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NATURAL RUBBER LATEX PROTEINS AND THEIR ALLERGY

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SUMMARY

Natural rubber latex obtained from *Hevea brasiliensis* contains 5–6% non – rubber substances out of which 2% is proteins. Latex protein allergy (Type I - Hypersensitivity) is mainly associated with only latex based products (mainly dipped products). Latex protein allergy is caused in sensitized persons due to non removal of extractable proteins in latex based products. Total extractable protein measurements of latex products are vital to ascertain the allerginicity of latex products. Extractable proteins in latex products are analyzed mainly using colourmetric methods. In order to minimize this allergy caused by latex products, it is important to remove as much extractable proteins as possible since even a minute quantity can cause allergic reactions in some sensitized persons. Proper leaching of latex products is the easiest and well known process for removing proteins from latex products. It is reported that techniques such as chlorination and polymer coating of latex gloves developed for the manufacture of powder free gloves have lowered the extractable proteins to extremely low levels. Radiation vulcanized natural rubber latex (RVNRL) could also be used to manufacture of extractable protein free gloves. However manufacture of latex gloves from RVNRL has not yet been commercialized mainly due to high investment cost.

INTRODUCTION

Natural rubber latex (NRL) is obtained by wounding the bark of *Hevea brasiliensis* which is known as tapping. NRL is a milky fluid comprising 25-35% rubber hydrocarbon in small particles suspended in a serum together with 5-6% non-rubber substances such as proteins, lipids, carbohydrates, sugars and some metal ions. The remaining major component is water.

Natural rubber latex collected from the tree is converted into two main types of raw materials namely centrifuged latex and dry rubber. Centrifuged latex is manufactured by centrifuging field latex in order to increase the dry rubber content to 60%. Centrifuged latex is the main raw material for the manufacture of all latex based products such as gloves, balloons, condoms, catheters, latex foam and latex thread. Dry rubber is manufactured by coagulation of NRL with weak acids. Coagulated rubber then converted into Crepe Rubber, Ribbed Smoked Sheets (RSS) or Technically Specified Rubber (TSR). Dry rubber is the main raw material for the manufacture of all dry rubber products such as tyres, tubings, hoses, footwear, rubber bands, bridge bearings, engineering parts, adhesive and household appliances.

Latex allergy (specially protein allergy) is mainly associated with only latex based products (mainly dipped-products). Latex allergy in dipped products particularly gloves, condoms and catheters is mainly attributed due to non-removal of extractable proteins and the residual chemicals. However latex protein allergy problem is hardly reported from the products made out of dry rubber. Dry raw rubber manufacturing process includes extensive milling and washing and hence most of the extractable proteins are removed during this process.

As shown in figure 1 natural rubber production in the country has been declined dramatically from the year 1996. However domestic consumption of centrifuged latex is regularly increased and recorded as 23,000 tonnes for the year 2000 (Plantation Sector Statistical Pocket Book, 2000). High domestic consumption of centrifuged latex indicates that latex based products manufacturing sector is growing rapidly. Latex gloves are the main category of dipped products that are produced in the country. According to the predictions of glove manufacturers 25,000 tonnes of rubber latex will be converted into dipped products by the year 2005. These figures give a clear indication that glove manufacturing industry will be expanded rapidly in the country. However latex protein allergy is one of the major threats faced by latex glove producers since Europe and USA are largely concerned with the above allergy associated problems. This paper discusses, what latex allergy is, types of allergies and methods for analysis of latex proteins. This paper also discusses the methods available to reduce the latex proteins from latex products in order to minimize latex protein allergy.

