EFFECT OF MAILLARD REACTION ON THE PROPERTIES AND EXTRACTABLE PROTEIN CONTENT OF CAST NATURAL RUBBER LATEX FILMS

by

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Thesis submitted in fulfillment of the
requirements for the degree
of Master of Science

MARCH 2007

ACKNOWLEDGEMENTS

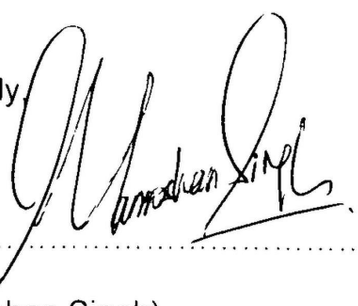
I would like to take this opportunity to thank my supervisor, Prof. Madya Dr. Baharin Azahari for his continuous support, guidance and supervision throughout the project.

I would also like to thank Mr. Zandar Md. Saman, Mr. Mohammad and Mr. Segaran for their excellent technical support during the course of this project as without their help, this project would not be very successful.

Pusat Pengajian Kejuruteraan Bahan dan Sumber Mineral (PPKBSM) is a nice place to work. I would like to thank everybody (too many to mention) for being helpful, encouraging and cheerful, making the work more enjoyable. For my dearest friends, Mr. Ramani, Abbas and Faizul and Mahathir, thank you for the wonderful nights having nasi lemak and teh tarik at Rias Nasi Kandar. I wish everyone all the best.

Finally, but not least, I would like to thank my bellowed father, mother, sisters and brother for their patience and support towards completion of this project.

Sincerely,



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(Manroshan Singh)

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LIST OF ABBREVIATIONS

AP	-	Antigenic protein
AR	-	Ammonia reduced
AR NRL	-	Ammonia reduced natural rubber latex
DI	-	De – Ionized
EP	-	Extractable protein
GLU	-	Glucose
HA	-	High ammonia
HA NRL	-	High ammonia natural rubber latex
M100	-	Modulus at 100% elongation
M300	-	Modulus at 300% elongation
M500	-	Modulus at 500% elongation
RIB	-	Ribose

KESAN TINDAK BALAS MAILLARD KEATAS SIFAT – SIFAT DAN KANDUNGAN PROTIN TEREKSTRAK FILEM – FILEM TUANGAN LATEKS GETAH ASLI

ABSTRAK

Lateks getah asli pada dasarnya digunakan dalam pelbagai aplikasi kerana ia mempunyai sifat –sifat yang amat baik tetapi disebabkan oleh masalah alergi protin, industri sarung tangan getah asli berada dalam dilema. Pelbagai kaedah telah digunakan untuk mengurangkan jumlah protin terekstrak dalam lateks getah asli. Walaupun berkesan, kaedah – kaedah ini tidak digemari disebabkan oleh kos tambahan bagi menghasilkan lateks tersebut. Dalam kajian ini, satu pendekatan lain untuk menanggani masalah protin lateks telah digunakan. Ini dilakukan melalui penyambung – silangan protin dalam lateks menggunakan gula penurun. Dua lateks berlainan (lateks berammonia tinggi dan lateks kurang ammonia) telah digunakan. Lateks tersebut dicampur dengan 5% gula penurun (glukosa dan ribosa) dalam kuantiti yang sama dan dipanaskan selama 1 dan 2 jam pada suhu 70, 80 dan 90 °C dalam kukus air. Tahap sambung – silang protin dalam lateks diukur melalui perubahan pada pH dan warna (nilai L) selama 10 hari. Filem – filem kemudiannya disediakan dengan menggunakan lateks yang telah bertindak balas melalui kaedah tuangan dan sifat – sifat mekanikal, kandungan protin terekstrak dan anitigenik filem – filem yang terhasil diukur. Keputusan yang diperolehi menunjukkan bahawa pH dan nilai L lateks menurun dengan peningkatan dalam masa pra – pemanasan, suhu pra – pemanasan dan kereaktifan gula. Filem – filem terubahsuai yang dihasilkan juga menunjukkan penurunan dalam indeks pembengkakan. Walaupun begitu, kandungan gel filem – filem tersebut meningkat. Tidak ada perubahan ketara dalam sifat mekanikal filem – filem diperhatikan sebelum pelarut – lelehan dilakukan tetapi selepas pelarut – lelehan dilakukan, peningkatan signifikan dalam sifat mekanikal filem – filem HA terubahsuai diperhatikan. Modulus filem – filem tersebut meningkat dua kali ganda dan kekuatan

hijau filem – filem juga meningkat enam kali ganda. Sedikit peningkatan dalam pemanjangan pada takat putus filem – filem juga diperhatian. Peningkatan dalam sifat mekanikal lateks AR terubahsuai pula hanya diperhatikan pada filem – filem AR NRL/RIB 5%. Kesan daripada tindak balas Maillard, kandungan protin terekstrak dan antigenik filem – filem terubahsuai meningkat lapan kali ganda apabila dibandingkan dengan filem – filem kawalan.

EFFECT OF MAILLARD REACTION ON THE PROPERTIES AND EXTRACTABLE PROTEIN CONTENT OF CAST NATURAL RUBBER LATEX FILMS

ABSTRACT

Natural rubber latex is basically used in many applications as it has excellent properties but due to the protein allergy problem, the natural rubber glove industry is currently in dilemma. Numerous methods have been used to reduce the amount of extractable proteins in natural rubber latex. Even though effective, these methods are not popular due to the additional cost of production of these latices. In this study, a different approach is used to tackle the latex protein problem. This is done by crosslinking the proteins in natural rubber latex with reducing sugars. Two different types of latex (high ammonia and ammonia reduced latices) were used. They were mixed with equivalent amount of 5% reducing sugar (glucose and ribose) and preheated for 1 and 2 hours at 70, 80 and 90 °C in a water bath. The extent of crosslinking of protein in the latex was monitored by measuring the pH and colour change (L value) over a period of 10 days Films were prepared from the reacted latex by casting and the properties, extractable and antigenic protein content of the resulting films were evaluated. Results showed that the pH and L values of the latices reduced with increasing preheating time, preheating temperature and sugar reactivity. The modified films produced also showed a reduction in the swelling index. On the other hand, the gel fraction of the films increased. There were no significant improvements in the mechanical properties of the modified films before leaching. However, after leaching, significant improvements in the mechanical properties of HA modified films were observed. The modulus of the films increased two times and the green strength increased six times when compared to similar unleached films. Slight improvements were also seen in the elongation at break of the films The improvements in mechanical properties in the AR modified films were however only significant in the AR NRL/RIB 5% films. As a result of Maillard reaction, the extractable

and antigenic protein contents of both HA and AR modified films were eight times higher compared to their respective controls.

