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Production of Soluble Protein Free Latex by Radiation Process

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Abstract

Generally for radiation vulcanization, centrifuged latex is first irradiated and the prevulcanized latex thus obtained is used for making products. During irradiation the latex proteins undergo disintegration which leaves a high soluble proteins in latex products. As reported recently these latex proteins cause widespread life threatening latex associated allergies. In order to follow up the radiation effects of NR proteins during irradiation, field latex was irradiated with γ-rays and the protein concentration in the rubber phase and serum phase were analyzed. It is found that the water solubility of proteins in the latex increases with increasing dose. The 27 kD protein which has lysozyme activity, is reported to be the one causing allergy in latex products. SDS-PAGE analysis revealed that, the 27 kD protein together with 14 kD appears in the radiation vulcanized latex up to a radiation dose of 160 kGy, and at 320 kGy it disappears due to the disintegration by radiation. Based on these results a new process for the preparation of protein free latex has been developed. In the new process the radiation-prevulcanized centrifuged latex is subjected to dilution and then centrifuged. In the case of field latex, it is irradiated first and then centrifuged after dilution. The new process can results in prevulcanized latex almost free from soluble proteins. Tensile strength of samples produced from the new process was comparable with that of the conventional radiation process

1. INTRODUCTION.

Latex from rubber trees (hevea brasiliensis) is the source of virtually all commercial natural rubber (cis-1,4-polyisoprene). Natural rubber latex is used in a wide variety of products that come in contact with human skin and other body surfaces. In the health care field, latex products include gloves, catheters, condoms and hundreds of different medical devices. However, recent reports of wide spread life threatening latex associated Type I allergies have focused attention on latex proteins as serious allergens[1-4]. About 1% of the general population is believed to be allergic to latex. Over 1000 allergic and anaphylactic reactions and 15 anaphylactic deaths related to the use of latex derived medical devices had been reported. The most common manifestation of latex allergy is non immunologic irritant dermatitis of the hand. An estimated 7 to 10% of health care workers regularly exposed to latex and 25 to 67% of children with spina bifida have positive skin results for latex proteins[5]. It has been well established that residual protein from natural rubber latex in a finished product may cause allergic reactions of immediate Type I hypersensitivity in some sensitized individuals[6]. There are several proteins present in fresh field latex. Analytical studies have shown that the protein content of latex is about 1-2 % by weight of latex, part of which is adsorbed on the surface of rubber particles while the remainder is distributed between the aqueous serum phase of the latex and the lutoids[7]. Hevea latex allergens include both soluble and particle bound proteins[8]. It should be noted that while the total amount of proteins present in natural rubber latex is relatively constant, the amount of extractable protein in a latex product may be

highly variable. This arises from the fact that only part of the non rubbers, the most important group of which is the proteins, in the aqueous serum of the latex have been removed during processing. The final quantity of extractable protein may depend upon variation in processing and manufacturing. Various methods are being examined to reduce the extractable protein content in latex products. These include the use of a enzyme-deprotienised natural rubber latex, leaching and chlorination of natural rubber latex products and an on-line or off-line enzyme treatment of gloves[9].

In view of this, the production of latex articles with low soluble protein content is highly desirable. Suitable methods for quantifying total soluble protein in NR latex products are therefore of great interest to the rubber industry. This paper reports the results of a recent study to reduce the extractable protein content of radiation vulcanized natural rubber latex. We followed the radiation changes of latex proteins and described a method for the preparation of protein free NR latex by radiation vulcanization.