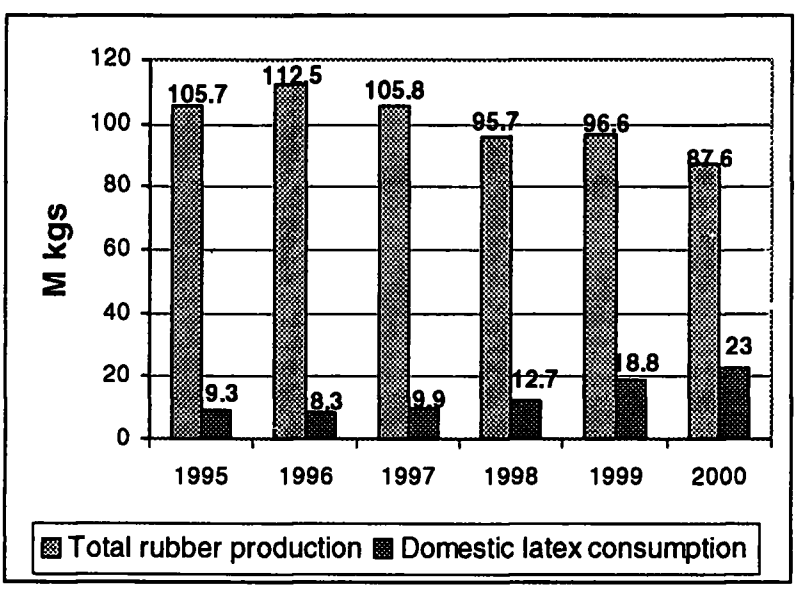

Fig. 1. Total rubber Production and domestic latex consumption

What is allergic reaction

An Austrian doctor, Clemens Von Pirquet devised the word "allergy" in 1905. He discovered that the human body sometimes treats harmless substances in an unusual way. Normally the immune system ignores these harmless substances, but in allergic people it reacts to them producing antibodies. Antibodies are proteins which protect the body from harmful invaders. However antibodies, which are produced in allergic reactions, cause distressing symptoms and harm the body rather than protecting it. Substances, which may cause allergic reactions, are known as allergens. A substance that is an allergen for one person may not be an allergen for another.

Latex allergy

Dipped products are manufactured by adding different types of chemicals into concentrated latex and a former is dipped into it and taken out and then dried and vulcanized. Latex allergy can occur due to residual extractable proteins remaining in the products as well as due to the effect of chemicals added for vulcanization. However corn-starch which is added to the glove surface to prevent the stickiness, itself is not an allergen. But corn-starch particles absorbed by extractable proteins in latex gloves become airborne and inhalation of such contaminated starch particles may cause sensitization. There are three types of adverse reactions associated with latex based products (*i.e.* mainly latex gloves) namely Irritant contact dermatitis (non-allergic), Type IV allergic reaction and Type I allergic reaction. Table 1 gives the three types of reactions their symptoms and the cause for each reaction (Long O E *et al.*, 1998).

Table 1. *Types of latex allergy*

Reaction type	Symptoms	Cause
Irritant contact dermatitis (non-allergic)	skin rash, dry flaky skin with papules, cracks and sores.	powder, temperature and pH extremes, incomplete hand rinsing after wearing
Type IV – chemical Hypersensitivity (Cell mediated allergy)	Eczema, appears at 48-96 hours post exposure by skin contact.	Residues of chemicals used for processing of latex products particularly the thiurams and carbamates.
Type I – Latex protein Hypersensitivity (IgE mediated allergy)	Immediate localized itching, burning or discomfort, urticaria (hives) within 5 to 60 minutes, rhinitis, asthma and in Very serious case, anaphylaxis (happens only rarely)	Residual extractable proteins found in natural rubber latex

However only Type I and Type IV hypersensitivity are considered as allergic reactions. While the irritant contact dermatitis and Type IV hypersensitivity have been known for many years, that of Type I hypersensitivity emerged only in the late eighties. It is important to point out that none of the above reactions is caused purely by the rubber hydrocarbon itself.

Latex protein allergy (Type - I Hypersensitivity)

This type I hypersensitive is attributed due to the residual extractable proteins found in natural rubber latex products (*i.e.* gloves, condoms, catheters). Although latex products have been used in large scale for more than thirty years, this latex protein allergy has been detected recently. Various hypothesis have been suggested for this sudden appearance of protein allergy, including electric charges in the protein produced by *Hevea brasiliensis*, changes in latex processing or products manufacturing, increase in the usage of gloves, improved methods for the diagnosis of protein allergic reaction *etc.* However none of these hypotheses fully explain the global out break of latex protein allergy. The actual explanation for the out break of protein allergy is likely multifactoral (Beezhold, 1995).

