

Commercial natural rubber can be classified into two major groups, solid natural rubber and latex concentrate (Kajornchaiyakul, 1986). Solid natural rubber has been produced from fresh field latex and skim latex. Solid natural rubber produced from skim latex can be divided into skim block and skim crepe. Solid natural rubber 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 1.5). 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.

1.7 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 be divided into two groups. The first is NRP made from concentrated latex such as gloves, condom, tip of catheters, endotracheal tubing, latex balloon, teats, and dental cofferdams. The second is NRP made from solid rubber such as tire, shoes, adhesives, elastic rubber, and medical products (Figure 1.6).

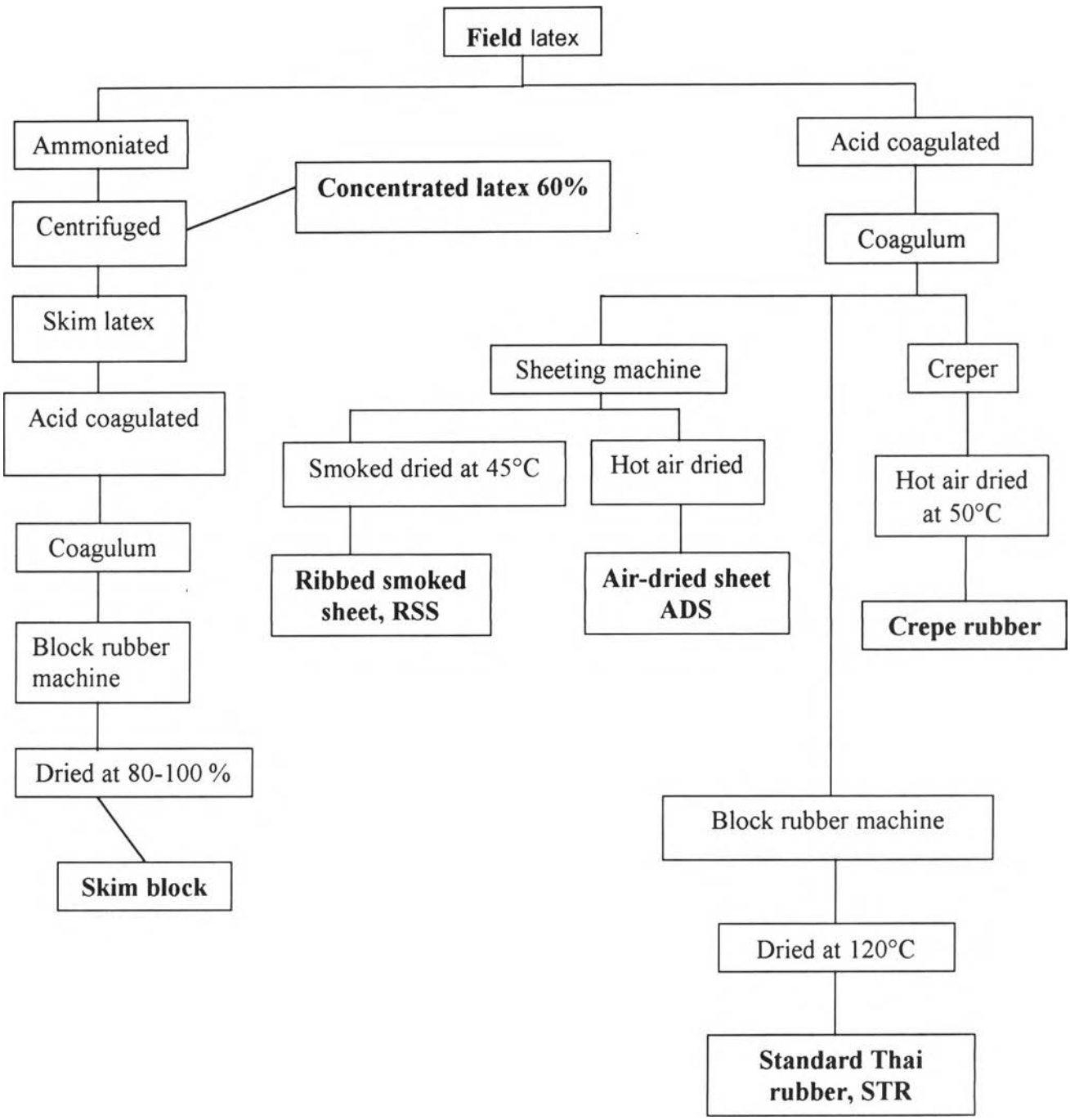


Figure 1.5 Natural rubber production process in Thailand (From Para Rubber Bulletin, Thailand 7,1986)

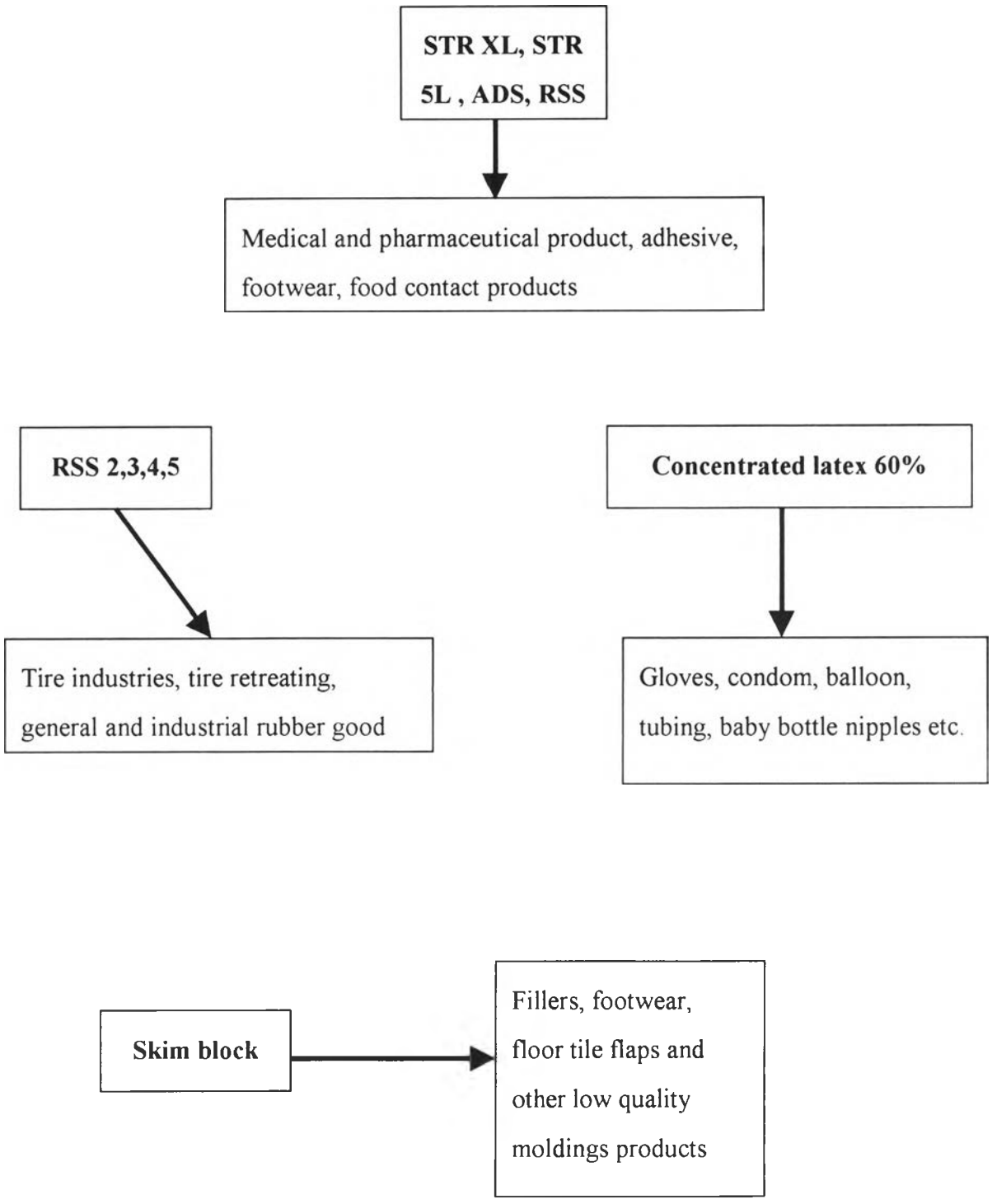


Figure 1.6 Natural rubber products in Thailand (From Para Rubber Bulletin, Thailand 7,1986)

1.8 Allergy

Natural rubber latex products have been reported to cause delayed and immediate hypersensitivity. Delayed hypersensitivity reaction of eczematous contact dermatitis is not caused by latex itself, but by chemicals added during rubber manufacture; such as accelerators (mercapto-benzothiazole and derivatives: tetramethyl thiurums and dithiocarbamates) and antioxidants (p-phenylenediamine). Immediate hypersensitivity to latex are more serious because manifestation within minutes may be contact urticaria, angioedema, rhinitis, and respiratory symptoms, including dyspnea and ashma attacks. Patients subject to immediate reaction are at risk to severe and fatal consequence anaphylaxis if the hypersensitivity is unrecognized when they are examined or operated on by physicians or surgeons wearing latex gloves. An increase in use of condoms, especially by those at risk of contacting HIV infection, further exposes the population to latex hypersensitivity reactions (Akasawa et al., 1993).