2. MATERIALS AND METHODS

Radiation vulcanized films were prepared from centrifuged latex supplied by Dunlop, Malaysia. The latex is first diluted to 50% total solids with 1% ammonia solution followed by 0.2phr of 10% KOH solution. The sensitiser n-butyl acrylate was then added with constant stirring. The irradiation was carried out by γ- rays from a source of Co-60 at a dose rate of 10 kGy/h for 1.5 h. The films were then cast on raised glass plates. It is then air dried till transparent. The films were leached wherever applicable, in 1% ammonia solution for 24h, washed with water and dried in air till transparent and again dried in oven at 80°C for 1h. Protein measurement was carried out as per ASTM D 5712. One gram of latex specimen was extracted with 10 ml of distilled water at room temperature for two hours. Interfering substances were removed from the test protein extract by protein precipitation. The precipitated protein was redissolved in minimum volume of 0.1N NaOH and then analyzed using BCA method. Liquid latex proteins, redissolved in 0.1N NaOH were diluted 1:2 in distilled water and then added to 1:1 sample buffer Tris- HCl, 0.125 mM; pH 6.8, 4% (w/v) bromophenol blue. Dithiotreitol (DTT) 0.1 M, was added when reduction of the sample was needed. The protein concentration per lane was 2 mg. SDS-PAGE analysis of extracts was performed on a 8-18 % precasted polyacrylamide gel. High and low molecular weight markers (Biorad, California, USA) were included. Electrophoresis was performed at 450V till the marker dye reached the bottom of the gel. A laboratory model centrifuging machine obtained from Saito Ltd. Japan, model SPL-100 with a capacity of 5 liters was used for concentrating the latex. Each time 2 liters of field latex were used for centrifuging.

3. RESULTS AND DISCUSSION

It is found that during irradiation with γ-rays, the solubility of proteins in natural rubber latex increases (figure 1).

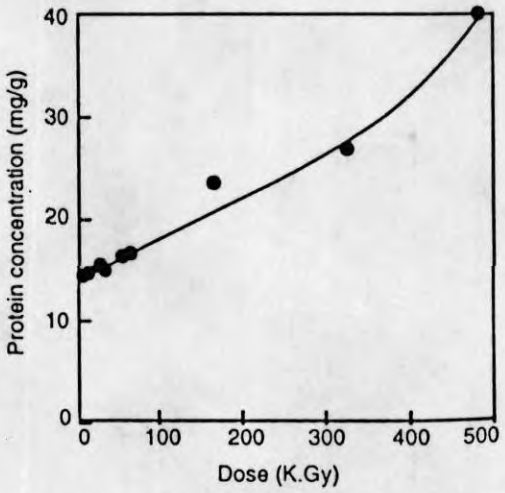

Figure 1. Variation of soluble protein content with radiation dose in field latex

This can be explained by the destructive effect irradiation has on latex proteins[10]. After irradiation, substantially more smearing is observed in the SDS-PAGE. Usually the smear is due to the disintegration or denaturing of the higher proteins. This

means that γ-radiation can cause protein disintegration, which might cause a reduction of Type I allergic reactions. In order to conform this data the rubber phase of irradiated latex was removed by centrifuging and the protein concentrations in the serum phase and rubber phase were determined separately (figure 2).

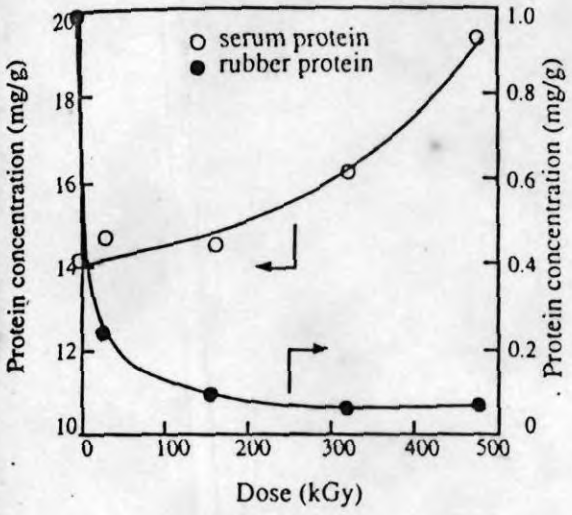

Figure 2. Variation of soluble protein content with radiation dose in the serum and rubber phases of field latex.

The drastic increase of proteins with radiation in the serum phase suggest that, as the radiation dose increases more proteins undergo disintegration. These low molecular weight protein cause a smear in the bottom portion of the SDS-PAGE. Contrary to the radiation change of latex serum proteins, the rubber phase shows a decrease of soluble protein content. The major latex allergens bound to the rubber particle are 30, 35 and 46 kD proteins.[10] These high molecular weight proteins undergo disintegration by radiation which result in low molecular weight ones, the migration of the same to the serum phase may be the reason for the low protein concentration in the rubber phase followed by a corresponding increase in the serum phase.