Protein allergic reactions are caused when latex proteins interact with specific IgE antibodies in blood sera of latex sensitized individuals. Symptoms of protein allergy are normally appeared immediately and some of them show within minute's exposure to the allergens. However this allergic reaction is not only specific to NR latex products but also to some individuals by penicillin, other antibiotics and some foods such as fruits and peanuts.

Residual extractable proteins in latex based products:

Natural rubber latex contains about 1-2% of total proteins out of which 25% is associated with rubber particles. The remaining 75% of the proteins are in the serum and they are water-soluble. When NR latex is converted into centrifuged latex considerable amount of these soluble proteins are removed with the skim latex. Further, in the process of conversion of centrifuged latex into latex products, more soluble proteins are removed during leaching and washing steps, so that the remaining level in the end product is very low.

This small amount of residual proteins in latex products is the main reason for protein allergy caused to some sensitized persons. However not all residual extractable proteins cause allergic reactions. There are about nine proteins having different molecular weight in latex products have been identified as potential allergens.

However, amount of residual extractable proteins in latex products may vary, depending on the manufacturing process. For example, gloves that have been subjected to more thorough leaching during the process will have less extractable proteins compared to gloves that have not been leached properly. Therefore analysis of extractable proteins in latex products is very important in latex protein allergy studies.

Analysis of extractable proteins

Following three types of identifications and measurements are currently being adopted in latex protein allergy studies of latex products.

i. Measurement of total extractable proteins.
ii. Identification of extractable proteins.
iii. Measurement of allergenicity.

Measurement of total extractable proteins

Following colourimetric and chromatographic techniques are commonly used in the determination of extractable proteins in latex products.

(a). Colourimetric measurements.

i. Modified lowry method.
ii. Bradford dye method.
iii. Bicinchonic acid (BCA) method.

All these methods are involved with developing a colour by reacting extractable proteins with a suitable reagent and the absorbance of the coloured complex produced is measured at a specific wave length using a Standard Colourimetric apparatus.

(b). Chromatographic measurement.

i. RRIM SE-HPLC method.
ii. Amino acid analysis by HPLC.

However, there is no common standard reference for all these test methods. As such, extractable protein values obtained from above methods for a given sample may vary from each of the test. Hence, it is important to mention the test method adopted along with the measured protein value for a particular sample.

Identification of extractable proteins

NR latex contains different types of proteins having different molecular weights and all these proteins are not responsible for protein allergy. Therefore it is important to identify each type of protein and which causes allergic problem.

Sodium dodecyl sulfate polyaccrelamide gel electrophoresis (SDS PAGE) technique could be used for the identification of extractable proteins. Extractable proteins are separated according to the molecular weight of each protein in this SDS PAGE method.

Measurement of allerginicity

Allerginicity measurements are necessary for the detection of allergenic proteins. There are few methods available to check the allerginicity of the proteins.

a. Skim prick test.

b. IgE latex specific RAST inhibition.
c. IgE latex specific ELISA – inhibition.

These tests are more specific for latex allergens than those of the extractable protein measurements. Skin prick test is the most appropriate since it evaluates the allergic reaction in-vitro. One drawback of this test is the availability of latex hypersensitivity individuals and their willingness to be tested.

Are there safe extractable protein levels in latex products

Although the threshold level of extractable proteins for protein allergic reaction is not known, it is possible to obtain an indication by measuring the total extractable protein level in relation to the number of latex hypersensitivity individuals. When latex hypersensitive people were tested for skin prick test, about 60% of them were negatively responded at extractable protein level less than 400 µg/g (RRIM modified lowry method). However very high degree of negative allergic reaction was observed when extractable proteins content is 100 µg/g (RRIM modified lowry method) or less (Yip, 1995).

Production of low protein latex products

Since there is no threshold level of extractable proteins for protein allergy, it is important to remove extractable proteins as much as possible. Removal of extractable proteins could be done by either during the manufacturing process of latex-based products or during the manufacturing process of centrifuged latex. The following describes the various methods and procedures used for removing extractable proteins from latex based products.