CHAPTER 1
INTRODUCTION

1.1 Introduction

The latex industry has been established in Malaysia for a long time. Malaysia, being one of the main rubber producing countries, was ranked fourth after Thailand, Indonesia and India with a production of 615, 400 tonnes of natural rubber in the year 2000 (Statistics on Commodities, 2001). Currently, Malaysia is the top natural rubber latex glove producing country contributing 80% of world glove market. Malaysia also produces other latex products such as latex threads, catheters, adhesive tapes, elastic bandages, rubber pads, stethoscope tubing, condoms and balloons.

Currently, the latex industry, especially the natural rubber glove industry is in dilemma due to allergic reactions caused by proteins present in the latex. The problem affects a certain group of people and was first reported in 1979 and by 1988, 50 such cases were reported in the European Journal of Health.

Latex allergy which is caused by exposure towards the protein in the natural rubber gloves effects health workers (2.8 – 16.9%), *spina bifida* (multi – operated children) (32 – 50.6%), hairdressers and housekeepers (8 – 9%) and rubber industry workers (2 – 11%).

Many methods have been used to tackle the allergic problem which is caused by the protein in the gloves. One method in particular which has proven to give good results is the leaching method where the gloves are washed in flowing water to remove the protein particles which may be on the surface of the gloves. Other methods include substage and double – centrifugation of latex and deprotenisation by enzyme treatment. Even though these methods reduce the protein content, they do not totally

remove the proteins from the gloves or latex as the proteins will be regenerated due to degradation as heat is applied to dry the gloves.

Therefore, a different approach is required. One way which has been identified to overcome the problem is by crosslinking the proteins in the latex so that it forms a network structure strong enough to withstand the degradation of proteins through heating. The method is known as Maillard reaction and is used extensively in food technology. This method is explained in detail in this thesis.

1.2. Research Objectives

The aims of this research are:

1. To crosslink the proteins in natural rubber latex by using the Maillard reaction;
2. To study the effect of different combinations of reducing sugars on the effectiveness of protein crosslinking;
3. To study the effect of ammonia on the browning rate of latex;
4. To study the effect of Maillard reaction on the properties of natural rubber latex; and
5. To study the effect of Maillard reaction on the extractable and antigenic proteins in natural rubber latex.

CHAPTER 2
LITERATURE REVIEW

2.1 Overview

More than two thousand plant species, many of which are tropical, produce natural rubber (Cornish, 2001). Most, however do not produce the high molecular weight polymers required for high performance commercial products (Swanson *et al.*, 1979). The first type of latex used in industry was the natural rubber latex obtained from the *Hevea brasiliensis* species tree from the *Euphorbiaceae* family found in the tropical region in Amazon, South America.

The world production of natural rubber is about 6.7 million tons per year. Thailand, Indonesia and Malaysia, in 2004, remained the three leading world natural rubber producers. Thailand produced 2.9 million tons of natural rubber, Indonesia produced 2.1 million tons of natural rubber whereas Malaysia produced 1.2 million tons. The total combined production from these three countries constituted about 80 % of the world natural rubber production in 2004. Natural rubber consumption, on the other hand, was mainly in the tyre industry (70%) and the latex glove industry (5%).

Even though Malaysia is ranked third among the major natural rubber producing countries, it is currently the top producer of natural rubber latex gloves. According to the Malaysian Department of Statistics, Malaysia's export of natural rubber latex gloves was 12.2 billion pairs in the year 2002, 13 billion pairs in 2003 and 18.5 billion pairs in the year 2004. Natural rubber gloves are used in hospitals, dental practices, restaurants and in the food industry.

Recently, the natural rubber industry especially the natural rubber glove industry is in a dilemma due to the glove protein allergy problem which causes allergic reactions

towards a certain group of people. People highly at risk due to the allergy problem are the healthcare workers (2.8 – 16.9%), *spina bifida* or multi – operated children (32 – 50.6%), hairdressers and housekeepers (8 – 9.7%) and rubber industry workers (2 – 11%) (Esah Yip, 1997).

Numerous methods, such as modified formulations, single non – allergic accelerators and substage or double – centrifugation of latex (Aprem and Pal, 2002) have been used to reduce the amount of extractable proteins in natural rubber latex. One method currently gaining much attention is the leaching method. Gloves are washed in flowing water to wash the protein layer which maybe available on the glove surface. This method has been found to be ineffective in minimizing the percentage of protein. This is because when the gloves are vulcanized in the oven, the higher molecular weight proteins degrade into lower molecular weight proteins which then migrate to the surface of the gloves. The gloves are next washed (leached) in flowing water and again dried in the oven. The proteins attached to the latex particles will once again degrade into lower molecular weight proteins and the whole process repeats again and again (Manroshan, 2002).

Other methods used in order to reduce the allergy problem are substage and double – centrifugation of latex and deprotenisation of latex proteins by enzyme treatment. These methods are not popular due to the additional cost of production of these latices (Aprem and Pal, 2002).

In this project, a novel method is used to address the allergic problem. In this method, the proteins are crosslinked with sugar so that the proteins will not degrade or tend to form water soluble proteins. This method of crosslinking the protein is known as the Maillard reaction and is used extensively in the food industry.

2.2 Natural Rubber Latex

Natural rubber latex or *cis* – 1,4 – polyisoprene is obtained from the *Hevea brasiliensis* species. Primarily, due to its molecular structure and high molecular weight (M_r > 10^6 Dalton (Da)) (Cornish, 2001), natural rubber latex has high performance properties that cannot be matched by synthetic latices.