1.8.1 Definition of allergy and allergic classification

The term allergy refers to certain diseases in which immune responses to environmental antigens cause tissue inflammation and organ dysfunction. The clinical features of each allergic disease reflect the immunologically induce inflammatory response in the organ or tissue involved. These features are generally independent of the chemical or physical properties of the antigen. Diversity of allergic responses arises from the involvement of different immunologic effector pathways, each of which generates a unique pattern of inflammation. The classification of allergic diseases is based on the type of immunologic mechanism involved. According to Coombs and Gell's classification from 1963 (Gell and Coombs, 1974), allergic reactions can be divided into four types (type I – IV).

1.8.1.1 *Anaphylactic Type Hypersensitivity (Type I)*

Special class of antibody (Cytotropic antibody, mainly IgE) binds to mast cells and basophils through the Fc fragment. When antigen reacts with this antibody, vasoactive amines and other mediators are liberated and elicit the reaction.

1.8.1.2 *Cytotoxic Type Hypersensitivity (Type II)*

Antigen on the cell surface combine with antibody. This may lead to opsonization and phagocytosis without complement, may facilitate attack by T cells, or may lead to binding of complement, which promotes immune adherence to phagocytes; or lytic effect may result in membrane by complement.

1.8.1.3 *Complex Mediated Hypersensitivity (Type III)*

Antigens combine with antibody to form complexes that in turn activates complement and Hagement factor (factor XII in blood coagulation) and aggregate platelets.

1.8.1.4 *Cell Mediated Hypersensitivity (Type IV)*

T-lymphocytes carrying specific antigen receptors become activated by contacting with that antigen, proliferate, transform, and release a variety of mediators (Lymphokines) that in turn act on macrophages, lymphocytes, and other cells to yield the reaction of delayed type hypersensitivity.

1.8.2 Allergen

An allergen is any antigen that causes allergy. The term is used to denote either the antigenic molecule itself or its source, such as pollen grain, animal dander, insect venom, food product or other natural products. Hypersensitivity and sensitivity are often used as synonyms for allergy. Immediate hypersensitivity and delayed hypersensitivity are

the terms formerly used to define antibody-mediated allergy and T lymphocyte mediated allergy.

1.8.3 Antibody molecules

The antigen goes to a body at first, its induces the body to produce a protein α-globulin, so-called immunoglobulin (Ig) or antibody. Antibody molecules are immunoglobulin and composed of four polypeptide chains, comprised of two identical copies of each of two nonidentical polypeptide chains, chains L and H giving $(LH)_2$. There are five classes of immunoglobulin, IgG, IgA, IgD, IgE, and IgM, depend on its H chain types. The H chain types are called γ in IgG, μ in IgM, α in IgA, δ in IgD and ε in IgE. In the most common immunoglobulin , IgG, the two H or heavy chains have approximately 440 amino acids (MW 50,000). The smaller L or light chains, always belong to one of two types: kappa and lambda, contain about one-haft the number of amino acids of the H chain (MW 25,000). The four polypeptide chains are covalently interconnected by disulfide bonds.

Immuoglobulin that produces allergic reaction is IgE. An IgE which is specific for antigen combined with the receptor on the mast cell or basophill. The combination between antigen and IgE results in the change of the surface of the mast cell to the signal transduction. It's effector causes destruction of the granule in the cytoplasm of the mast cell. Cell wall is changed and produces mediator substance or vasoactive amine from granule and cell wall such as histamine and serotonin. Histamine and serotonin, cause the mechanism of hypersensitivity such as asthma, vasodilator, edema and rash etc. Anaphylactic hypersensitivity is the most important hypersensitivity that causes very fast symptoms after contacting or receiving the antigen within 5-30 minutes. Some people were dead from anaphylactic hypersensitivity.

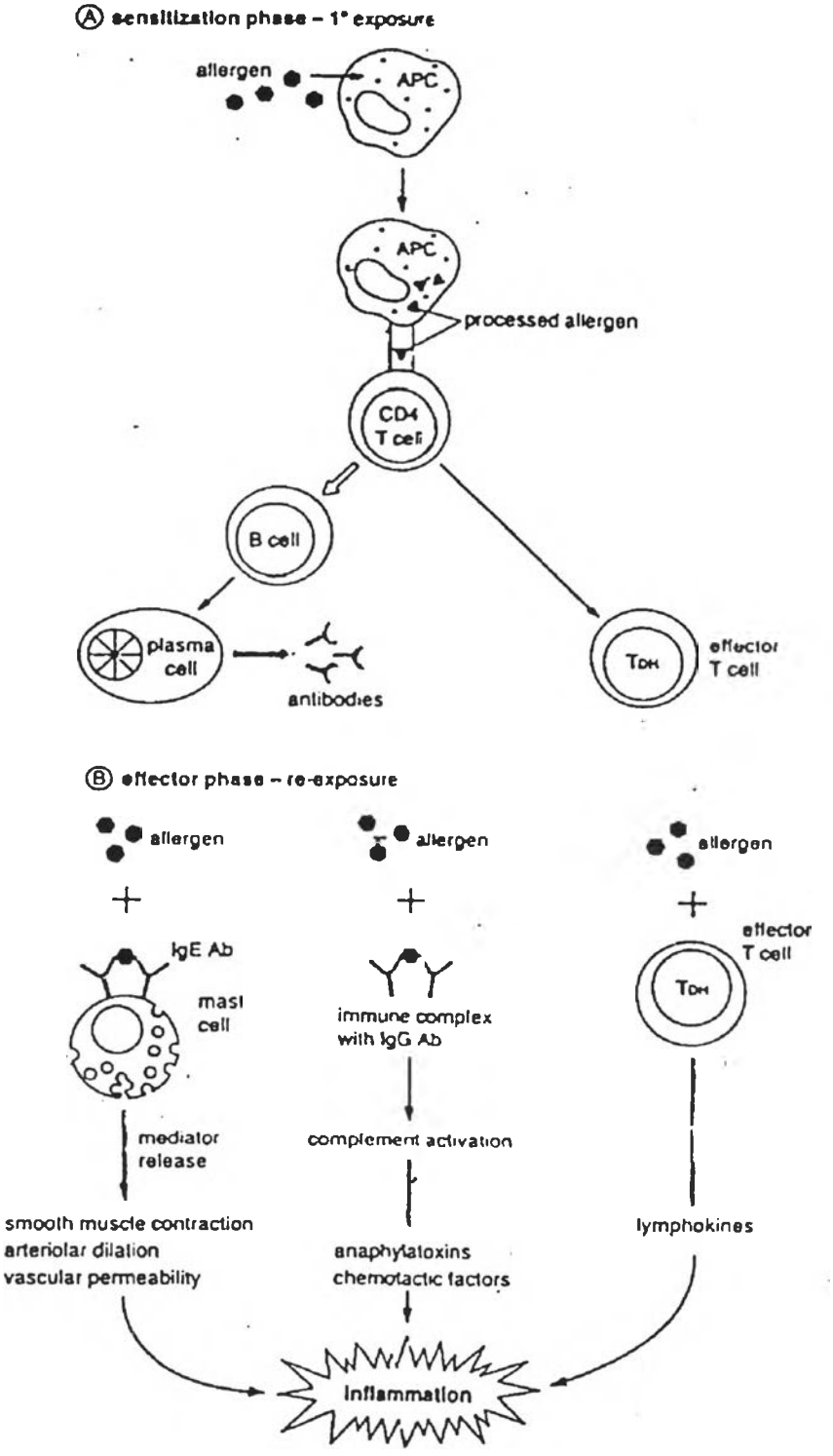


Figure 1.7 Role of the immune system in allergy. A: sensitization phase, showing immunologic response to allergen from unsensitized (nonallergic) state to sensitized (allergic) state. B: Effector phases, showing reaction on reexposure of allergen to specific antibody or to specifically sensitized effector T cell (Harncharoen, 1996)

