Rosamma Alex*, Joy Joseph, and Eldo. K. Abraham

Gamma ray irradiation can reduce proteins in NR

Exposure of NR latex to γ radiation is a simple and less time-consuming technique to produce deproteinised natural rubber with good raw rubber characteristics, and radiation vulcanised natural rubber latex that can be used to produce latex products with very low leachable proteins.

Abstract: *An attempt is made to reduce protein content of natural rubber by exposure to different doses of gamma radiation. Exposure of natural rubber (NR) latex to low doses of γ radiation results in partial degradation of proteins. By treating the γ ray irradiated latex with protein hydrolysing enzyme, it is possible to further reduce the protein content of NR equivalent to that of deproteinised natural rubber (DPNR), along with raw rubber characteristics acceptable as per graded natural rubber. Generally, DPNR produced by enzymatic hydrolysis of proteins have a lower initial plasticity (Po) and lower plasticity retention index (PRI) while a combination of gamma ray exposure and enzyme treatment results in DPNR of high Po and PRI.*

Exposure of creamed latex which is obtained from latex that is irradiated by low doses of γ radiation and compounded with suitable sensitiser, to higher doses of gamma radiation results in radiation vulcanised natural rubber latex (RVNRL). Such RVNRL has an advantage that it is suitable to produce latex based products with very low leachable proteins after suitable leaching operations, as Type 1 allergy has been reported with latex products that have high amount of leachable proteins.

On exposure of latex to varying doses of γ radiation the following chemical changes are expected to take place (1) degradation of proteins (2) formation of micro gel that enhances green strength and initial plasticity of rubber (3) cross linking of rubber molecules inside the rubber particles. Most of the degraded proteins can be removed easily during further processing of latex.

Exposure of NR latex to γ radiation is a simple and less time consuming technique to produce deproteinised natural rubber with good raw rubber characteristics and radiation vulcanised natural rubber latex that can be used to produce latex products

*Corresponding author is former Joint Director (RT), Rubber Research Institute of India.
Email: rosammaalex2000@yahoo.com

with very low leachable proteins.

***Key words**: Deporteinisation, natural rubber latex, gamma ray irradiation, gel content, initial plasticity.*

Natural rubber latex is a colloidal dispersion of rubber particles in an aqueous serum. In addition to rubber hydrocarbon, natural rubber latex contains non-rubber ingredients. The amount of non-rubber ingredients is as follows: Resins 1-2%, proteins 2-2.5%, sugar 1-1.5% and ash 0.7-0.9%.

Latex allergy

The non-rubber ingredients, specifically proteins, affect the properties of both latex and dry rubber. In latex it is well-known that the colloidal stability is attributed to the presence of proteins that adsorb on rubber particles making them negatively charged. In dry rubber, proteins are known to have antioxidant property as they can improve plasticity reduction index (PRI) of rubber. In dry rubber-based products dynamic properties such as resilience, stress relaxation and heat build-up are affected adversely by proteins. Further proteins present in dry rubber, absorb moisture leading to a reduction in modulus and electrical resistivity which are required to be stable in rubber products used in electrical and engineering applications. Due to this, deproteinised natural rubber (DPNR) finds application in products that require precision in modulus and dynamic properties as in vibration isolators, engine mounts, bridge bearing pads etc and in products that require electrical resistivity like rubber parts in submarines and in underground tubes, duct etc [1,2]

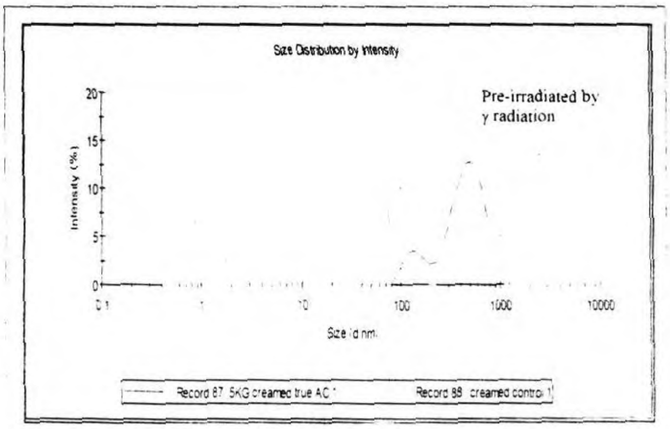

Figure 1. Particle size of creamed latex before and after subjecting them to γ ray exposure to a dose of 5 kGy