Freshly tapped natural rubber latex is known as field latex. The composition of field latex is as shown in Table 2.1. The substances present in fresh natural rubber latex are distributed in three phases; the rubber phase (35% m/m of the latex), the aqueous phase (55% m/m of the latex) and the lutoids phase (10% m/m of the latex).

Table 2.1: Typical composition of fresh natural rubber latex (Blackley, 1997)

Constituent	**Ratio (% m/m total latex)**
Total solids	36
Dry rubber	33
Proteinaceous substances	1 – 1.5
Resinous substances	1 – 2.5
Ash	Up to 1
Sugars	1
Water	The rest

2.2.1 The Rubber Phase

The first important microscopic study of natural rubber latex was reported by Hauser (1926). He concluded that rubber particles are predominantly pear – shaped and they consist of a tough and hard elastic shell which encloses viscous liquid. In certain cases the shape seems to be a clonal character. Pear shape is reported to be frequently encountered in clones such as Tjir 1 and PR 107 (Southorn, 1961). A typical composition for the rubber phase in fresh natural rubber latex is shown in Table 2.2. The rubber hydrocarbon in natural rubber latex is predominantly linear *cis* – 1, 4 – polyisoprene (Figure 2.1).

Table 2.2: Components in the rubber phase of fresh natural rubber latex (Blackley, 1997)

Component	**Proportion / % m/m on whole latex**
Rubber	30 – 40
Protein	1 – 1.5
Resin	1 – 2.5
Sugar	1
Ash	< 1
Water	55 – 60

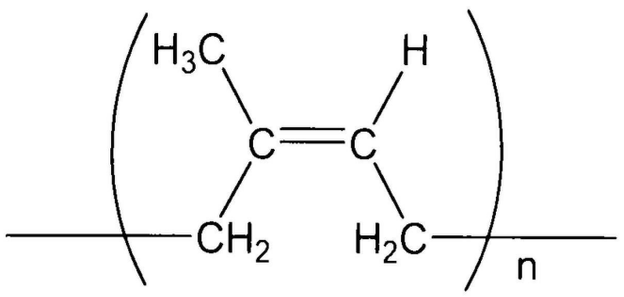

Figure 2.1: Typical latex, *cis* – 1, 4 polyisoprene structure

The outer regions of the surface of the particles in fresh natural rubber latex are believed to be proteinaceous in nature. It is this layer which determines the electric charge carried by the particles, the electrophoretic behavior of the particles and the behavior of the latex when it is colloidally destabilized (Figure 2.2).

Na – Ranong *et al.* (1995) in their study stated that the rubber particles are wrapped in a monomembrane made of negatively charged glycolipo – phosphoproteins. The negative charges allow stability of the latex colloidal suspension (Figure 2.2) whereas the membrane protects the *cis* – polyisoprene molecules against oxidative degradation.

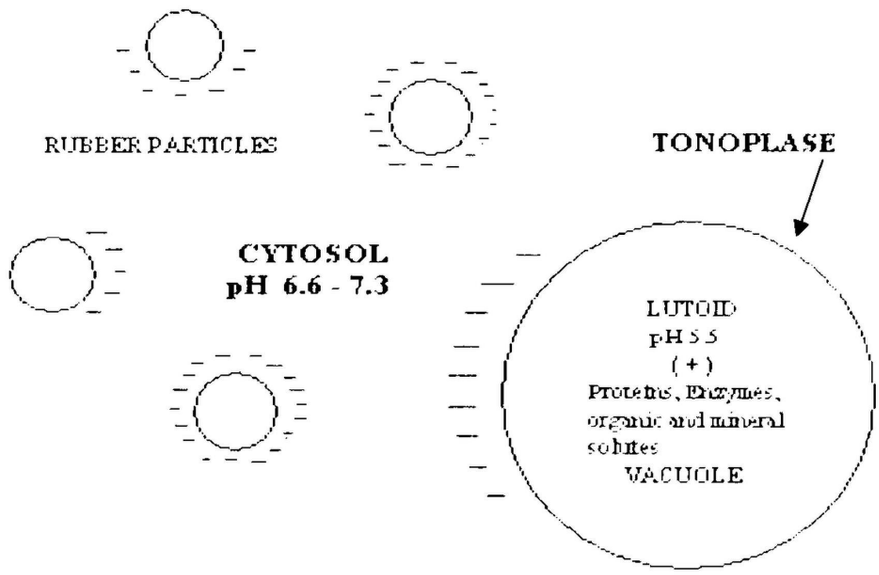

Figure 2.2: Latex as a colloidal suspension (d'Auzac, 1997)

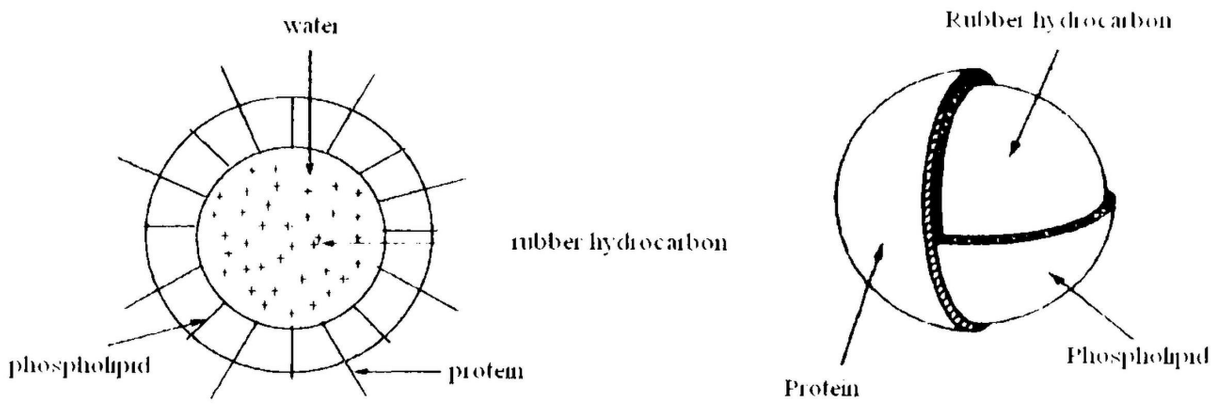

Figure 2.3: Presumed structure of natural rubber particle (Aprem and Pal, 2002)

The phospholipids are strongly adsorbed at the surface of the rubber particles. It has been suggested that they function as intermediaries by which the proteins are strongly anchored to the particle surface (Figure 2.3). The principal phospholipids associated with the particles in fresh natural rubber latex are of the lecithin type (Blackley, 1997). The chemical structure of lecithin is as shown in Figure 2.4 with R representing long chain hydrocarbon moieties.