1.9 Latex Allergy

Allergy to natural rubber latex has been recently acknowledged as the most important form of contact urticaria syndrome in the European (Turjanmaa, 1997), mainly due to its connection to hazards in healthcare workers. The prevalence of natural rubber latex allergy is high (40-60%) in spina bifida children. The health care workers form the largest single occupational risk group (3-11%) both in Europe and in the USA. Two types of allergy caused by natural rubber products (NRP) are known: type I, immediate type and type IV delayed type hypersensitivity (Alenius et al., 1995).

1.9.1 Immediate allergic reactions (Type I)

This type occurs within an hour of exposure to NRP. The contact urticaria syndrome includes localized urticaria, angiodema, 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 reviewed 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 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).

1.9.2 Delayed type hypersensitivity (Type IV, DTH)

This type occurs about 48 hours after exposure to NRP. The sensitizing agents that caused this delayed type contact dermatitis are chemicals 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-benzothiazolin-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 populations is probably less than 2 % (Table 1.7). Studies using radioallergosorbent test (RAST) with serum samples from blood donors indicate higher rates of sensitization may be due to different test method or probably because health care workers, known to be at risk for latex allergy, are more likely to donate blood.

Table 1.7 Latex sensitivity in atopic and general patients, health care workers and blood donors

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
Blood donors	1000	RAST	6.5	Ownby et al., 1994
Hospital & Dental staff	202	SPT	3.5	Wrangsjo et al., 1994
Atopic patients seen for annual check-up	195	SPT/RAST	5.6/8.6	Porri et al., 1995
Non-atopic patients seen for annual check-up	170	SPT/RAST	1.2/2.3	Porri et al., 1995
Blood donors	1436	RAST	7.9	Merrett et al., 1995
Blood donors	352	EAST	4.5	Harncharoen K., 1996
Emergency medical provider	41	SPT	9.8	Safadi et al., 1996
Health care workers	224/405	SPT/Quest	3.13/12.4	Teeraratkul et al., 1997
Hospital employees	135	SPT	8.2	Kibby et al., 1997
Anaesthesiology staff	101	SPT	15.8	Konrad et al., 1997
Hospital employees	1326	SPT	12.1	Liss et al., 1997
Dental students	131	SPT	10	Danne et al., 1997
Nurses ward / ITU	140	SPT	22	Douglas et al., 1997
Latex glove manufactury workers	583	SPT	1.7	Chaiear et al., 2000
Latex tappers	475	SPT	1.3	Chaiear et al., 2000
College students	144	SPT	0	Chaiear et al., 2000

(Adapted from Warshaw et al., 1998)

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

1.10 Identification of latex protein allergens

As summarized in Table 1.8 immunobloting studies show that IgE from sera of latex-allergic patients binds heterogeneously to many different proteins ranging from 4 to 200 kD. Currently, there is no consensus on which proteins is the 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 1.8)

Table 1.8 Identified latex antigens

Author(s) (year)	<5	10	15	20	25	30	35	40	45	50	>50
Turjanmaa et al 1988	**2.5**					**30**					
Morales et al., 1989		**10**			**24**		**35**				**100**
Turjanmaa and Reunala, 1989	3 —— 10										
Turjanmaa et al 1990		**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 Wah I., 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**		**27**						
Alenius et al., 1994			14 ——	20	———						200
Slater and Trybul, 1994			**14.3**		**26.7**						
Alenius et al., 1995				**20**		**30**	**36**				
Aamir et al., 1996			**14**		**24**					**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

1.11 Deproteinization of natural rubber

To overcome these problems, there are several attempts to produce a rubber product with a very low protein content. This rubber with a low protein or nitrogen content has been generally known as "Deproteinized Natural Rubber",DPNR or "Low Nitrogen Natural Rubber",LNNR.

1.11.1Deproteinization Methods

There are three main methods that have been successfully investigated for removal of protein from *Hevea* rubber.

1.11.1.1 *By centrifugation or washing of latex with surfactant or detergent.* : Proteins are eluted from the rubber surface. However, Archer (1975) and Yapa (1984) reported that although the addition of surfactant improves the degree of deproteinization, it affects the plasticity retention index (PRI) value adversely.

1.11.1.2 *By chemical treatment*: The rubber is soaking with NaOH for 24 hrs, The rubber proteins are hydrolyzed by chemical reaction. However, chemical or alkaline hydrolysis is known to adversely affect the oxidative resistance and PRI property of the resulting rubber (Yapa, 1984).

1.11.1.3 *By biochemical treatment with proteolytic enzyme*: The proteolytic enzymes such as papain, trypsin, alcalase, superase etc. that hydrolyzes protein into small peptides and amino acids, which are more soluble for washing out.

The deproteinization scheme can be carried out by combination of these treatments in order to remove protein out as much as possible. However, enzyme treatment is the most suitable method because it can be carried out in mild condition and quite specific with fewer side effects on rubber molecules.

1.11.2 The development of deproteinized natural rubber (DPNR)

In 1940, Baker has reported that trypsin is the most effective among three enzymes, trypsin, pepsin and papain at 30°C. However, trypsin, an animal enzyme, is unlikely to be available as cheaply and conveniently as an enzyme from plant source, like papain which can be produced in the plantations by growing the papaya trees as an intercrop during the immature phase of rubber trees when replanting (Senanayake, 1968).

In 1955, Firestone Plantations produced skim rubber by alkali treatment to break 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% (Firestone, 1955).

In 1971, John produced solid rubber from latex with low prctein 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 ordinary acid coagulated rubber (John, 1971).

In 1974, Yapa and Balasingham reported the advantages of papain over superase that papain can be used in wider range of pH. The coagulation of latex by papain can be occurred at high pH values, thus giving light colored rubber even if ammonia is used at high dosages as preservative. On the contrary, papain is also active under acid conditions so it can be used as a deproteinizing agent as well as a coagulant for field latex (Nadarajah et al., 1973).

In 1975, Yapa had prepared low nitrogen content and constant viscosity rubber. The chemicals such as hydroxylamine sulphate, hydroxylamine hydrocholride, semicarbozide hydrocholride were used to stabilize the viscosity of solid rubber. In this case, he started from field latex, which was diluted, to ½ with water and papain (0.05% on volume) was added then. Followed by stabilized chemical solution (0.08% on volume), the latex was left overnight for the enzyme to act on the latex proteins. He found that papain and hydroxylamine were suitable for the manufacture of low nitrogen CV-rubber and reduced nitrogen content to about 40% (Yapa, 1975).

In 1977, 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 enzymolysis was carried out in a tank with a slow-speed stirrer for 24 hours. Then the treated latex was diluted to 3% total solids content and coagulated with 2% mixture of parts by weight of phosphoric and sulfuric acid. The nitrogen content was 0.12 g% (Chang et al., 1977).

In 1977, John, Nadarajah and Chang had prepared DPNR by papain treatment and surface active agent, Nonidet P-40 (John et al., 1977).

In 1977, Yapa had prepared solid DPNR and CV-DPNR by different 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 (Yapa, 1977).

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 (Yapa et al., 1978).

In 1984, Yapa et al. prepared 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 (Yapa et al., 1984).

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 ^{o}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 a shaker at 50 ^{o}C for 10 hours. The treated latex was diluted to 25% 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% (Visessanguan, 1992).

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 ^{o}C. The latex was then either centrifuged once or twice and coagulated by additions 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% (Eng et al., 1992).

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 ^{o}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% (Eng et al., 1993).

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 once or more. The nitrogen content of HDPNR was 0.013% (Nakade et al., 1997).