This can be further confirmed by the SDS-PAGE analysis of the serum proteins (figure 3). It can be seen that up to a radiation dose of 50 kGy, the protein bands are almost the same but the smear at the bottom increases, suggesting the denaturing or disintegration of higher proteins.

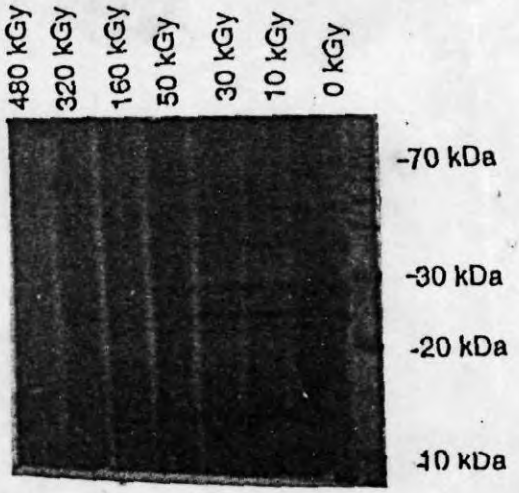

Figure 3. SDS-PAGE of latex serum proteins with radiation after precipitation

The major latex allergens such as 14 kD, 27 kD and 29 kD remain intact up to a radiation dose of 50 kGy. At a dose rate of 160 kGy the allergens start disappearing. At higher doses these allergens are found to disappear from the SDS band followed by the appearance of a smear at the bottom.

This suggests that vulcanization of latex at higher doses can result in protein free latex. However, the physical properties are found to be affected adversely due to breaking down of polymer chains. Based on the radiation induced solubility of latex proteins a new process (figure 4) for the preparation of protein free latex has been developed and the laboratory results were performed on a pilot plant scale with a centrifuging machine for the production of soluble protein free latex. In the new process field latex is irradiated first and the prevulcanized latex is then diluted to a dry rubber content of 20, which is then centrifuged. In the case of concentrated latex, it is first vulcanized and then subjected to a dilution of 30 dry rubber content which is then centrifuged. Lattices from Malaysia and Indonesia were radiation processed as per the new method and the results are given in Table 1. As we have seen, radiation can