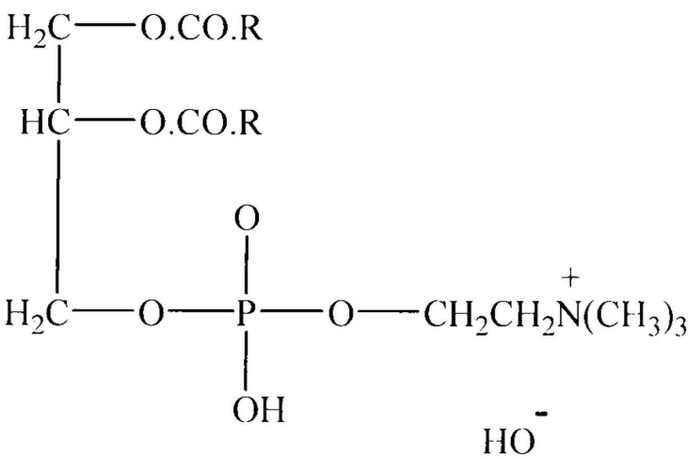

Figure 2.4: Lecithin structure (Blackley, 1997)

The presence of these long – chain R groups in a lecithin molecule makes it strongly surface – active and since they contain polar and permanently ionized sites, this makes the molecule strongly adsorbed at the rubber aqueous phase. The association between lecithin and protein occurs at the particle surface as a consequence of the lecithin molecule carrying a slight overall positive charge whereas the proteins are negatively charged. This was discovered by Du Pont *et al.* in 1976 when they confirmed the presence of phosphatidylcholine and small amounts of phosphatidyl ethanolamine in the lipids associated with rubber particles.

2.2.2 The Aqueous Phase

The aqueous phase of fresh natural rubber latex, which is sometimes referred to as C - serum is a dilute aqueous solution with a density slightly over 1.0 g / cm^3. The serum contains water and various non – rubber solids. Nitrogenous solids include 23.4% ammonium salts and 23.6% proteins and amino acids. The sugars include quebrachitol (32.7%), glucose (4.2%), fructose (2%) and galactose (1%) (Aprem and Pal, 2002). Sugars have little influence upon the properties of latex in which it contains. They become microbiologically oxidized to the so – called volatile fatty acids in the absence of adequate preservation.

The aqueous phase of fresh natural rubber latex also contains several proteins of differing isoelectric points. The principal proteins are known as α – globulin and hevein. α – globulin is a surface – active protein and is insoluble in distilled water but soluble in neutral salt solutions, acid solutions and alkaline solutions. It has an isoelectric point at pH 4.8 which is close to that for the particles of fresh natural rubber latex. Therefore, α – globulin is an important component of the protein layer which is bound to the surface of the rubber particles and is responsible for the colloidal stability (Aprem and Pal, 2002). However, it is denatured by heating and by storage in the dry state.

Hevein, on the other hand, has its isoelectric point at pH 4.5. It contains 5 % of sulphur as cysteine – type linkages. Even though it displays little surface – activity, it is soluble in water at all pH values and is not precipitated from water by boiling.

Several other proteins are present in the aqueous phase of fresh natural rubber latex besides α – globulin and hevein. Roe and Ewart (1942) in their studies of the aqueous phase of fresh natural rubber latex using electrophoretic analysis reported that there are at least seven distinct protein components having various isoelectric points.

Various polypeptides and simple amino acids have also been observed in the aqueous phase of fresh natural rubber latex, but whether these are the precursors or the degradation products of the latex proteins is not clear. The free amino acids that have been identified in the aqueous phase of fresh natural rubber latex until 1960 are shown in Table 2.3.

Table 2.3: Free amino acids that have been identified in the aqueous phase of fresh natural rubber latex (Blackley, 1997)

Amino acid	McGavack and Rumbold (1934)	Altman (1948)	Whitby and Greenberg (1941)	Drake (1951)	Ng (1960)
Glycine	No	Yes	No	Yes	Yes
Alanine	Yes	Yes	No	Yes	Yes
Valine	No	Yes	Yes	No	Trace
Leucine	No	Yes	Yes	Yes	Yes
Isoleusine	No	Yes	Yes	Yes	Yes
Phenylanaline	Yes	Yes	Yes	No	Yes
Tyrosine	No	Yes	Yes	No	Yes
Aspartic acid	No	No	Yes	Yes	Yes
Glutamic acid	No	Yes	No	Yes	Yes
Arganine	No	No	Yes	No	Trace
Proline	No	Yes	Yes	No	Trace
Lysine	No	No	No	No	Yes
Cysteine	No	Yes	No	No	Yes
Serine	No	No	No	Yes	Trace
Tryptophan	No	No	No	No	Yes
Theorine	No	No	No	No	Trace

2.2.3 Lutoids Phase

Lutoids or viscoids were first reported to be present in fresh natural rubber latex by Homans and Van Gils (1948). These workers centrifuged undiluted fresh latex at low speeds, thereby causing the latex to separate into two fractions; a white fraction which contained most of the rubber particles, and a bottom yellow fraction. At the boundary between the two fractions, a thin vivid yellow fraction comprised of ill – defined aggregates, known as lutoids were observed.

