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# Extractable proteins from field radiation vulcanized natural rubber latex

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**Abstract**

The type I allergy associated with the use of natural rubber latex (NRL) products is caused by the NRL proteins leached by the sweat or other body fluids. Makuuchi's group proposed for the first time the proteins removal by the addition of water-soluble polymers (WSP) on radiation vulcanization of natural rubber latex (RVNRL) that is a promising process under development in many countries. In this study, Brazilian field natural rubber was irradiated with a $^{60}$Co gamma source to reduce the content of WSP in the final product. WSP was used as additive to improve the extraction of protein. After irradiation the RVNRL was centrifuged to extract the WSP and proteins. The analytical methodology for protein content was based on the modified Lowry method according to ASTM D5712. Protein determination was carried out in serum of latex and in the extracts of the gloves. The concentration of extractable water-soluble proteins in serum of irradiated field NRL (NRL1), not irradiated one (NRL2); of twice centrifuged sample with polymer additive NRL (NRL3) and of the glove manufactured (NRLG) are compared with commercial glove (CG). The irradiation process increases the extractable water-soluble proteins, EP, as reported in the literature. In this study the use of polymeric additive on the bi-centrifugation process to remove protein was successful and the EP of the glove obtained in NRL3 was at around 40% of the commercial glove.


## 1. Introduction

The radiation induced vulcanization of natural rubber latex (RVNRL) has prompted mechanistic comparisons to be made between vulcanization work and other related radiation processes [1] such as radiation grafting, curing and vulcanization. Grafting is the copolymer, from a backbone polymer, and a monomer. Curing is the rapid polymerization of an oligomer monomer mixture to form a coating, which is essentially bonded by physical forces to the substrate. Vulcanization is essentially a crosslinking of macrochains.

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Radiation grafting has been performed for over thirty years [2,3]. Curing processes were developed twenty years ago. Works in RVNRL have only been in progress at the last twenty years. An important feature common to all three of these radiation induced processes is the use of sensitizers to either speed up the polymerization reaction or to enhance the degree of crosslinking. Common sensitizers used in all three processes are acrylic esters monomers.

However the bad smell of goods made of irradiated latex was due to the presence of trace amount of 2-ethylhexyl acrylate (2EHA), which was used as sensitizer for radiation vulcanization of natural rubber (NR) latex together with carbon tetrachloride ($CCl_4$) according to Devendra and Makuuchi [4]. Other monomers that have high vapor pressure gas other than 2EHA were used as *n*-butyl acrylate (*n*-BA) because *n*-BA has higher vapor pressure and sensitizing efficiency on RVNRL than 2EHA [5]. So far *n*-butylarylate (*n*-BA) has been accepted as the optimum RV accelerator due to its high accelerating efficiency and no residue in the final dipped products. Since the RVNR latex does not contain dithiocarbamates, sulfur and zinc oxide that are used in the conventional vulcanization, the RVNR latex has the following advantages over conventionally vulcanized NR latex with sulfur: (a) Absence of *N*-nitrosaminas, (b) very low cytotoxicity, (c) easy degradation in the environment, (d) transparency and softness and (e) less formation of $SO_2$ when burned.

Recently, life-threatening latex allergy caused by latex proteins is emerged as a serious problem for health care workers and others who use latex products. The extractable protein (EP) should be removed as much as possible from the latex products. It was expected that irradiation of NR latex may denature the NR proteins and not cause allergic reactions. However, the irradiated NR latex exhibited moderate allergenic [6]. Leaching of the latex product is not practical to remove the EP because it takes long time. Centrifugation of the RVNR latex was attempted to discharge the EP [7]. Leaching of the dried RVNR latex film is effective to remove EP when water soluble polymers (WSP) such as Poly(vinyl alcohol) were added into RVNR latex. In this paper, the effect of combined treatment of WSP addition and centrifugation of RVNR latex on the removal of EP in RVNR latex film will be reported.

## 2. Materials and methods

### 2.1. Preparation of latex films

High ammonia type commercial concentrated latex, 60.97% of total solid contents and 58.97% of dry rubber contents, has 10.08 pH and density of 0.98 kg/dm$^3$ at room temperature (ASTM1076-79). The latex formulation using 3 phr *n*-BA/0.2 phr KOH as sensitizer was the following: the total solid contents was reduced to 50%, 2/3 of 10% KOH were added to the latex and after 300 s the *n*-BA emulsion was added which has 1% emulsifier and 1/3 of 10% KOH. The formulated latex was irradiated without stirring at room temperature by gamma rays from $^{60}$Co source, which is industrial multipurpose use with carrier system design. The dose rate was 4 kGy/h and the vulcanization dose was 10 kGy.

The WSP used was prepared as 10% solution, and added to RVNR latex as much as 3 phr. WSP was added to the diluted RVNR latex followed by centrifugation. The centrifugation of the latex was carried out by Alfa Laval centrifuge machine.