Proper leaching of latex products

Leaching is the process of removal of hydrophilic materials from latex products by washing them with water. Leaching is the most easiest and well-known process for the removal of extractable proteins (Said *et al.*, 1996 and Zin *et al.*, 1996). The leaching process not only removes extractable proteins but also improves physical properties such as tensile strength and film clarity.

In the production of latex gloves, there are basically two methods of leaching namely wet-get leaching and dry-film leaching are being adopted. Wet gel leaching involves the washing of the wet gel, prior to drying and vulcanization. In contrast dry-film leaching involves the washing of the dried and vulcanized latex products after removing from the former. However wet gel leaching is often carried out for a period of several minutes (about 1-10 minutes) in the dipping line and it does not remove most of the extractable proteins. On the other hand dry-film leaching is carried out usually off line and from extended period of time. Dry – film leaching is more effective compared to wet gel leaching since the substantial amount of extractable proteins is generated upon drying and vulcanization. In order to remove

extractable proteins more effectively from latex products it has been found that a combination of wet gel leaching and dry – film leaching is most desirable.

The following describes various factors affecting the leaching efficiency in reducing extractable proteins from latex products.

(a). Leaching time:

During leaching, extraction of soluble proteins is rapid initially but decreases quickly with time. Therefore optimum practical leaching time appears to be about 5 to 10 minutes.

(b). Leaching temperatures:

The reduction of extractable proteins levels can be improved slightly by leaching at an elevated temperature.

(c). Use of used fresh water:

Extraction of proteins in used water is less effective. Extraction has to be carried out in fresh water.

(d). Latex film thickness:

Leaching is a diffusion-controlled process. Therefore thinner films would require a shorter leaching time to reduce the extractable proteins compared to thicker films.

Chlorination of latex products

Chlorination of latex gloves is done by washing gloves with chloirne water for the removal of tackiness. Chlorinated latex gloves have extremely low extractable protein contents. Therefore chlorination is the most effective method of reducing the extractable protein content of latex gloves. However one significant draw back of the use of chlorinated gloves is deterioration of physical properties of the chlorinated product on thermal aging.

Use of polymer coatings

Polymer coatings for latex gloves has been introduced to produce powder free gloves. Hydrogel polymers such as polyvinyl pyrolidone, poly hydroxyethyl acrylate or methacrylate are used as coating material for latex gloves. However it is reported that hydrogel-polymer coated latex gloves have low amount of extractable proteins.

Use of low protein latex

Low protein latex is produced by treating natural rubber latex with bio-enzymes which hydrolyses latex proteins into more soluble forms and these hydrolized proteins are removed more effectively during the manufacturing process. It was reported that if low protein centrifuged latex is used as a starting material for latex products, the final product will contain low level of extractable proteins.

Use of double centrifuged latex

It was observed that extractable protein content of gloves made from double – centrifuged high ammonia centrifuged latex is significantly lower than that of latex from single-centrifuged high ammonia centrifuged latex. However it is practically impossible to use double-centrifuged latex since it is more expensive compared to single-centrifuged latex.

Use of radiation vulcanized natural rubber latex (RVNRL)

Irradiating natural rubber latex with a suitable radiation accelerator and a suitable stabilizer produces RVNRL. The resultant radiation vulcanized natural rubber latex (RVNRL) can be used for the manufacture of latex products by conventional coagulant dipping process. The process has been developed to overcome few drawbacks existing in sulhpur vulcanization system. Irradiation of NRL degrades extractable proteins and converts them into low molecular weight proteins. They are more water soluble due to their low molecular weight. Hence, the use of improved leaching process of latex products manufactured from RVNRL shows a lesser amount of extractable proteins (Makuuchi K, 1997) compared to unleached products.

Use of water soluble polymers incorporated RVNRL

Several studies have been done to incorporate water soluble polymers into RVNRL to remove extractable proteins from RVNRL (Varghese *et al.*, 1997) It was found that extractable proteins could be effectively removed by the addition of water soluble polymers into RVNRL. Removal of extractable proteins could be further enhanced centrifugation by Ratnayake *et al.*, 1999 and Ratnayake *et al.*, 2001 of water soluble polymer mixed/RVNRL.

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