Chemically, lutoids appear to be mainly water. They contain small quantities of soluble protein (3% m/m), insoluble proteins (2% m/m) and phospholipids (0.5% m/m). Lutoids are very labile, therefore disappearing when the latex is ammoniated.

According to Dickenson (1965), lutoids contain dissolved substances, mainly fibrils of proteinaceous nature suspended in it. These fibrillar contents disappear as the latex particles age since they seem to be fairly specific to the latex obtained from young vessels.

The structures of lutoid particles have been studied in detail by using phase contrast microscopy and application of suitable staining procedures. Mainly two types of fibrillar structures have been described. The first type, known as microfibrils, is characteristic of latex vessels in young tissues (Dickenson, 1965, 1969; Audley, 1965, 1966). The microfibrils are freely suspended in the fluid content of the lutoid B-serum even though they are usually seen as grouped together in bundles. The second type of fibrillar structures, observed in lutoids of latex from mature bark of stimulated trees, are known as 'microhelices' (Gomez and Yip, 1975), so named because of their spring like shape. They are occasionally found in unstimulated trees and their number increases on dilution.

2.3 Natural Rubber Latex Concentration and Stabilization by Ammoniation

Immediately after natural rubber latex is tapped and collected from the trees, 0.2% ammonia solution is added to the latex as a temporary preservative before transporting it to the factory. The purpose of adding ammonia is to prevent the latex from coagulating. The coagulation process, also known as spontaneous coagulation is due to the hydrolysis of various lipid substances present in the latex which liberates fatty acid anions (Blackley, 1997). The anions which consist of formic, acetic and

propionic types are formed by the action of microorganisms, especially bacteria upon certain carbohydrates which are present in the aqueous phase of the latex. Therefore, the addition of ammonia retards the formation of fatty acids. At the factory, ammonia is further added to the latex to make up to 0.7% before concentrating it to 60% total solid content.

Among the four methods of concentration (creaming, centrifugation, evaporation and electrodecantation), centrifugation is the most popular concentration method. It is a rapid process as it utilizes centrifugal force to separate the rubber particles from the aqueous phase.

The concentrated natural rubber latex is then used or stored for a period of time before being utilized. During storage, a considerable change in the chemical constitution of natural rubber latex occurs. The change, which is an increase in the colloidal stability of the latex, is a direct effect of ammoniation on some of the non – rubbers present in the latex. Proteins are hydrolyzed to polypeptides and amino acids while the phospholipids are hydrolyzed to various substances such as glycerol, long chain carboxylate anions, phosphate anions and organic bases. The hydrolyzed products are next adsorbed at the particle surfaces, thereby enhancing their stability (Blackley, 1997).

2.4 Production of Latex Goods

Conversion of concentrated liquid natural rubber latex into finished natural rubber latex goods, such as surgical gloves, is complicated, variable, and, in part, a proprietary process. In general, the natural rubber latex is prepared for dipping by addition of a potpourri of chemicals necessary for processing and to ensure the desired physical properties in the finished product. The addition of accelerators and antioxidants, follows. In addition, emulsifiers, stabilizers, extenders, colorants,

retarders, stiffeners, biocides, ultraviolet light absorbers, and fragrances are also added depending on the desired product. The chemicals are compounded and the natural rubber latex is next pumped to a dipping machine.

Typically, gloves are manufactured on glove – shaped porcelain formers mounted on a continuous chain mechanism that moves through a series of processing step stations. The majority of gloves are made by a single – dipped process that requires the pretreatment of the former with a coagulant. The coagulant makes the uniform deposition of a liquid natural rubber latex film on the porcelain former possible. Pre – treatment of formers with a releasing agent can be accomplished simultaneously with the coagulant. The coagulation is completed by passing through ovens. Bead rolls are next created by automatically rolling down the proximal edge of the coagulated film. The former then passes through several warm baths to extract the leachable processing chemicals and water – soluble proteins.

Factors such as the length of time in leaching tanks, rate of water exchange, and water temperature are crucial variables that influence the degree of chemical removal. This is followed by crosslinking the *cis* – 1,4 – polyisoprene chains to one another with sulphur in a heat – catalyzed process known as vulcanization. Completeness and speed of vulcanization is dependent on the choice and concentration of chemical accelerators added during compounding.

Cornstarch powder is then applied to the exposed glove surface either in a wet emulsion dip or as a dry aerosolized powder. Removal from the former inverts the glove. Vulcanization and powder distribution are completed by applying heat during a cycle in an industry rotating dryer. To convert these products to powder free, a chlorination wash is necessary. An added benefit to chlorination is the additional

reduction of water – soluble protein. Chlorination also has a detrimental impact on the aging and physical properties of natural rubber latex products.

2.5 Effect of Non – Rubbers on Natural Rubber Latex Vulcanization

In natural rubber latex, the extent or rate of crosslinking is affected by the time taken for sulphurating agents to be formed by the reaction of the vulcanizing ingredients. The rate for these reactions to take place depends on the type of chemicals and the reaction temperature involved.

In contrast to dry rubber, the polar medium induced by the non – rubbers in the latex serum can accelerate the vulcanization reaction in the latex. Chong (1977), demonstrated this when he compared the crosslinking efficiency between the dry rubber that was vulcanized in a heat mould and cast latex films that were heated in the oven. From his results, Chong suggested that the active sulphurating agents can diffuse rapidly into the rubber particles to form crosslinks with the rubber hydrocarbons; therefore expecting the polar nature of the aqueous phase to accelerate such reactions.

Loh (1982) compared the crosslinking efficiency of similarly compounded synthetic polyisoprene latex and natural rubber latex and found that the rate of crosslinking was slower in synthetic latex than natural rubber latex. The difference in the rate between these latices of similar hydrocarbon structure was attributed to the natural non – rubber materials content of the natural rubber latex. It was also found that the films obtained from synthetic latex exhibited lower modulus and tensile strength values than natural rubber films.

Due to the less altered non – rubbers in natural rubber latex films, the non – rubbers are believed to activate both the rate of vulcanization as well as increasing the

crosslinking efficiency by increasing the stability of the sulphur crosslinking complexes in the rubber hydrocarbon.