In 1997, Tangpakdee and Tanaka had prepared latex DPNR from fresh field latex (FL-latex) by different treatment, i.e. enzymatic deproteinization, transesterification and saponification. 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 ^{o}C for 24 hours followed by centrifugation. The cream rubber was redispersed in 1% 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 ^{o}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 (5:1 v/v) in the presence of 0.1% w/v methanolic pyrogallol as an antioxidant. The reaction was refluxed at 70 ^{o}C for 2 hours under nitrogen atmosphere. The hot saponified 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% (Tangpakdee, 1997).

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 ^{o}C for 3 hours without stirring. The saponified rubber obtained has reduced the nitrogen content to 0.008% (Tanaka, 1998).

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 of 24 hours. Skim crumb rubber obtained has reduced nitrogen content to 0.52%. Skim latex treated with 5% NaOH technique at room temperature for 24 hours, 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 nitrogen content 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 nitrogen content to 0.37% (Rungvichaniwat et al., 1998).

The main drawback concerned for commercial implementation of papain treatment is the low availability and consequently high price of papain in natural rubber producing countries (Yapa, 1980). Papain can be used only once also be contaminated protein in rubber latex after enzymic treatment.

1.12 Proteases

Proteases are enzymes that degrade polypeptide chain of proteins. It was found in animals, plants and microorganisms. There are many kinds of proteases. Different proteases have specificity for different substrates, mechanism of catalyzation, active site, inhibitors, activators, amino acid sequence, pH and temperature optimum. Proteases may be classified as follows;

1.12.1 Source of enzyme, such as from animals, plants (papain) and microorganisms.

1.12.2 Site of cleavage or digestion of peptide bond, such as exopeptidase and endopeptidase (papain).

1.12.3 Character of active site of enzyme that can be further divided into four groups.

1.12.3.1 Serine protease

This group has serine and histidine residues in the active site that can divide to two small groups;

1.12.3.1.1Alkaline Protease

Enzyme in this group is endopeptidase. The optimal pH is above 7 such as Subtilisin etc.

1.12.3.1.2Trpsin-like Protease

Trypsin and Chymotrypsin etc.

1.12.3.2 Metallo Protease

This group has a metal ion in the active site such as Thermolysin that has Zn^{2+} in the active site. The enzyme is inhibited by metal chelating agent, such as EDTA, 1,10-phenanthroline. The optimal pH is 6.5-7.5 (Neutral Protease).

1.12.3.3 Acid Protease or Aspartic Protease

This group has aspartate residue in the active site that increases the catalytic activity in the pH range of 2-5, such as Rennin and Pepsin.

1.12.3.4 Thiol Protease or Cysteine Protease

This group has cysteine residue in the active site of enzyme such as Ficin, Bromelain and Papain.

1.13 Papain

Papain is the main protein constituent of latex of the green fruits, leaves and trunk of *Carica papaya*, a small soft wood tree which is native to tropical countries. Crude papain is collected from full-grown but still unripe fruits by making longitudinal scratches on the fruit, and allowing the collected latex to coagulate. The coagulum is dried to reduce moisture content to 5-8%. Papain is a single polypeptide containing 212 amino acids (7-cysteine residues). Six cysteine residues form three disulfide bonds and the other cysteine residues, which locates at the twenty-fifth amino acid position is in active site. (Figure 1.8, Lowe, 1970). Papain, a plant proteolytic enzyme, is a sulfhydryl protease having a sulfhydryl or thiol group (-SH) at its active site. Papain catalyzes the hydrolysis of a variety of peptide, ester and amide bonds of synthetic substrates, for example, Benzoyl-L-arginine-p-nitroanilide, (BAPNA), Benzoly-L-arginine ethyl ester, (BAEE), Benzoly-L-arginamide. Properties of papain are summarized in table 1.9. Thiol protease, papain, is activated by mild reducing agent, low molecular weight thiol compounds such as cysteine, sulfide, sulfite and cyanide (Arnon, 1970). Since papain activity depends on a free –SH group, it is inactivated by reagents or conditions that modify this functional group.α-Halogen acid or amides and N-ethyl-maleimide irreversibly inhibit the thiol group while heavy metal ions and organic mercurial salts inhibit in a fashion that can be reversed by low molecular weight thiols, particularly in the presence of EDTA which chelates such metals (Liener, 1974).

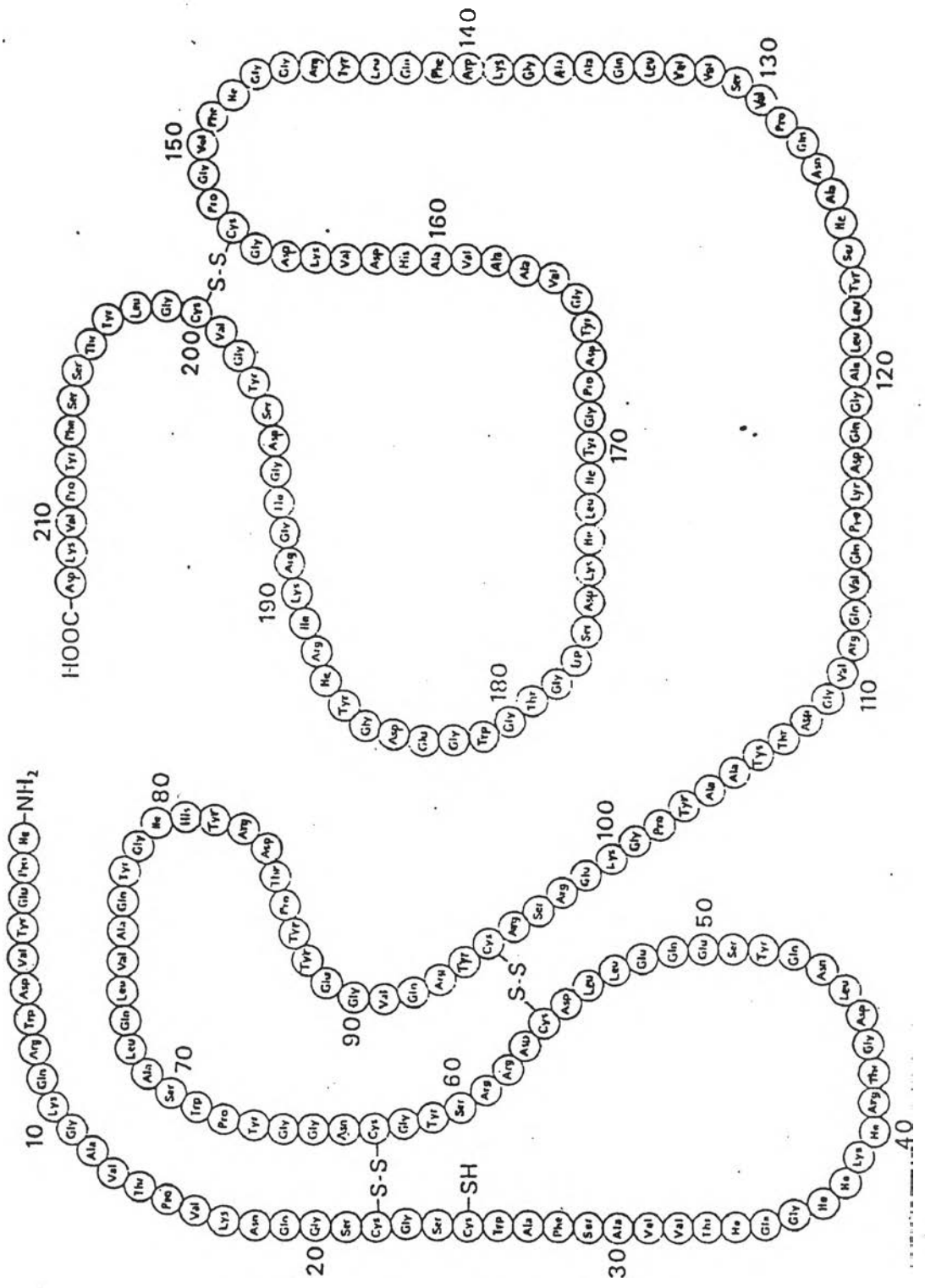


Figure 1.8 The sequence of the amino acid of Papain (Husain and Lowe, 1970)

Table 1.9 Properties of papain

Property	Papain
Enzyme Commision (E.C)	3.4.4.10
Source	*Carica papaya*
PI	8.75
Molecular weight	20,700-24,000
Working pH	5-8
Working temperature (^{o}C)	40-75
Specificity for hydrolysis of peptide bond	Wide
Activator	Reducing agent, thiol-compound and cysteine.
Inhibitor	Oxidizing agent and metal ions.

