Determination of Protein Content in Gamma (γ)-ray Irradiated and Non-irradiated Natural Rubber Latex Film

Md. Rakibul Hasan1, Md. Ashraful Islam Molla1, Mithun Sarker1, Shah Md. Masum1, Ashequl Alam Rana1, Salma Sultana2, Md. Emdadul Haque2 and Mohammad Mainul Karim1

1Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka-1000, Bangladesh.
2Nuclear and Radiation Chemistry Division, Institute of Nuclear Science and Technology, Bangladesh Atomic Energy Commission, Dhaka, Bangladesh.

Abstract - With the recent increase and awareness of natural rubber latex protein allergies, it is very important for all health care workers to understand and recognize the need to decrease and/or eliminate latex exposure in the rubber gloves and other rubber products for those individuals who are allergic to natural rubber latex. This study is based on a series of Lowry method of protein analysis. The field latex was concentrated by centrifugation method. A laboratory scale latex centrifuge machine (model SPL-100, Satio Separator Ltd, Japan) with a capacity of 5 liters was used for concentrating the latex. The process increased the solid content in rubber latex from 29% to approximately 60%, and also reduced the percentage of non-rubber content. The experimental results of UV spectrophotometer (UV-2401PC, Shimadzu Corporation, Japan) showed that rubber films of higher TSC percentage got significant amount of water leachable protein. Furthermore, they showed different protein content for different dipping time in water. Another experiment was carried out for irradiated latex of different doses. The change of protein content was significant.

Index Term -- immunoglobuline, irradiated latex, tetramethylthiuram.

I. INTRODUCTION

Natural Rubber Latex (NRL) is formed in the cytoplasm of laticiferous cells which occur beneath the bark of the rubber tree, Hevea Brasiliensis. Fresh NRL consists of rubber hydrocarbon particles in an aqueous solution commonly called as the “serum phase”[1]. Rubber hydrocarbon (cis-1,4-polyisoprene) is surrounded by proteins and lipids, and these together form the rubber particles. Non-rubber substances include proteins (1-1.8%), carbohydrates, lipids and inorganic constituents. Allergy to NRL, which contains a complex blend of water-soluble plant proteins, has become a major source of concern in clinical practice[2]. The Food and Drug Administration (FDA) has received incident reports of thousands of allergic reactions involving latex-containing medical products, including anaphylaxis, cardiac arrests and deaths. As the exposure to latex products continued to increase, so did the incidence and severity of reported allergic reactions. Latex is ubiquitous in medical equipment and devices. Patients and health care providers are at risk for developing sensitization to latex and possibly serious allergic reactions following exposure to any of this products[3]. The level of exposure to proteins is quantified by measuring leachable protein levels per product or per surface area or weight of the products. Exposure is a prerequisite for the risk of sensitization, but the actual sensitizing risk depends on many factors, including exposure site, amount of allergen, frequency and duration of contact, and genetic background (in particular atopy) of the individual. The methods used for quantification may not always reflect the biological availability of the proteins during product use, but merely indicate the potential release of the proteins present. A possible direct relationship between exposure to leachable protein levels and risk of latex allergy and/or sensitization can only be documented on experimental animals, where exposure to latex proteins can be controlled. This is not achievable with humans due to ethical restrictions[4].

Kristina Turjanmaa et al.[5] studied the allergy to natural rubber latex as a major occupational problem in the health care sector and a problem even in other occupations in which protective gloves are used. There is little information available about the long-term outcome at work in large patient populations. The use of low-allergen latex or non-latex gloves throughout the health care sector seems to be an adequate step for health care workers who have natural rubber latex allergy; non-health care workers get along with personal avoidance of latex gloves if they are not working directly with natural rubber latex containing materials in production. Since increased exposure to latex proteins leads to a higher prevalence of sensitization, the logical conclusion is that less protein reduces the incidence of sensitization. Indeed low protein/allergen gloves seem to have reduced the prevalence of sensitization. However, the risk of allergy cannot be completely controlled by reducing the amount of leachable protein, even though the reduction will be of benefit to many. There are major and minor allergens among the leachable proteins and the analytical methods available today cannot totally rule out the possibility of any residual allergen in latex products. At the moment measurement of total protein is the best available way to monitor the allergenic properties of latex.
products, but it may not be sufficient. The total protein content is not necessarily equivalent to allergen content. The leaching process during production reduces the amount of proteins, but the reduction in specific or total allergenic protein is not necessarily in the same proportion as the reduction in proteins. Nevertheless, the risk for latex sensitization and/or allergic reactions can be reduced by minimizing the amount of leachable proteins. It is known that only a limited number of proteins from latex source material are recognized by immunoglobuline E(IgE) antibodies from sensitized subjects[6]. In this respect little is known about extracts from latex products. Most studies have focused on the water-leachable protein moiety as being the sensitizing agent. Many of the proteins have been characterized[7], but there seems to be little information about other possible antigenic or allergenic substances in the latex, e.g. the carbohydrate or lipid moiety of the proteins. The leaching process during production is performed with aqueous solutions and any lipophilic or hydrophobic substances will remain in the latex products, so in theory these substances may contribute to the subsequent clinical exposure to antigenic/allergenic substances[8-10].