In highly purified natural rubber latex where some of the non – rubbers are removed from the latex mix by surfactant treatment and purification by multiple centrifugation, the vulcanization rates are reduced and films obtained from the vulcanized mixture appear softer, cleaner and tackier from unpurified mix (Gorton, 1977). Furthermore, the physical and processing behavior appears to approach synthetic latex properties. This again demonstrates the unique role of natural rubber non – rubbers as vulcanizing activators in latex vulcanization.

2.6 Reinforcement of the Rubber Matrix in Natural Rubber Latex Film

The non – rubbers in natural rubber latex vulcanizates have been shown to be responsible for reinforcement of the rubber matrix (Chong, 1977). The reinforcing structure in latex films is believed to consist of hard domains of protein molecules held together by hydrogen bonds. These domains arise during the evaporation of water by agglomeration of the proteins present on the surface of each individual rubber particle of the latex. However, the effect diminishes when latex film is treated with alkaline, swollen in water or pre – stretched (Chong, 1977).

The reinforcing effect of these non – rubbers seem to diminish in purified dry natural rubber. Purified natural rubber is prepared from multiple centrifuge enzyme – treated natural rubber latex. The purpose of the enzyme treatment is to denature or breakdown the proteins and the multiple centrifuging is to remove these denatured proteins as well as other non – rubbers. It was found that by removing these non – rubbers, the stiffness of the dry natural rubber was reduced compared to normal dry natural rubber vulcanizates (Knight and Tan, 1975; Metherell, 1980)

2.7 Latex Allergy

According to the Oxford dictionary, allergy is defined as an adverse reaction by the body to a substance to which it has become hypersensitive. Therefore, latex allergy could be termed as an allergy that develops after some sensitizing contact with latex. Hypersensitivity is usually induced by rubber products such as rubber gloves, condoms, catheters, dental dams, balloons and sporting equipment.

Latex allergy involves more than one latex allergen. At least six distinct allergens have been found to bind to IgE, which is involved in allergic reactions. The allergic reactions are divided into two types, Type I and Type IV. While Type IV hypersensitivity has been known for many years, that of Type I emerged only in the late eighties (Nutter, 1979).

Type I allergy is sometimes referred to as immediate hypersensitivity because the response on exposure to the allergen is fast. Type I allergic reaction is due to the water – soluble low molecular weight protein (LTP) which is found in natural rubber latex only. Individuals with Type I allergy to latex goods are in a state of hypersensitivity brought about repeated contact with latex proteins and have antibodies to these proteins in their blood. At some point, further exposure to these protein antigens (allergens) can cause immediate response such as contact urticaria or anaphylactic shock.

Contact urticaria or hives, is an eczematous reaction characterized by the formation of wheals or flares at the contact site, with itching or stinging and the effect usually disappears within 0.5 to 2 hours. In the case of anaphylactic shock, the state of hypersensitivity is known as anaphylaxis. The shock reaction is characterized by a severe fall in blood pressure, difficulty in breathing, speeding heart rate and unconsciousness. Some other effects seen are conjunctivitis, rhinitis, and local or

general urticaria. The effects are directly due to the release of histamine and other substances from cells of the immune system affected by the interaction of the allergens and their antibodies.

Type IV allergy or classical dermatitis is an allergic reaction due to the chemical additives such as accelerators and antioxidants used in the manufacture of latex products such as gloves. Symptoms of Type IV allergy includes rash, usually of eczemas or keratonic form, confined to the skin. The reaction is a relatively slow response with irritation appearing 48 hours after exposure.

Healthcare workers, *spina bifida* or multi – operated children, hairdressers, housekeepers and rubber industry workers are the groups of people at risk to the latex allergy. (Esah Yip, 1997). Since these groups of people have already been sensitized, the only way to avoid further allergic reactions would be by avoiding further contact with natural rubber latex. For the rest of the population, threshold levels for sensitization is not known, but it is possible to obtain indications on extractable protein levels of low risk by identifying levels at which great number of latex hypersensitive individuals do not react.

The Rubber Research Institute of Malaysia with collaboration with Dr. K. Turjanmaa of the Department of Dermatology, Tampere University of Finland, have shown that when a group of latex hypersensitive subjects were skin tested with latex gloves at a varying content of extractable proteins, about 60 % of them indicated no allergic response at levels less than 400 µg/g. Up to 100 % of negative responses were observed at extractable protein lower than 100 µg/g in the study (Esah Yip *et al.*, 1995).

The Food and Drugs Administration (FDA) of the USA has allowed 'Low Protein Labeling' claim for the 510 (K) submission by glove manufacturer's since March 1995.

However, no maximum extractable protein levels have yet been specified, although claim on label below 50 μg/g is the sensitivity limit of the ASTM modified Lowry method.

2.8 Production of Latex with Low Extractable Protein Content

There is little information available about the duration of exposure to and the amount of EP required to bring about sensitization. On the other hand, the amount of EP required to trigger off an allergic response in sensitized subjects can be very small (Kelly *et al.*, 1993) and it is probably not practical and economical to produce latex goods with such low levels. However there is a general agreement that to manufacture goods with low EP is highly desirable, especially to prevent more people acquiring the allergy. The need for producing low protein latex lies in the following facts:

- To prevent latex products of excessively high protein content getting into the market and then sensitizing further individuals;
- To ensure that medical devices are safe for use;
- Gloves of low protein content are likely to give low allergic response.

In general, the following methods are recommended to reduce extractable protein levels:

- Reduce protein levels in raw latex;
- Modify formulation;
- Use single non-allergic accelerators;
- More leaching/washing of latex film
- Smaller batch of product to allow for adequate treatment.

2.9 Minimization of Protein Levels

Extractable protein (EP) in natural rubber latex products can be minimized at two stages. The first stage is the latex stage itself, in which the raw material is treated to reduce the amount of proteins. Substage and double – centrifugation or deproteinisation by enzyme treatment onto the latex is usually done in this stage.

The second stage is the product stage in which the latex products are subjected to washing off in order to reduce the protein content. The processes involved in this stage include leaching, slurry dip and chlorination.