Source: Ward (1983) and Arnon (1970)

1.13.1 Mechanism of Papain

The overall reaction pathway for the catalytic activity of papain, is best described by the scheme shown in Figure 1.9. This mechanism shows the formation of an enzyme-substrate complex which results in the acylation of the enzyme (to form a thiol ester) and its subsequent deacylation, the overall reaction leading to a regeneration of the enzyme and the elimination of the products of hydrolysis.

Papain is used extensively in the food industry for chilled-proofing, the tenderization of meat and has application in tanning and textile industries and is used medicinally as digestive aids.

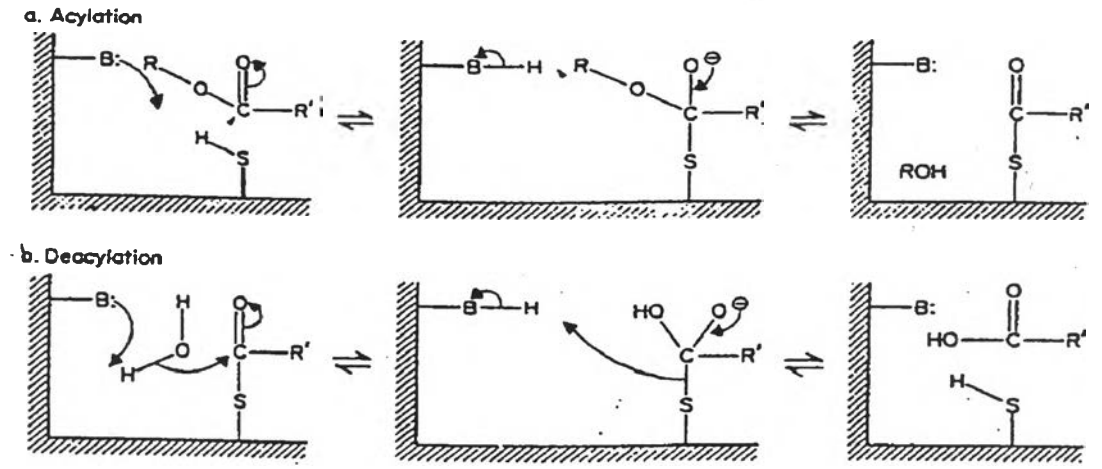


Figure 1.9 A mechanism of action for papain catalyzed hydrolysis (Linener,1974)

Papain has a sulfydryl group (-SH) and an imidazole group on the active site. Imidazole group (B:) acts as a general base that pulls hydrogen ions (H^+) from –SH group. Then sulfur (S) in –SH group was combined with carbonyl group (-C-) of the substrate easier and faster. It forms an enzyme-substrate complex (ES complex) and get of the R residue. The retained substance is formed in acyl-enzyme thiol ester (E-S-C-R). This reaction was called "Acylation". The E-S-C-R has led to deacylation by imidazole group (B:) that acts as a general base that pulls hydrogen ion (H^+) from H_2O then cause the combination between hydroxide ions (OH^-) of water and carbonyl group (-C-) of acyl-enzyme. This causes the free of acyl group (R-C-OH), and the enzyme (E-SH) has returned to the normal form (Lowe, 1975).

1.14 Deproteinization of natural rubber by protease and microwave energy

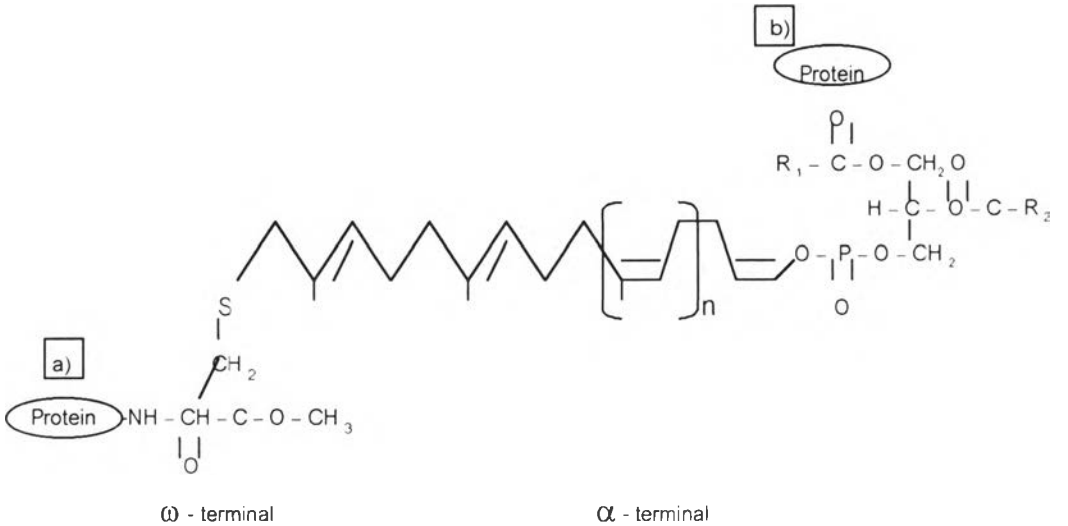
Figure 1.10 a and Figure 1.10 b show the presumed structure of linkage between protein and NR that can be removed by deproteinization. The presumed structure of linkage between protein and NR are:

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

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

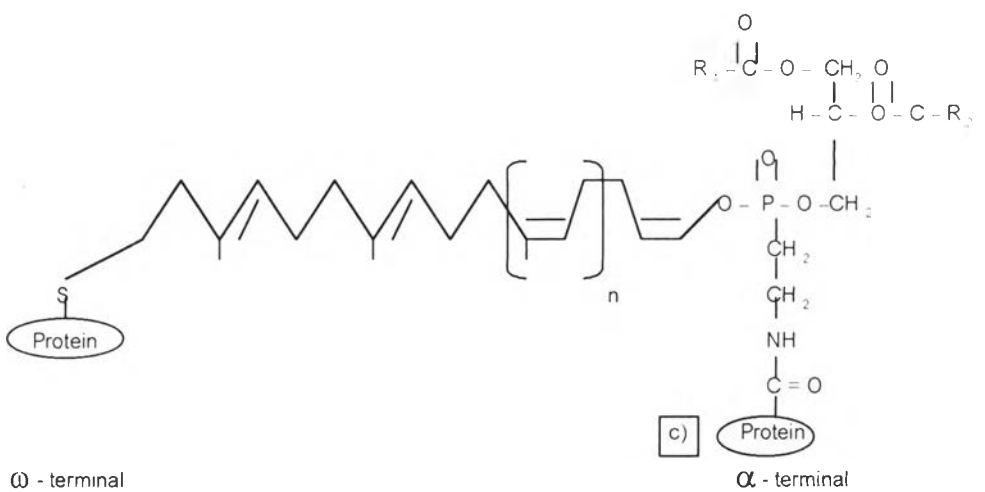
3. Proteins may covalently link to α-terminal of NR through phospholipids such as phospho ethanolamine .