### 2.2. Protein assay

Proteins were precipitated according to standard ASTM D5712-99 to separate interference substances [8].

Ovoalbumin standards were prepared in the concentration of 0.1% (1 mg/mL) and the absorbance at 280 nm using a UV spectrophotometer. Table 1 shows the dilutions used to calculate the calibration curve ($\mu$ = 750 nm).

Latex serum and precipitate were separated using acetic acid 2% to the NRL. The serum was frozen and the precipitate was left at room temperature for at least 1 day to dry.

Latex precipitate and gloves were taken as single test specimen, weighted (minimum 1 g) and were determined the surface area (*S*) in dm$^2$. The

Table 1
Dilutions used to prepare the standard curve

| Sample | Dilution (μg/mL) | Absorbance interval (reference only) |
|---|---|---|
| A | 200.0 | 0.60–0.66 |
| B | 100.0 | 0.35–0.38 |
| C | 50.0 | 0.19–0.21 |
| D | 25.0 | 0.10–0.12 |
| E | 12.5 | 0.05–0.06 |
| F (blank) | 0.0 | 0.0 |

test specimens were placed in extraction vessels so that all surfaces of the test specimens were exposed to the extraction solution. The reagents needed for the analysis are the following:

a. Sodium deoxycholate "DOC" – 0.15% (m/V) solution in water.
b. Trichloroacetic acid "TCA" – 72% (m/V) solution in water.
c. Phosphotungstic acid "PTA" – 72% (m/V) solution in water.
d. Reagent A (Alkaline tartrate).

$$\begin{cases} 2.22\ \text{g} - \text{Sodium carbonate} \\ 0.44\ \text{g} - \text{Sodium hydroxide} \\ 0.18\ \text{g} - \text{Sodium tartrate} \\ 100\ \text{mL with distilledorde ionized water} \\ (\text{d}H_2O) \end{cases}$$

e. Reagent B (Copper sulfate)
*Prepare just before use.*

$$\begin{cases} 0.70\ \text{g} - \text{Cupricsulfatepentahydrate} \\ 100\ \text{mL with distilled or deionized water} \\ (\text{d}H_2O) \end{cases}$$

f. Reagent C (Alkaline copper tartrate)

$$\begin{cases} 100\ \text{mL} - \text{Reagent A} \\ 0.67\ \text{mL} - \text{Reagent B} \\ (100.67\ \text{mL final volume}) \end{cases}$$

g. Reagent D (Folin)

$$\begin{cases} 20\ \text{mL} - \text{Reagent Folin}(2N) \\ 20\ \text{mL} - H_2O \\ (40\ \text{mL final volume}) \end{cases}$$

Make the dilutions of the standards and the test specimens in duplicates, using five points plus the blank. The final volume for each tube must be exactly 0.8 mL.

In each tube of 0.8 mL of final volume add (standard and test specimen) reagents C and D and analyze at the spectrophotometer. Then calculate the calibration curve and find the protein concentration in the test specimens.

## 3. Results and discussion

Table 2 shows the changes in extractable water soluble proteins (EP) contents in serum and rubber phases by radiation of field latex. EP in solid contains about 0.21945 mg/g it increasing to 0.40901 mg/g when the latex was irradiated. This confirms that water solubility of proteins in the latex increases due to radiation degradation of proteins. Considering these results it was proposed to discharge EP by centrifugation after irradiation. The dilution used to WSP of irradiated latex before centrifugation is effective to discharge the

Table 2
Water extractable soluble proteins (EP) contents in serum and rubber phases by radiation of field latex

| Test specimen | Quantity | Protein precipitate $A$ (mg/mL) | Volume of extract $B$ (mL) | Surface area of specimen $C$ ($dm^2$) | Aqueous extractable protein $D = A*B/C$ ($mg/dm^2$) | Protein precipitate (mg/g) |
|---|---|---|---|---|---|---|
| RVNR latex solid | 1.08 g | 0.02208 | 20.00 | 0.0718 | 6.152 | 0.409 |
| RVNR latex serum | 10 mL | 2.02073 | – | – | – | – |
| With WSP + centrifugation solid | 1.35 g | 0.01652 | 20.00 | 0.1386 | 2.383 | 0.244 |
| With WSP + centrifugation serum | 10 mL | 1.16561 | – | – | – | – |
| NR latex solid | 1.15 g | 0.01262 | 20.00 | 0.0792 | 3.186 | 0.219 |
| NR latex serum | 10 mL | 3.81373 | – | – | – | – |

EP by centrifugation and reduced the amount of EP in solid to 0.24468 mg/g. Presumably the dilution affects the configuration of EP due to the change in ionic strength on the surface of rubber particles. Since WSP and EP have polar sites along their chains, that may produce water-soluble protein–WSP complexes. The proteins adsorbed on the surface of rubber particles are taken away by WSP into serum phase. Thus, the WSP–protein complexes were discharged together with water by centrifugation.