A significant progress has been observed in developing rubber material using radiation technology. The technology is called radiation vulcanization of natural rubber latex, or RVNRL. It uses high-energy gamma (γ) radiation (it also can use electron beams) to initiate vulcanization, a process that chemically bonds molecules to produce rubber elasticity and strength. In the RVNRL process, radiation energy replaces the use of a sulphur-based process and produces a material that retains all properties of the conventional product. However, it has some additional remarkable qualities: the absence of carcinogenic nitrosamines; extremely low cytotoxicity; absence of sulphur and zinc oxide; and high transparency and softness. These properties are important for many products, particularly catheters, protective gloves, and other medical and hospital supplies. For such uses, it is important that products are free of contaminants, and toxic and carcinogenic components to avoid harmful effects in people. In this work, the Lowry method of protein analysis was used to determine the protein content for both gamma (γ)-irradiated and non-irradiated latexes. The prepared thin rubber films of various total solids content as well as various film thicknesses using these two types of latexes. Then leaching time for the rubber films was varied for determination of the extractable residual proteins. The samples showed significant change in protein concentration while subjected to UV spectrophotometric experiments.

II. EXPERIMENT

Natural rubber latex was collected from the rubber garden adjacent to Bangladesh Atomic Energy Research Establishment (BAERE) vicinity area. After collection of field latex it was treated with ammonia solution to avoid coagulation and putrefaction. A laboratory scale latex centrifuge machine (model SPL-100, Saito Separator Ltd., Japan) with a capacity of 5 liters was used for concentrating the latex. Approximately 50% total solids content natural rubber latex sample was taken in a beaker and then placed on a magnetic stirrer (model 78-1, UK). Ammonium laurate was used as the stabilizing agent and it was prepared by mixing 5% aqueous ammonia solution with lactic acid obtained from Fluka, Switzerland. Normal butyl acrylate (n-BA) from Kanto chemical Co. Inc. Japan was used as radiation vulcanization accelerator (RVA). The emulsion of RVA was prepared by mixing the RVA with the stabilizing agent for one hour. This procedure was carried out for irradiation of different total solid content (TSC) rubber.

For protein measurement, several total solid content latexes were prepared. This was carried out by adding required amount of 1.5% ammonia solution. Seven different categories of %TSC i.e. 20, 30, 40, 45, 50, 55 and 58 were examined in this study. A certain thickness was maintained while preparing latex film e.g. 20ml latex was poured in a glass plate of 200cm² through a 200 mesh aluminium sieve to avoid suspended particles and bubbles in the latex. The plate was dried at room temperature for 24 hours. The film thus prepared was removed from the plate and was taken under air drying until it is clear in appearance. Again 25ml and 30ml latex were poured in the same area. Thus three different thicknesses of films were maintained. Then one part of the films was dipped in water for 24 hours. Other parts of the films were made dipping it for 48 hours. Again air drying was accomplished after picking up from water. After drying, the films were kept in oven at 70°C for one hour. Then the films were stored in desiccators. Thus, six sample films were prepared for each %TSC latexes. All the films were prepared as double leached films. First, the films were leached in water for two different leaching time- one sample for 24 hours and other for 48 hours. Then all the films were subjected to second leaching which were carried out in the water bath at 36-37°C (the temperature resembles the human body temperature).