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



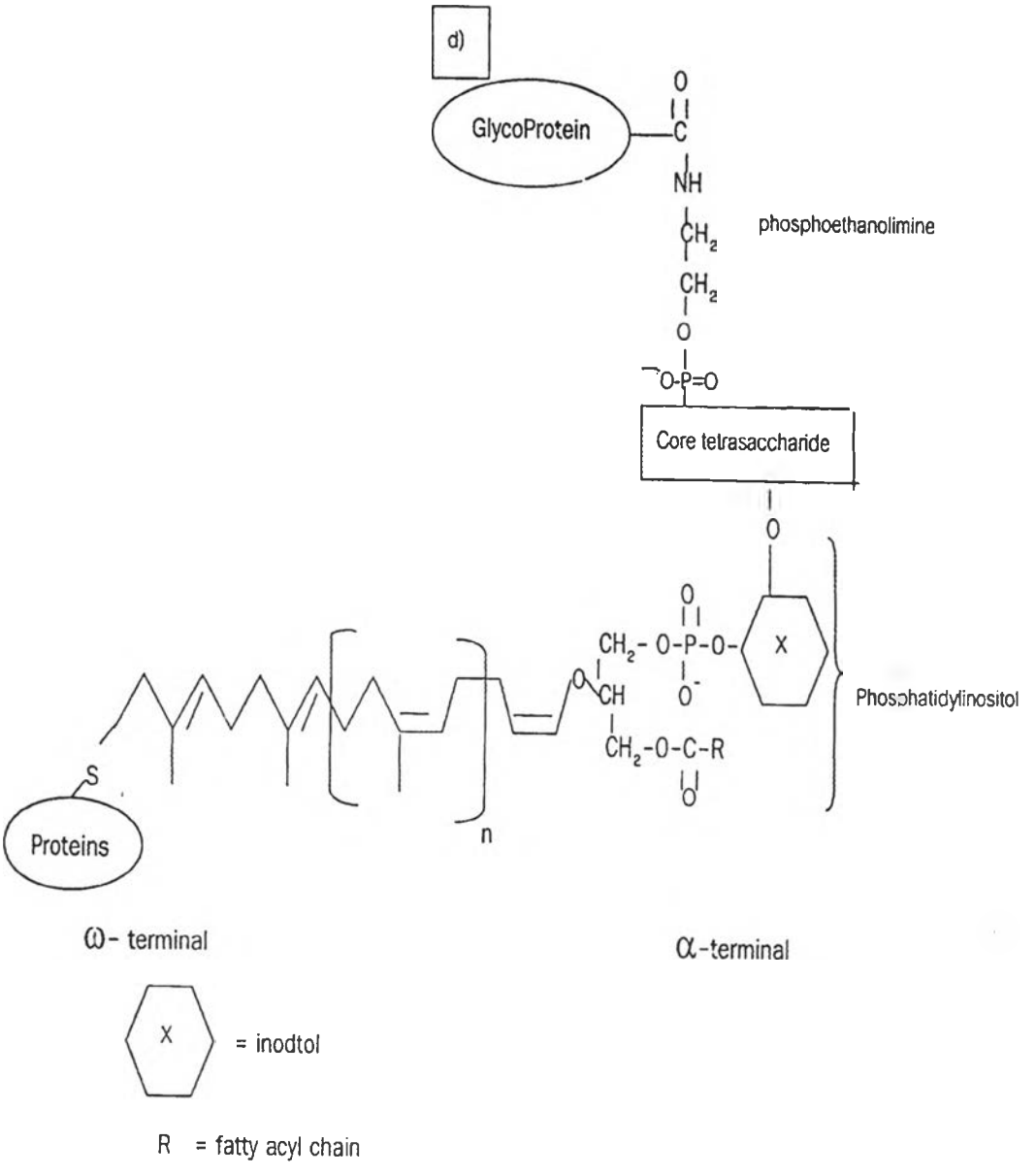
a) Proteins may covalently link to isoprene group in ω-terminal of NR by S-cystein methyl ester so called prenylated proteins

b) Proteins may associate with phospholipids by hydrophobic interactions



c) Proteins link to α-terminal through phosphoethanolamine

Figure 1.10 The presumed structure of linkage between protein and NR



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

Figure 1.10 The presumed structure of linkage between protein and NR (continue)

1.15 Microwave Energy

1.15.1 A primer for the use of microwaves in the rubber industry

Over the last five years the explosive growth of the domestic microwave oven to over 65% of the American households has made the majority of industrial processors in the United States aware of this unique form of energy. Even though microwaves have been used successfully in industry for well over two decades, their full potential and growth have not been realized due to a lack of awareness of the resulting benefits and cost savings. The rubber industry, for example driven by competitive pressures for quality and cost, is rapidly accepting microwave vulcanization. There are now about 500 continuous microwave lines worldwide. In this note, a brief explanation of microwave heating will be presented along with guidelines for the application of microwaves to the rubber industry.

1.15.2 Principle of microwave

Microwaves are invisible radio frequency or electromagnetic waves, which have properties that enable them to be generated and sent trough space and received or absorbed at a distance. Electromagnetic waves are basically identical to the familiar radio and TV transmissions, which we receive daily, and to aircraft radar (Figure 1.11). All electromagnetic waves are similar. They differ only in the number of alternatives or oscillations of their field per second. These oscillations are measured in megahertz or millions of oscillations per second (abbreviated MHz). Each electromagnetic wave has a characteristic spatial dimension called wavelength. The higher the frequency of a radio waves the shorter the length of the wave.

The FCC (Federal Communications Commission) has assigned a number of bands for industrial microwave ovens, which will not conflict with communications frequencies. All present day home microwave ovens in use are at 2450 MHz. At this frequency the length of a wave is 4.8" in air.

Table 1.10 Properties of fields

Characteristics	Wind	EM field
Optical:	Invisible	Invisible
Direction:	N, S, E, W	Up, Down, Right, Left
Strength:	Miles per Hour	Volts per inch
Power:	Horsepower	Kilowatts
Effects objects:	Transport dust, debris,etc.	Transports ions, electrons
Rotates and Lines-up Objects:	Weather vanes, grass, etc.	Molecular dipoles
Flow	Steady, gusty Gusts/Minute	Gusty-Gigahertz
Frequency Spectrum	Bass, baritone, tenor, soprano	RF, MW, UV, etc.

Source: Allen et al., 1994

Microwaves are a combination of electric and magnetic fields perpendicular to each other. It is difficult to visualize a field, however, a good analogy would be to the wind which is invisible, has direction, can vary in strength, be gusty, etc. (Table 1.10)

Another aspect of microwaves is power, which is usually measured in kilowatts. Power is the expression of how much work microwaves can perform. A kilowatt is approximately 1 BTU per second or 3400 BTUs per hour or about 3.4 pounds of steam per hour, or about ¼ calorie per second. One kilowatt of microwave power is able to boil away about three pounds of water at one atmosphere pressure and at room temperature in 1 hour. Microwave ovens used in rubber processing range from approximately 1 kilowatt in power for small preheaters, to about 50 kilowatts for large continuous systems.

Microwave fields have certain properties (Figure 1.12). They are reflected off metals that they do not heat. Therefore, metals are used as conduits for microwaves. These conduits are called waveguides. Metals are also used for the walls of the microwave oven where they confine the microwave to a usable region.

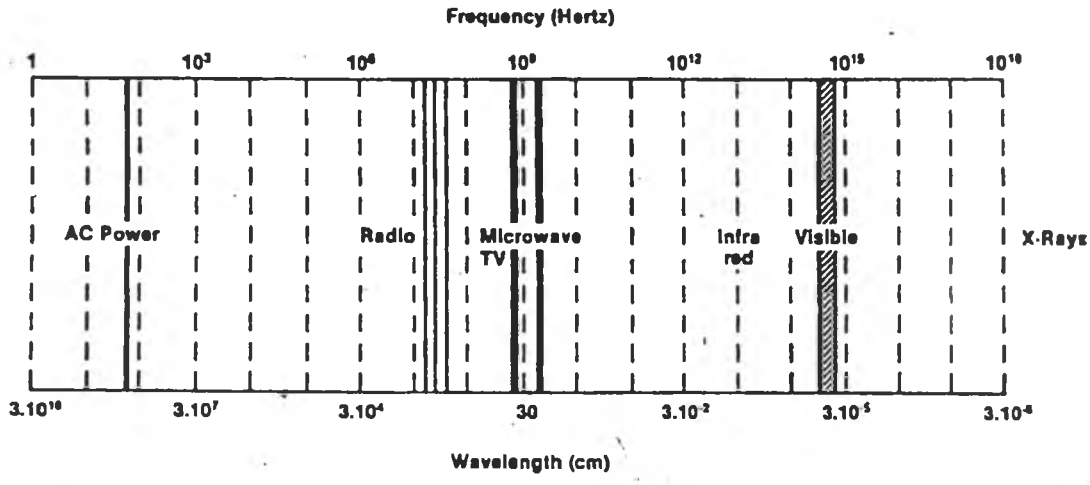


Figure 1.11 The electromagnetic spectrum (Allen et al., 1994)