Following the procedure above the calibration curve, Table 3, was showed in Fig. 1: where $R^2$ next to 1 show us the points are aligned. The equation $y = 0.2511x - 0.0073$ was used to calculate the concentration of the test specimens, based on the dilutions made, where $y$ is the concentration in mg/mL we want to determine, and $x$ is the absorbance obtained at the spectrophotometer, at 750 nm wavelength, as described in the procedure.

Table 4 shows the absorbance for the serum of not irradiated latex test specimen.

Applying the dilution factor to the test specimen we find five points with its real protein concentration. The average gives us the real protein concentration for the serum of not irradiated latex test specimen. See Table 5.

Table 3
Calibration curve for ovalbumin standards

| Latex | Absorbance | Concentration mg/mL |
|---|---|---|
| A | 0.794 | 0.196 |
| B | 0.441 | 0.098 |
| C | 0.248 | 0.049 |
| D | 0.110 | 0.025 |
| E | 0.066 | 0.012 |

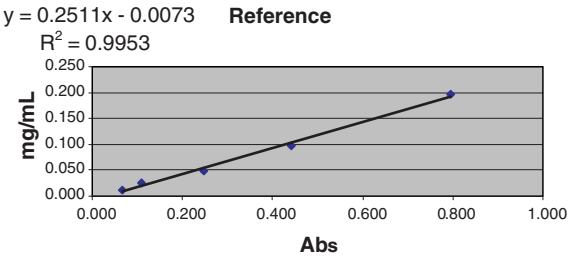


Fig. 1. Calibration curve for ovalbumin standards.

Table 4
Absorbance and concentration for the serum of not irradiated latex test specimen

| Latex | Corrected absorbance | Concentration mg/mL |
|---|---|---|
| Blank | 0.019 | −0.007 |
| A | 1.048 | 0.251 |
| A′ | 1.040 | 0.249 |
| B | 0.631 | 0.146 |
| B′ | 0.634 | 0.147 |
| C | 0.408 | 0.091 |
| C′ | 0.401 | 0.089 |
| D | 0.226 | 0.045 |
| D′ | 0.252 | 0.051 |
| E | 0.131 | 0.021 |
| E′ | 0.136 | 0.022 |

Table 5
Final results analysis for the serum of not irradiated latex test specimen

| Latex | Corrected absorbance | Concentration mg/mL | Dilution | Concentration mg/mL |
|---|---|---|---|---|
| Blank | 0.000 | −0.007 | | 0.000 |
| A | 1.026 | 0.250 | 110 | 2.575 |
| B | 0.614 | 0.147 | 1/20 | 3.084 |
| C | 0.386 | 0.090 | 1/40 | 3.877 |
| D | 0.221 | 0.048 | 1/80 | 4.429 |
| E | 0.115 | 0.022 | 1/160 | 4.620 |
| Average | | | | 4.003 |

## 4. Conclusion

Combination of dilution, WSP addition and centrifugation of RVNR latex can reduce the amount of EP in the rubber films (0.24 mg/g at around 50% in respect to the RVNR solid latex used to commercial gloves) reducing the leaching time of the process to 20–30 min.

## References

[1] J.L. Garnet, P.A. Dworjanyn, S.J. Bett, H.P. Dang, New developments and trends in radiation chemistry and technology, in: Proc. IAEA Meeting, Tokyo, 1989.
[2] A. Chapiro, Radiation Chemistry of Polymeric Systems, Pergamon Press, Oxford, 1960.
[3] J.C. Arthur Jr., in: G. Allen (Ed.), Graft Polymerisation of Lignocellulose Fibres, Pergamon, New York, 1989, p. 317, Chapter 4.
[4] R. Devendra, K. Makuuchi, Development of new sensitizer for radiation vulcanization of natural rubber latex, Final Report to IAEA and JAERI, 1987.

[5] K. Makuuchi, K. Tsushima, Radiation vulcanization of natural rubber latex with acrylic monomers, in: International Rubber Conference, Kuala Lupur, Malaysia, 1985.
[6] K. Makuuchi, F. Yoshii, K. Hyakutake, T. Kume, K. Suzuki, Allergic response of radiation vulcanized natural rubber latex, J. Soc. Rubber Ind. Jpn. 68 (1995) 263.
[7] S. Varghese, Y. Katsumura, K. Makuuchi, F. Yoshii, Effect of water soluble polymers on radiation vulcanized natural rubber latex, Rubber Chem. Technol. 72 (1999) 308.
[8] ASTM D 5712-99, The analysis of aqueous extractable protein in natural rubber and its products using the modified lowry method, 1999.