2.9.1 Latex Stage

2.9.1.1 Substage and Double – Centrifuged Latex

There are several methods available for concentration of field latex but centrifugation is the most important among them. Reduction of the soluble protein content of ammoniated concentrates can be done by initial dilution of centrifuged latex and a further centrifugation known as substage double – centrifugation. The EP content obtained by Subramaniam *et al.* (1993) for unleached gloves produced using single and double centrifuged HA prevulcanized latex is shown in Table 2.4. Even though the EP content is reduced significantly as the centrifugation is repeated, there does not seem to be any advantages in using the latices prepared by this process as there could be additional increase in the cost of manufacturing.

Table 2.4: Extractable Protein (EP) Content of Unleached Gloves Made from Different Prevulcanized Latices (Subramaniam *et al.*, 1993)

Latex Type	Extractable Protein (mg/g)
HA PV	1.146
HADC PV	0.324
RC HA PV	0.282
RC HADC PV	0.169

HA PV = *Single – centrifuged high ammonia pre – vulcanized latex;*
HADC PV = *Double – centrifuged high ammonia pre – vulcanized latex;*
RC HA PV = *Recentrifuged HA PV;*
RC HADC PV = *Recentrifuged HADC PV.*

2.9.1.2 Deproteinisation by Enzyme Treatment

Studies on deproteinisation were started in the mid – twentieth century. Attempts were made to deproteinise *Hevea* latex (Morris, 1954; Nadarajah *et al.*, 1973 and Ong, 1974) using substantial number of proteolytic enzymes such as ficin, trysin, bromelan and papain. Certain drawbacks were reported for the enzymatic method of deprotenisation. They were:

- Enzymatic hydrolysis of proteins under normal conditions removes only about 40 – 50% of the latex proteins.
- Enzymes like superase and BPN (Bacterial Protein Novo) have broad specificity. Therefore, the type of peptide linkage attacked can differ. Thus, deproteinised rubber obtained by this process may not show consistent behavior.
- Deproteinised rubber with low nitrogen content can be obtained by starting with skimmed concentrated latex while treating with enzyme and surfactants. In this manner, the deproteinised rubber becomes uneconomical.

New developments in enzyme treated natural rubber latex had been made by Peralla (2001). In her study Peralla showed that the enzymatic process reduced the size of stainable proteins from the 10 kDa to 200 kDa range to 10 kDa or less.

Table 2.5: Residual antigenic protein content of natural rubber and gloves (Peralla, 2001)

Test Method	RAST		ELISA	Lowry
	(µg/ml)	(µg/g)	(µg/g)	(µg/g)
Sample	**NRL**	**Gloves**	**Gloves**	**Gloves**
Untreated control (a)	3310.0 ± 680.1	364.6 ± 129.6	211.2 ± 43.9	89.0 ± 30.6
Enzyme treated Expt 1 (b1)	330 ± 50.5 (n = 5)	13.4 ± 9.8 (n = 5)	0.8 ± 0.8 (n = 5)	101.6 ± 13.8 (n = 5)
Expt 2 (b2)	-	5.8 ± 3.6 (n = 12)	1.0 ± 0.7 (n = 24)	-
% reduction (100 x (b/a))	> 99	> 96	> 99	- 14

Peralla's studies indicated that enzyme treated natural rubber latex was less immunogenic than control natural rubber latex and the proteolytic digestion of natural rubber latex associated proteins produced less allergic natural rubber latex. The Lowry assay, however, was inaccurate as it overestimated the amount of proteins in natural rubber latex due to the interference of chemicals present in the latex. Furthermore, the Lowry assay could not distinguish between the intact and digested protein fragments, making it difficult to evaluate the significance of enzyme treated natural rubber latex with respect to allergenicity.

Nevertheless, enzymes so far are not an answer to the latex protein problem. Controlling enzymatic reactions, maintaining consistency of product and enveloping enough historical data still remains as the major obstacle.

An enzymically produced low protein latex (Loprol) concentrate, had been developed at the Rubber Research Institute of Malaysia. Examination and surgical gloves with satisfactory properties were made from it (Ghazaly, 1993). However, it seemed unlikely for a variety of reasons (added cost, requirement for extra centrifuging capacity) that Loprol, or similar latices which are commercially available, will become major materials for the production of gloves and other high volume articles.

2.9.1.3 Irradiation on Deproteinisation

Natural rubber latex could be prevulcanized using γ – radiation. Since γ – radiation causes denaturation of latex proteins, it was expected that irradiated latex might not cause allergic reactions. However, the irradiated natural rubber latex showed moderate allergic responses as evident from PCA (passive cutaneous anaphylaxis) test in mice (Tsuchiya *et al.*, 1992). The extractable protein content of natural rubber latex also increased with radiation dose (Aprem and Pal, 2002). As mentioned before, 25% of the proteins, including enzyme required for rubber biosynthesis, are bound to the latex particle surface. This particle bound, high molecular weight proteins may undergo disintegration during irradiation, which would result in low molecular weight proteins.

The radiation – induced solubility of latex proteins might be the cause for the higher extractable protein in the serum phase of the latex after irradiation. Processing at lower radiation doses (< 160 kGy) is advisable as higher doses of radiation have been found to affect the physical properties adversely due to the breaking down of polymer chains.

2.9.2 Product Stage

2.9.2.1 Leaching Process

Leaching is the removal of hydrophilic materials from latex dipped products by washing them in water. It is an essential process in the production of latex dipped products. The removal of excess calcium nitrate and water soluble non – rubber materials such as proteins and added compounding ingredients results in improvement of physical properties such as tensile strength and film clarity, provocation of surface blooms and reduction in water of latex dipped products. Therefore, the leaching process is critical in determination of the overall quality of gloves produced.

There are basically two methods of leaching; wet – gel leaching and dry – film leaching. The wet – gel leaching involves the washing of the wet – gel, prior to drying and vulcanization. Wet – gel leaching is usually carried out on – line. In contrast, dry – film leaching consists washing of the dried, vulcanized latex product after removal from the former and is an off – line process.