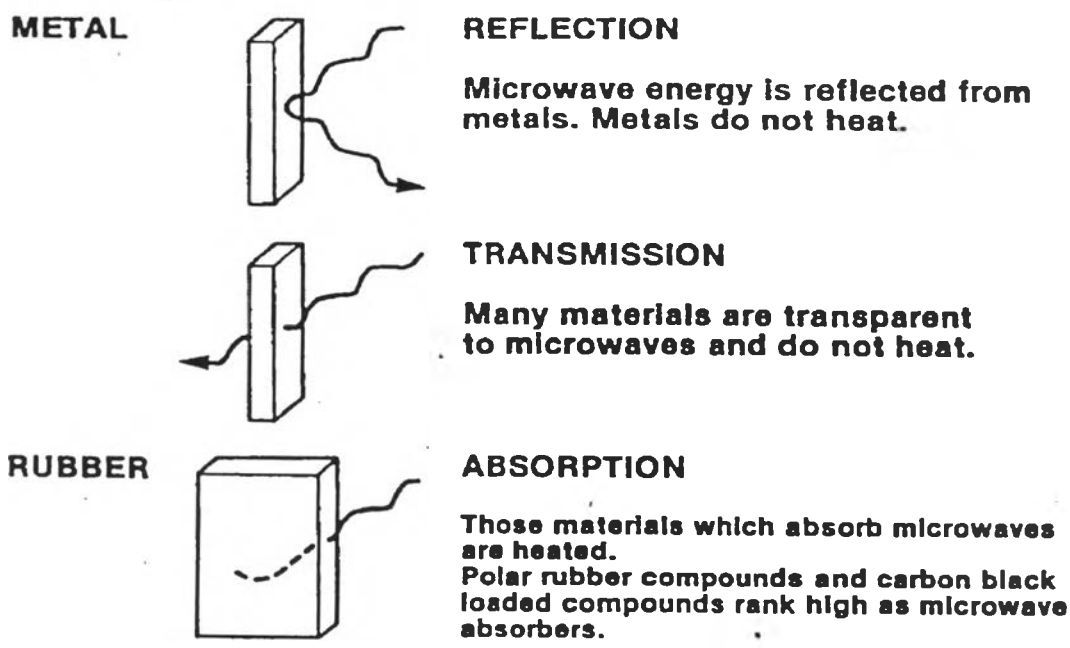


Figure 1.12 Microwave properties (Allen et al., 1994)

Some materials have the property of transmission in a microwave field. That is, they are transparent to the microwave fields and the fields will pass through as light will pass through glass. Microwave transparent materials neither reflect microwave fields nor do they heat. Such materials are used in microwave ovens as support structure where absorption is not desired.

The most important property of materials in a microwave field is the property of absorption. Materials that absorb microwaves are heated.

1.15.3 How do microwaves heat materials that absorb microwave field?

There are essentially two mechanisms for the absorption of microwave power by materials. One is that of dipole rotation that would apply to polar materials such as nitrile and neoprene polymers. The molecules of these polar compounds are electrically neutral, but have a spatially separated positive and a negative electric charge. They appear as molecular electric "compass needles" or dipoles which react to field changes and orient and reorient themselves as the amplitude of the field increases from zero in one direction, reaches a maximum, decreases back to zero and than increases to a maximum in the opposite direction. The field has both amplitude and direction. At 2450 MHz, the field is alternating or reversing its direction at a rate of 2450 million times per second. The polar rubber molecule in this microwave field will attempt to rotate its negative pole in the direction of the field. It will then return to its normal state of disorder as the amplitude move to zero, and then will attempt to rotate its positive pole to the opposite direction of the field and do all this activity at a frequency of 2450 MHz (Figure 1.13).

The force exerted on the polar molecule is by definition the field strength, which is related to the amount of microwave power available. This action of molecular rotation to orderly align with the microwave field and then return to the normal state of disorder of the molecule forms part of the mechanism of microwave heating. It is instantaneous, uniform and penetrating throughout the material. The microwave field has transferred energy to the rubber molecule and the rubber molecule has transformed the energy into heat within itself. This instantaneous and deep penetrating effect is the great

advantage to microwave processing of rubber since rubber is a thermal insulator. To conduct heat into the rubber in a conventional manner such as hot air is considerably slower than the microwave effect of molecular rotation.

Different materials have different abilities to absorb microwave energy. This ability is expressed in terms of dielectric loss factor. Table 1.11 shows the dielectric loss factors for various common materials (Allen et al. 1994 has not used polar rubbers in this illustration since there are so many varieties that it would make the illustration overly complex). The interesting thing to note in table 1.11 that is the characteristic of water where it can be seen that the dielectric loss factor decreases as the temperature of the water increases. To put it another way, water becomes less receptive to microwaves as it is being heated. Polar rubber polymers, on the other hand, generally have the opposite characteristic, that is, in most cases they tend to become more receptive to microwave energy as they are heated.

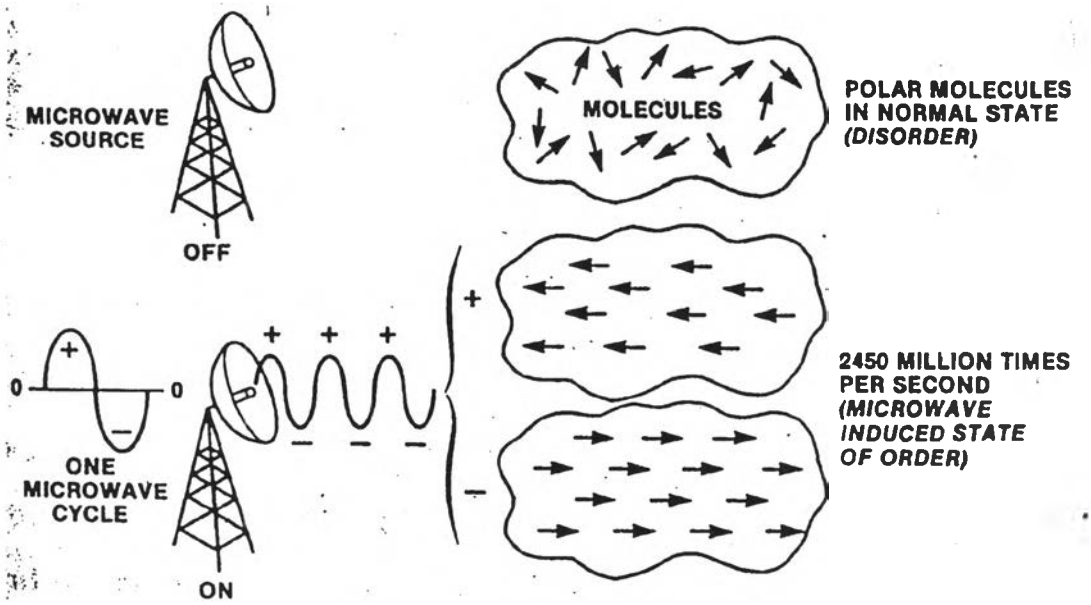


Figure 1.13 Dipole rotation of polar molecules in a microwave field (Allen et al., 1994)

Table 1.11 Dielectric loss factor at 300 MHz

Material	Degrees (^{o}C)	Dielectric constant	Dielectric loss tangent (x 10^{4})	Dielectric loss factor (x 10^{4})
Water	15	78.8	2050	161,000
Water	45	70.7	1060	75,000
Water	95	52.0	470	24,000
0.1 M. NaCl solution	25	75.5	2400	181,000
0.5 M. NaCl solution	25	67.0	6250	418,000
Fused Quartz	25	3.78	0.6	22.6
Nylon 66	25	3.03	128.0	388.0
Teflon	22	2.1	1.5	3.1
Polystyrene	25	2.55	3.3	8.4

Source: Allen et al., 1994

Water, a liquid, and monomers have small molecules, whereas polymers (polar rubbers) have quite large molecules. The efficiency, or amount of energy converted into heat by each cycle of dipole rotation is optimum when the time intervals of application and removal of the electric field (the microwave frequency) coincides with the time required for the build-up and decay of the induced order. The higher the temperature, the faster the build-up and decay of order imposed by the microwave field. The temperature dependent and the molecular-size dependent time for build-up and decay are called the relaxation frequency. In small water molecules the relaxation frequency is already higher than the microwave frequency and it moves further from the microwave frequency as the temperature increases, causing a slow down of energy conversion. On the other hand, large molecules, (e.g. polar rubber), have a relaxation frequency that is lower than the microwave frequency, but which gets closer to it as the temperature climbs, resulting in faster energy conversion at higher temperatures.