Earlier reports show that extractable proteins of latex could be reduced by proteolytic enzymes in the presence of surfactants to less than their detection limit[5,6] and other methods like double centrifugation, chlorination etc

In the case of latex products the main concern is that certain latex proteins are adsorbed by human body and cause allergy problems in certain susceptible individuals. The immune system of such individuals produces antibodies that react immunologically with these antigenic proteins. The most serious and rare form of latex allergy, Type 1 hypersensitivity can cause an immediate and potentially life-threatening reaction.[3,4] .In 1991, the FDA stipulated that rubber products made from NR latex (e.g., gloves and condoms) should be treated to remove extractable proteins. Earlier reports show that extractable proteins of latex could be reduced by proteolytic enzymes in the presence of surfactants to less than their detection limit[5,6] and other methods like double centrifugation, chlorination etc. Papain is a proteolytic enzyme preparation derived from papaya plants (*Carica papaya*) and had been used earlier successfully for production of deproteinised rubber and deproteinised latex.[7,8] These methods have been subjected to modification. However, it is expected that the enzyme residues retained in rubber could result in allergic responses and the methods reported for protein reduction are generally time-consuming.

Easier and less time consuming method

It will be useful if there is an easier and less time consuming method available to reduce the protein content of latex and rubber for use in dry rubber and latex-based products. There are several reports of simultaneous protein degradation and cross linking of latex by exposure of latex to gamma radiation[9]

It is expected that suitably processed γ radiation exposed latex could be an ideal raw material for production of gloves, medical tubes, balloons etc. Generally, products made from radiation vulcanized natural rubber latex (RVNRL) have a lower modulus. If RVNRL is used as such, then leachable proteins present in latex could be high. Hence processing conditions need to be standardized to meet these requirements. Natural rubber latex that has rubber of high green strength will be a suitable material to obtain both rubber of low protein content and radiation vulcanized latex that gives films of superior mechanical properties. The leachable proteins retained on latex films could be reduced by suitable leaching operations. If fresh NR latex is exposed to small doses of gamma radiation in the absence of sensitizers the green strength of rubber can be improved. The work presented here is an attempt in this line.

Table 1. Raw Rubber Propoerties of NR recovered after creaming from latex before and after exposure of to γ radiation (LP rubber)

Parameter	Before exposure	After exposure (LP rubber)
Nitrogen content,%	0.33	0.23
PO	29	33
Gel content,%	2	36
Acetone extractable,%	3.87	5.95

Table 2. Raw Rubber Propoerties of DPNR in comparison with ISNR 5

Parameter	DPNR		ISNR 5
	γ radiation exposed	Control	
Dirt %	0.02	0.02	0.02
Volatile matter,%	0.47	0.49	0.48
Ash, %	0.09	0.09	0.1
Nitrogen,%	0.09	0.09	5.95
PO	39	31	39
PRI	54	19	75

Materials and method

Field latex collected from the RRII Experiment Station was used as the starting material. Stabilized Liquid Papain (LP) supplied by M/s. Senthil Papain and Food products (P) Ltd; Coimbatore, India, was used for deproteinisation. Its activity was 208.15 TU/mg, mineral content 2.16 % and pH of aqueous solution [5.4]. Five per cent solution of the enzyme in water treated with potassium oleate was used for the study. All other chemicals used were of laboratory reagent grade.

Fresh NR latex, stabilised with ammonia at a concentration of 1% (w/w), was exposed to γ radiation (5 kGy) using Gamma Chamber - 5000 from Co60 source, to get latex with rubber of high green strength. The irradiated latex was subjected to the following processing operations

1. The irradiated latex was diluted to 20% DRC and then subjected to creaming with ammonium alginate (0.25% w/v on latex as 3% solution) and potassium oleate (1% w/v as 10% solution). Fresh NR latex stabilised with ammonia was also subjected to creaming process. (Control). The creamed latex was compounded with 0.3 phr potassium hydroxide as stabiliser and 5 phr n-butyl acrylate (n-BA) as sensitiser . The compounded latex was then exposed to gamma radiation to a dose of 15 kGy to get radiation vulcanised natural rubber latex (modified RVNRL) A control RVNRL was prepared from fresh creamed latex in the same manner without any pre irradiation (conventional RVNRL)

2. The irradiated latex subjected to creaming as cited in (1) was coagulated using 2% acetic acid , washed and dried at 70°C. The rubber so obtained was expected to have low levels of protein (This was designated as LP rubber).