III. RESULT & DISCUSSION

The main cause of latex sensitivity is the presence of soluble proteins in natural rubber products[11-16]. Removal of proteins from natural rubber may be essentially concerned with methods of how to control interactions between the rubber and proteins in the latex stage, i.e. chemical and physical interactions. In this experiment, a change in the amount of proteins of irradiated and non-irradiated natural rubber was investigated under two different leaching times (24 hours and 48 hours). The total solids content (%TSC) of concentrated natural rubber latex was found 58.77 ± 0.2. The experiment was carried out for the total solid content of 45, 50, 55, and 58. This is because the lower %TSC than 45% TSC content i.e. 20%, 30% and 40% showed no protein concentration for non-irradiated films.
Fig. 1. Protein concentrations of 20, 25 and 30ml volume non-irradiated latex films against different %TSC at different leaching time.

For the non-irradiated latex films, the double leaching period was performed and protein content was measured by spectrophotometric method. The fig. 1 shows, protein content is increased with the increase of total solid content. The protein content differs a little with respect to leaching time. It is also found that, the films which were dipped in water for 24 hours, they contain more protein than the films which were dipped in water for 48 hours. Yip et al.[17] showed that high extractable protein (EP) levels are associated with positive skin prick test responses. EP level lower than 400 μg/g of glove tested in individuals with latex allergy showed that 60% did not have a positive response, and in levels of about 100 μg/g and less, the percentage of non responders reached about 100%. It is seen that the curve is almost the same as for the 20ml sample. 25ml sample shows the higher protein content in this experiment. It is assumed that, in the thickness range for 25ml sample 0.23−0.59mm, water penetration is intact and leachable protein content is greater. Although 30ml film density is greater, it shows lower quantity of protein than 25ml film protein. For example, at 50%TSC sample of 25ml 24 hours contains 4.63ppm whereas sample of 30ml 24 hours contains 2.99ppm. Thus those samples lost more proteins than the samples of 24 hours dipping time. But in case of 30ml samples, as their film thickness was greater (thickness range 0.38−0.87mm), water penetration was not easy even though the samples were dipped in water for 48 hours. So, after double leaching, they bear small quantity of protein.

Fig. 2. Protein concentrations of 20, 25 and 30ml volume irradiated latex films against different %TSC.

The fig. 2 shows all protein concentrations found after irradiation. The latex was irradiated at a dose of 5 kGy. Normal butyl acrylate (n-BA) was used as radiation vulcanization accelerator (RVA) because it might destabilize the natural rubber latex[18]. n-BA increases the viscosity of the latex and the latex may coagulate upon storage for a short time period. It is assumed that, after radiation most protein is degraded and since the polymer was grafting with n-BA and leaching was difficult. Here from the fig. 2, it is seen that, five samples out of eight samples show protein content. But this time, they reduced to a very small quantity. The previous highest quantity for 25ml sample was 8.53ppm. The sample was dipped in water for 24 hours whereas; the irradiated sample contains only 2.44 ppm. Protein content is reduced to almost one-fourth from the previous one. Again, five samples are the maximum number out of eight samples which retain their protein content even after irradiation. Here, only two samples of 55 and 58 total solids content bear a trace protein after irradiation. Total two samples out of eight shows the quantity and this is quite less than that for 25 ml samples. The samples of thickness range 0.38−0.59 mm that is 25ml volume samples contain most protein. Again, the samples which were dipped in water for 24 hours contain more protein amount than the samples of 48 hours dipping time.

Here, only two samples of 55 and 58 total solids content bear a trace protein after irradiation. Total two samples out of eight shows the quantity and this is quite less than that for 25 ml samples. This may be happened due to greater thickness and grafting of rubber particles with n-BA.

IV. CONCLUSION

The amounts of latex in plants and rubber content in the latex vary depending on the physiological conditions of the
plants. The total solids content of the field latex varies with the range of 29–32. The optimum radiation dose for different properties ranges from 12 to 14 kGy. The interaction of gamma radiation with rubber latex produces various radicals, either the cleavage of the C-H bond or the C-C bond of polyisoprene molecule. The radical attacks the double bond of a polymer to form a polymer radical or macro radical. On the other hand, the radical abstracts a hydrogen atom from a polymer molecule and produces another polymer radical containing a C=C (double bond). The macromolecules might either recombine or attack a double bond or another polymer molecule to produce cross-links. Protein content increased gradually with increasing %TSC. Irradiated latex had less protein content than the non-irradiated latex due to the protein molecules get broken under irradiation.

REFERENCES