In the production of latex examination gloves, wet gel leaching is often carried out for a period of several minutes, usually 1 – 10 minutes, in a continuous chain dipping line. The actual leaching time is very much dependent upon the design of the dipping unit. It was previously established that a substantial amount of water – soluble proteins are generated upon drying and vulcanization of dipped product (Amir – Hashim, 1993) and that the proteins are drawn towards the surface away from the former during this stage, giving rise to asymmetry of extractable protein distribution (Yeang *et al.*, 1993). Any form of leaching or washing, including slurry dip, after drying is therefore expected to further remove the extractable proteins (Figure 2.5 and Figure 2.6).

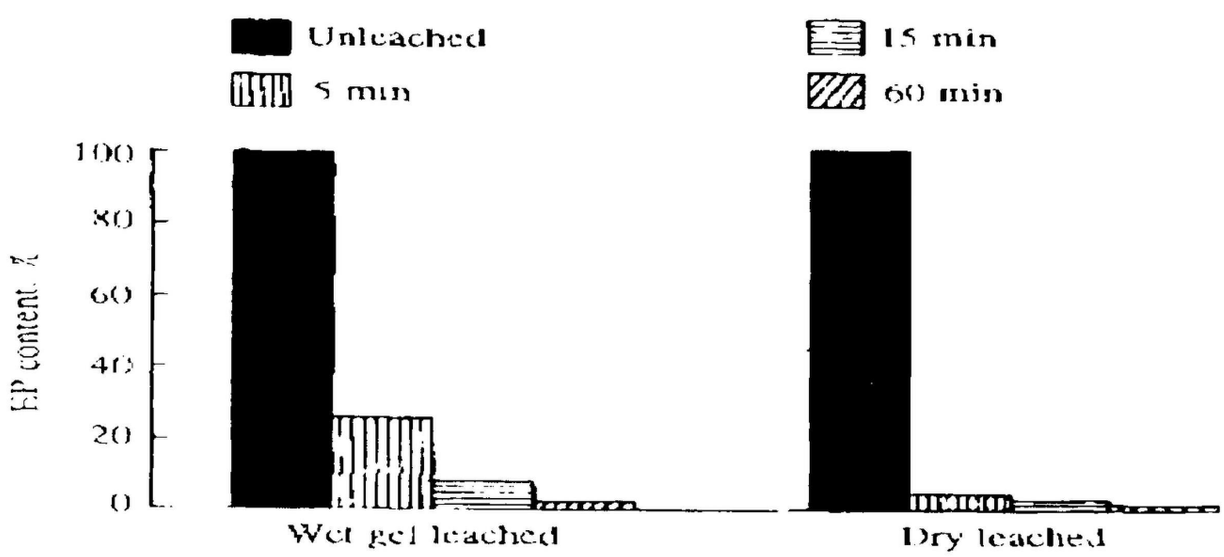

Figure 2.5: Effect of wet and dry leaching of dipped films from pre – vulcanized mix. (Amir – Hashim, 1993)

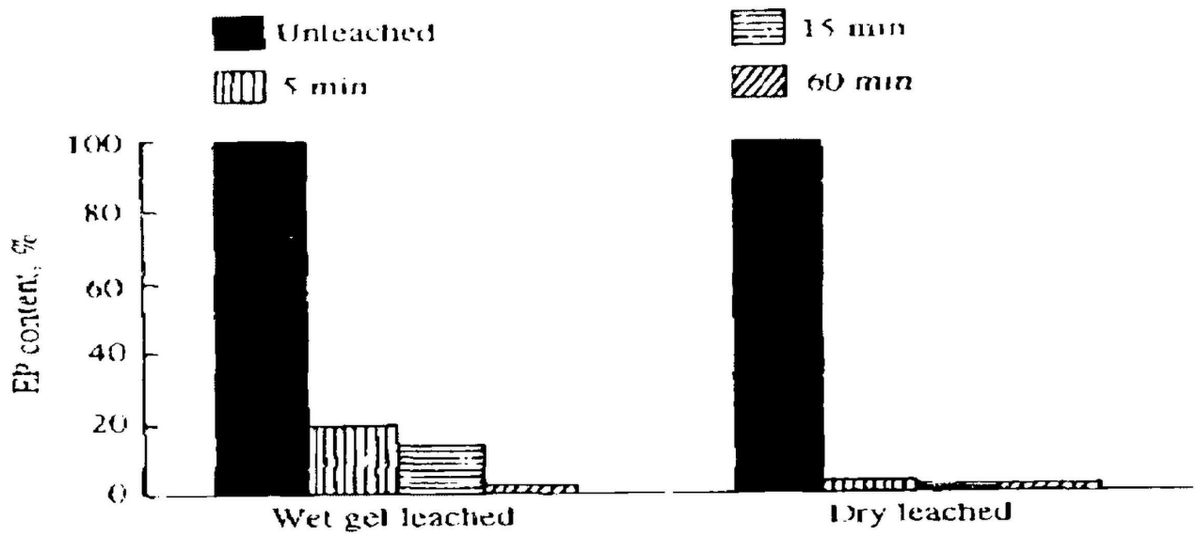

Figure 2.6: Effect of wet and dry leaching of post – vulcanized dipped latex films. (Amir – Hashim, 1993)

2.9.2.2 Use of Steam

It has been found that a combination of washing and autoclaving in steam is very effective in reducing both EP and responses to skin tests in sensitized subjects (Leynadier *et al.*, 1991). Other work has demonstrated that EP can be generally reduced under less severe conditions of autoclaving, but no dermatological test was carried out (Yeang *et al.*, 1996). Steam autoclaving can affect physical properties of the products but this can be guarded against by appropriate formulation. It does not seem likely that autoclaving can be used on a large scale to produce articles of low allergenicity.

2.9.2.3 Post Washing and Chlorination

The washing and surface treatment of natural rubber latex gloves with chlorine ions is an effective means of reducing the extractable protein content on the glove surface (Truscott, 1992; Aziz, 1993; Lai and Ng, 1995 and Subramaniam *et al.*, 1993) (Table 2.6).