The second mechanism of microwave heating is that of ionic conduction. It is commonly known that non-polar rubber such as natural rubber, EPDM, SBR, etc., are not receptive to microwave energy but are made receptive by the addition of carbon black. The heating effect of non-polar rubber with carbon black is due to ionic conduction. Free ions exist at the interface of semi-conductor materials, the interface between the carbon particle and the polymer. These ions are not electrically neutral, but rather are either positively or negatively charged. As such, they are attracted by electric fields and their movement in such field constitutes a flow of current. Their velocity represents kinetic energy given to them by the microwave field. The free ions do not travel very long in the microwave field before they collide with un-ionized molecules giving up their kinetic energy in a randomized billiard ball fashion almost as fast as they obtain it. You will note in table 1.11 the dramatic effect on microwave receptivity when salt is added to the polar water to induce ionic conduction.

It should be noted, as distinguished from dipole rotation, the ionic conduction heating process is not dependent to any great degree on either temperature or microwave frequency. Non-polar rubbers, therefore, which have only the addition of carbon black will tend to remain constant in their ability to receive microwave energy as they are

heated. In actuality, however, rubber compounds contain many ingredients and chemicals. There is, therefore, a complex mixture of materials, some of which are being heated by ionic conduction and other by dipole rotation within a given receipe.

1.15.4 Applications of microwave in the rubber industry

1.15.4.1 Continuous vulcanization

Microwave continuous vulcanization (MCV) ovens have the ability to continuously vulcanize extruded rubber weather-stripping and similar mechanical goods at extremely high speeds. The microwave oven brings the rubber up to vulcanization temperature and a subsequent hot air tunnel provides the necessary residence time at the temperature to complete the cure.

It is obvious from the above discussion of microwave properties that the rubber can be heated in the microwave oven as fast as its properties can stand the temperature rise, since the temperature rise is purely a factor of the amount of microwave power applied.

Approximately 90% of the microwave energy that is introduced into the microwave cavity can be transferred to the rubber. Overall system efficiency of the microwave oven starting with AC from the wall is about 55% when the losses in converting AC to DC and DC to microwaves are considered. This, however, is approximately fives times more efficient than conventional conductive processes. The speeds, of course, are very many times greater than the conventional process and the advantage increases as the thickness of the product is increased.

1.15.4.2 Preheating

Microwave energy is used to preheat rubber slugs and performs prior to compression or transfer molding. It offers the advantage of cutting process time approximately in half and increasing yield since there is less flash and backrinding because preheated rubber flows more easily in the press. It yields a better product since

the cure is more uniform throughout the past; it cuts down on scrap and it prolongs tooling life because of the shortened process time. The disadvantage is that the preheater is an extra step in the manufacturing process. However, its overall benefits are very positive.

Microwave energy has shorter lamda (0.001-1.000 m) and higher frequency (300 MHz-300 GHz) than the radio waves. At the present, the most frequency of the microwave had been used at 2450 MHz.

1.16 Enhancement of deproteinization by microwave energy

In a suspension system, the particles are dispersed in a solution. The action of microwave energy was to disperse the particles and vibrate of the rubber particles to make the optimal temperature faster than used the water bath.

Rubber particles consist of long length of the polyisoprene chain and non-rubber such as protein and lipid. The proteins are adhered on the rubber particles or between the polyisoprene chain. The proteins adhered to the rubber particles are easily removed by papain because they are on the surface of the particles. But the proteins between the polymer chain are difficult to remove. (Figure 1.14)

In the fresh field latex there are three major components: water, serum protein and rubber particle. When microwave was used to preheat the latex.

1. Polar water molecules which are much smaller than proteins and rubber particles were rapidly rotated in the electromagnetic field at the frequency of 2450 million times per second.

2. Protein molecules (4-100 kD) and rubber particles (10^4-10^7 kD) are heated by ionic conduction.

3. Microwave heating should result in conformational change of serum proteins and rubber associated proteins so that they are more susceptible to digestion by papain.

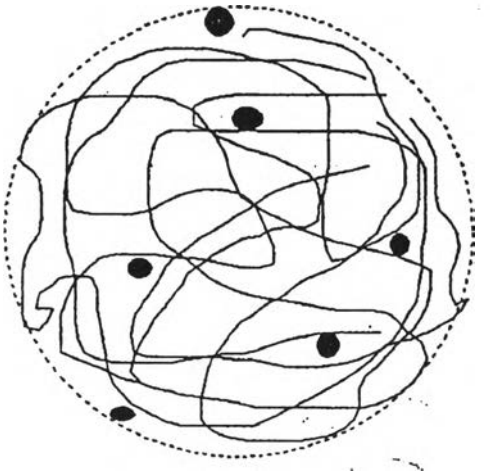
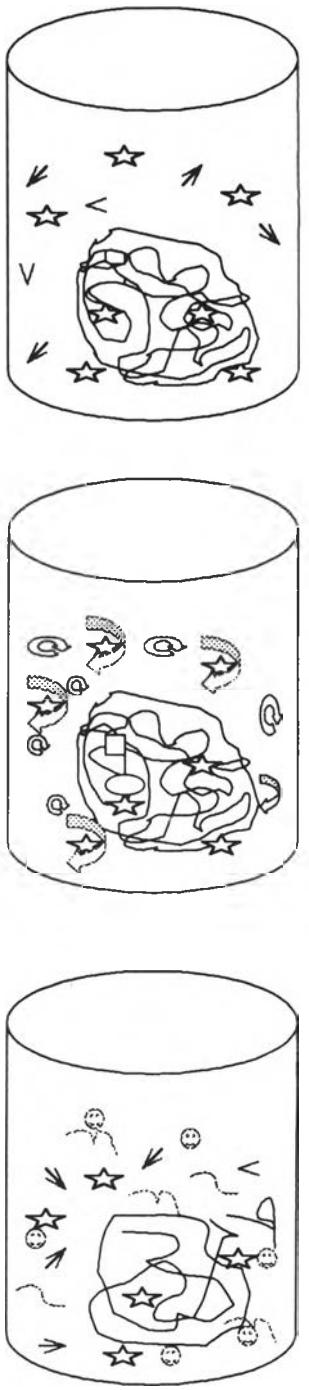


Figure 1.14 Rubber Particle

——— Polyisoprene chain

● Proteins



a. Before preheat by microwave

b. During preheat by microwave

(microwave treatment)

c. During papain treatment

Figure 1.15 The presumed effect of microwave energy and papain on the latex

: Rubber particle

: Water

: Protein

: Papain

: Phospholipid

1.17 The rationale and purposes of this study

It is well known that Thailand is an important natural rubber producer in the world and natural rubber is one of the most important export goods of Thailand. But the raw rubber doesn' t cost as much as its rubber products.

Since the spread of HIV infection, some of the rubber products such as gloves and condoms are more frequently used and become a "must" for all healthcare workers report that the therefore more people are sensitized by NRP, although the percentage of people who are hypersensitivity is lower than 2-3%, the problem of latex allergy has been recognized in Europe and USA.

Looking in the 21 century, the rationale of this research is to produce rubber goods at the grade of "allergen-free" rubber products from allergen-free solid rubber.

The first and primary objective is to produce a new grade of solid rubber products. It is hoped that this will lead to the production of highly qualified, allergen-free 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 protease and microwave energy is the approach of this research. The other objective is to characterize this new solid rubber product for its raw rubber properties, processability and last but not least protein impurities and allergenic properties.

The scope and experimental protocol of this research and the followings:

1. To optimize the condition for latex deproteinization by protease and microwave energy.
2. To deproteinize fresh field latex by protease and microwave energy under the optimized latex deproteinization conditions.
3. To study physical properties of raw solid rubber under these conditions.
4. To identify water extractable protein in solid rubber obtained before and after deproteinization by SDS-PAGE.
5. To study prevalence of latex allergy in general population, and compare for the allergenic response between control and deproteinized natural rubber by skin prick test (SPT) and enzyme allergosorbent test (EAST) or enzyme-linked immunosorbent test (ELISA).