3. The irradiated latex as cited in (1) was mixed with liquid papain, a protein hydrolysing enzyme as 5 % solution so as to have a dose of 0.25 phr and kept at room temperature for 48 h and then subjected to creaming. The creamed latex was coagulated using 2% acetic acid, washed and dried at 70°C to get deproteinised natural rubber (γ radiation exposed DPNR) A control DPNR was prepared by mixing fresh NR latex stabilised with ammonia with liquid papain enzyme as 5 % solution so as to have a dose of 0.25 phr and further processing in the same method as used for producing γ radiation exposed DPNR.

The particle size distribution of latex samples was determined using a particle sie analyser (Malvern Zetasizer, Nano Z, UK). The dry rubber was tested for raw rubber characteristics as per ASTM method. The properties of RVNRL were evaluated by measuring quality parameters and strength of films by casting and dipping techniques. The dried films obtained by casting were leached in distilled water at room temperature for 24 hours and then further dried at 70°C in an air oven. The tensile strength modulus and elongation at break were determined by using a Universal Testing Machine (Houns field HSK-S). The presence of nitrogen (N) and extractable proteins (EP) content of the films were determined by ASTM D 3533 and ASTM D 5712 respectively. The gloves were prepared using RVNRL in a commercial unit using commercial processing operations like leaching and chlorination.

Table 3. Properties of Modified RVNRL in comparison with conventional RVNRL (*samples leached in distilled water for 24 hours) immersed in calcium nitrate solution (2.5%) followed by leaching in distilled water for 24 hours**

Parameter	Conventional RVNRL*	Modified RVNRL*	Modified RVNRL*
Modulus 500%, MPa	1.14	1.6	1.65
Tensile strength, Mpa	22.0	24.05	25.5
Elongation at break, %	1380	1285	1280

RESULTS AND DISCUSSION

1. Particle size distribution of latex

The particle size distribution of fresh and latex exposed to γ radiation to a dose of 5 kGy is shown in Figure 1. Latex creamed after irradiation recorded a reduction in size of rubber particles compared to fresh NR latex that is creamed without irradiation as reported earlier [10]

Table 4 Leachable proteins

	Leachable protein µg/g
RVNRL films	312
RVNRL films leached in distilled water for 24 h	40

It is observed that fresh latex after creaming has about 87.8 % particles with average diameter of 496 nm and about 12.2 % particles with average diameter of 121 nm. Irradiated and creamed latex has 90.2 % particles with average diameter of 446 nm and about 9.8% particles with average diameter of 86.8 nm. During irradiation it is possible that some chemical changes take place for the proteinaceous materials and this may be contributing to reduction in size of rubber particles after creaming. This also reveal that there is no agglomeration of rubber particles even after partial degradation of proteins as proteins are the main non rubber ingredient that provide colloidal stability to latex.

2. Properties of rubber recovered from irradiated and creamed latex (L P rubber)

The properties of rubber recovered from latex before and after exposure to γ radiation followed by creaming (LP rubber) are shown in Table 1. It is observed that the nitrogen content reduces by a small extent while the gel content and the initial plasticity (Po) increase after γ ray irradiation. The acetone extractable content also change considerably .

On exposure to γ radiation it is expected that two chemical changes take place: (1) the proteins present in latex can undergo degradation to lower molecular weight fractions [9,11] and (2) the rubber chains can undergo cross linking. The latter is expected to result in an increase of gel content and initial plasticity. The gel content is also related to other types of cross linking of NR molecules as they are presumed to be linked to phospholipids that associate with proteins to form cross slinking [12,13]

The degraded low molecular weight proteins are washed away during the process of dilution and washing. In ammonia preservation process the phospholipids hydrolyses to form long chain fatty acids that combine with ammonia to form fatty acid soaps[14]. The change in content of acetone extractable could be attributed to the chemical changes that take place in latex during γ ray irradiation and also during preservation of latex

3. Properties of rubber recovered from γ ray irradiated, protein hydrolyzing enzyme treated and creamed latex (γ ray irradiated DPNR)

The raw rubber properties of rubber prepared by γ ray exposure, enzyme treatment and creaming in comparison with conventional dry rubber (Indian Standards Natural Rubber (ISNR5) is shown in Table 2.