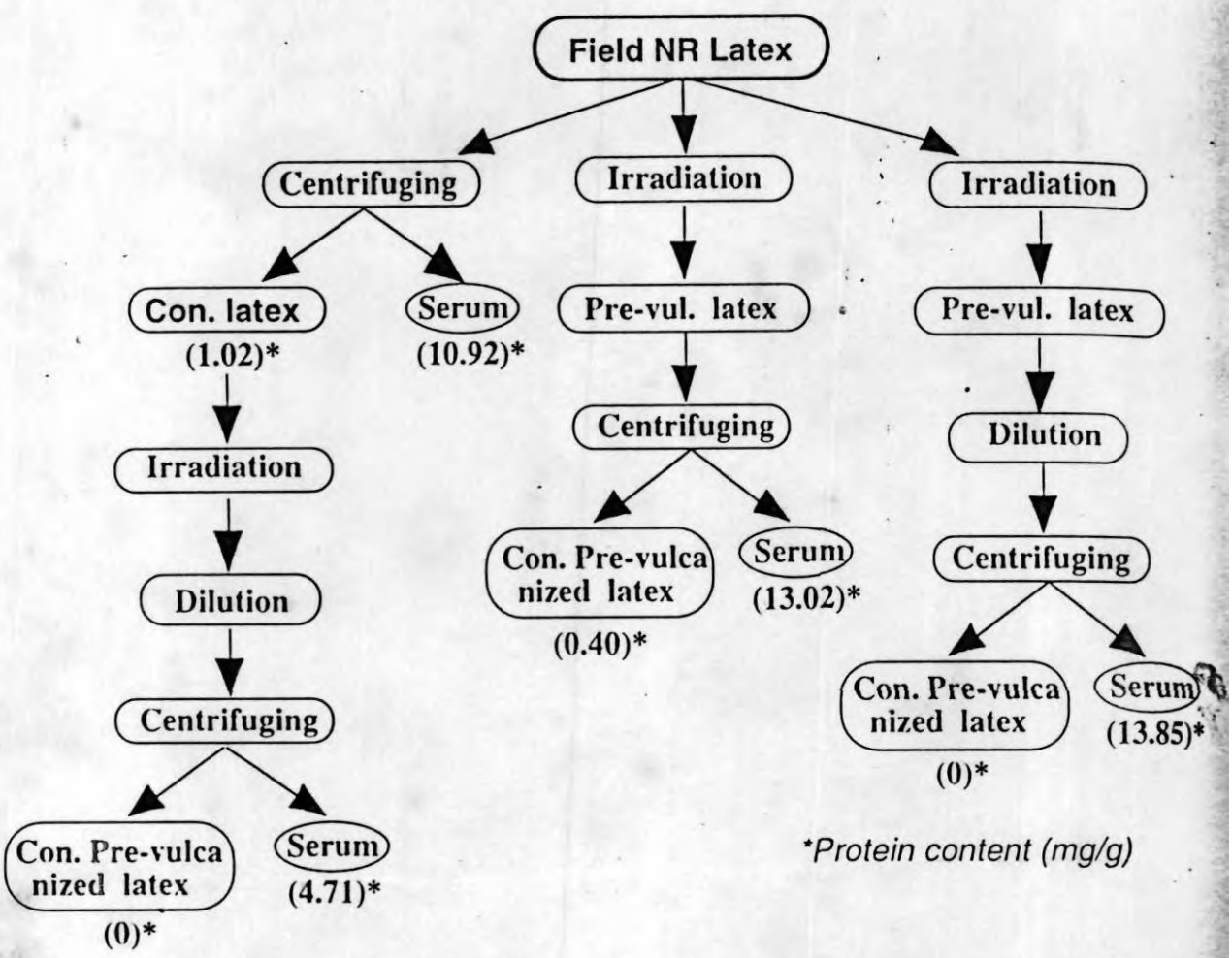

Figure 4. Schematic representation of radiation processing of natural rubber latex

TABLE I. VARIATION OF PROTEIN CONTENT AND TENSILE STRENGTH WITH DIFFERENT PROCESSING STEPS

Sample (Process)	Protein content (mg/g)		Tb (Mpa)
	Rubber	Serum	
Field latex	1.20	10.92	8.26
(after centrifuging)	(1.20)	(11.30)	(8.16)
Concentrated latex	3.20	--	34.63
(after irradiation)	(4.40)	--	(33.09)
Field latex	0.40	13.02	31.26
(after irradiation & centrifuging)	(0.60)	(13.86)	(31.43)
Field latex	not detected	13.85	29.86
(after irradiation, dilution & centrifuging)	(not detected)	(14.01)	(29.91)

Figures in brackets show the values for Indonesian latex

cause an increase of soluble protein, the products made from the above said process contains appreciable amount of soluble protein. To avoid this here we irradiated the latex first and then centrifuged it. This process washes away the soluble protein produced during irradiation, from the rubber phase to the serum phase which makes the products almost free from soluble proteins. The tensile strength of RVNRL films produced from the new process are comparable with those of the conventional process.

4. CONCLUSIONS

As radiation dose increases, the soluble protein content in the serum phase increases where as that in the rubber phase decreases. SDS-PAGE analysis revealed that, the 27 kD protein together with 14 kD appears in the radiation vulcanized latex up to a radiation dose of 160 ,kGy and at 320 kGy, it disappears due to the disintegration by radiation. Based on these results a new process for preparation of protein free latex has been developed. In the new process the radiation-prevulcanized centrifuged latex is subjected to dilution and then centrifuged. In the case of field latex, it is irradiated first and then centrifuged after dilution. The new process can results in prevulcanized latex almost free from soluble proteins. Tensile strength of samples produced from the new process was comparable with that of the conventional radiation process.

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