As a general observation this rubber has comparatively very low protein content (as measured by nitrogen content) in relation to ISNR 5 along with comparable low initial plasticity (Po) and plasticity retention index (PRI). Deproteinised natural rubber prepared by enzyme hydrolysis alone (control DPNR) shows low Po and PRI. The protein hydrolyzing enzyme papain hydrolyses the proteins to low molecular weight fractions [7,15] . Due to removal of proteins DPNR produced by enzymatic hydrolysis alone has a low initial plasticity and plasticity retention index. These two parameters can create problems during the drying operations of the fresh deproteinised wet coagulum and other processing operations like compounding and further processing [7]. However exposure of latex to low dose of gamma radiation followed by enzyme hydrolysis of proteins improves the Po and PRI of the recovered rubber. This is attributed the gel content introduced during γ ray irradiation.

Table 5. Mechanical properties of gloves made in a commercial glove *unit using RVNRL courtesy: M/s Primus Gloves Pvt. Ltd., Ernakulam, Kerala, India. All gloves made with TiO2 and Wingstay L anitoxidant, I minute leaching is the maximum leaching time available during glove production

Parameter	Chlorinated gloves	Washed gloves*
Modulus 500%, MPa	2.51	2.32
Tensile strength, Mpa	19.78	18.66
Elongation at break, %	654	816
Protein content **µg/g**	47	100

4. Mechanical properties of Radiation Vulcanised Natural Rubber Films (RVNRL)

The mechanical properties of RVNRL films prepared from pre-irradiated creamed latex in comparison with pure creamed latex is shown in Table 3. RVNRL films prepared after pre-irradiation showed a higher modulus tensile strength and elongation at break. These properties are further improved after immersion of dried films in calcium nitrate solution (2.5% for 4 hours)

During pre-irradiation as shown earlier several changes take place This includes chain entanglements, microgel formation and partial removal of proteins These factors are expected to contribute to an enhancement in physical properties Earlier reports show that during immersion in calcium nitrate solution there is formation of cross links between rubber molecules involving calcium ions [9]. It is expected that inter particle cross linking will improve tensile strength in presence of calcium ions due to ionic bonding involving calcium ions.

5 Leachable proteins In RVNRL

The leachable proteins after leaching of dried films in water for 24 hours is below specified level for commercial surgical gloves (Table 4). During irradiation the protein content of latex is disintegrated resulting in an increase in water extractable proteins content. Consequently, after leaching the residual water extractible protein content in the films decrease. Earlier reports show that proteins are more readily extracted from pre-vulcanised films than post-vulcanised ones [15]

6. Leachable proteins in gloves prepared from a commercial glove manufacturing unit

The leachable proteins and mechanical properties of latex gloves made in a typical commercial glove manufacturing unit is give in Table 5. It is observed that leachable proteins are in a level as per the specifications of gloves as per international standards. Here the leaching time was low. In spite of this after chlorination the leachable protein content is low.

Conclusions

Exposure of latex to low dose of 5 kGy gamma radiation followed by enzymatic hydrolysis of proteins followed by creaming and coagulation results in deproteinised natural rubber with good raw rubber properties like initial plasticity and plasticity retention index. Exposure of pre-irradiated (to a dose of 5 kGy) and creamed latex to γ radiation to a dose of 15 kGy results in radiation vulcanized natural rubber latex, that has films of good modulus and tensile strength. Leaching of the casted films in distilled water for 24 hours results in leachable proteins retained on rubber film to a negligible extent. Exposure of NR latex to γ radiation is a simple and less time consuming technique to produce deproteinised natural rubber with good raw rubber characteristics, and radiation vulcanised natural rubber latex that can be used to produce latex products with very low leachable proteins. ■

CONTINUED ON PAGE 131