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A STUDY ON FACTORS AFFECTING EXTRACTABLE PROTEIN CONTENT OF LATEX FILMS

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I. INTRODUCTION

Proteins which constitute about 1-1.5% of natural rubber latex concentrate consist of a wide range of components both water solubles and water insolubles. It has now been well established that residual proteins from natural rubber latex remaining in a finished product may cause allergic reactions of immediate hypersensitivity such as contact urticaria and anaphylaxis in some sensitised individuals.[1] Therefore, it is generally agreed that it is desirable to lower the extractable protein content in latex products as far as practically possible.

The residual proteins in latex products arises from the fact that only part of the proteins are removed during the manufacture of latex products. The final quantity of extractable protein content depends on variations in processing and manufacturing steps. The manufacturers of gloves, condoms and other dipped products are, therefore, attempting to modify their manufacturing processes in order to ensure minimal quantities of extractable protein content in their products.

Various methods have been proposed to reduce the extractable protein content of latex products. These include the use of double centrifuged latex, enzyme deproteinised natural rubber latex,[2] leaching,[3,4] and chlorination[5] of latex products.

This paper reports the effects of wet gel leaching, dry film washing and chlorination under various conditions in reducing the extractable protein content in dipped latex films. The extractable protein content is determined by a modified Lowry micro assay with bovine serum albumin as standard.[6] The level of proteins in examination gloves produced by various units in our country has also been studied.

EXPERIMENTAL

Raw Materials

Centrifuged natural rubber latices (LA-TZ type) were procured from M/s Pilot Latex Processing Centre, Chethackal. Coagulant solution was 7.5% calcium nitrate in 70/30 water/ methanol mixture. Latex compounds were prepared as per the recipe given in Table 1. Prevulcanised latex was prepared by heating the latex compound at 60°C for 4 hours.

Table 1

FORMULATION OF LATEX COMPOUND

	Wet (Parts by weight)
60% Centrifuged Latex	167
10% Potassium hydroxide solution	1
50% Sulphur dispersion	2.5
50% ZDC dispersion	2
50% Zinc Oxide dispersion	0.5

B. Preparation of dipped films

Films (0.15-0.25 mm thick) were prepared by coagulant dipping of glass plates and vulcanising at 100°C for 1 hour. The films were lightly dusted with talc, stripped off from the formers, stored in polythene bag and analysed for extractable protein within a few days of preparation.

C. Treatments

1. Wet gel leaching

The effects of varying time and temperature of wet gel leaching on extractable protein content were studied for prevulcanised and post vulcanised films. Wet gel leaching was carried out on films dried for 2 minutes at 70°C and the ratio of rubber:leaching water was 1 : 400. The water was gently stirred occasionally during the leaching process.

2. Dry film washing

Dry film washing was carried out by immersing the dry vulcanised films in distilled water at different temperatures for various periods of time. The proportion of rubber to washing water was 1 : 400 and the water was gently stirred occasionally during the process.

3. Wet gel leaching/dry film washing using 1:1 water/methanol mixture

Wet gel leaching/dry film washing was carried out as described using 1:1 water/methanol mixture instead of distilled water.

Chlorination

To study the effects of chlorination on extractable protein, films which were subjected to wet gel leaching at 60°C for 5 minutes were used as control. Films were prepared using centrifuged latex and doubly centrifuged latex. The films were immersed in chlorine solutions of different concentration for 3 minutes at room temperature with agitation. Chlorine solution was prepared by acidifying sodium hypochlorite solution. The ratio of film to chlorine water was 1: 50. After chlorination, the films were washed, dipped in 1% ammonia to neutralise any acid and immediately washed in water to remove excess ammonia. The films were then dried at a temperature between 50-60°C and tested for extractable protein content.

Estimation of Extractable Protein Content

Extraction of total soluble proteins

Samples of two pieces measuring about 7 cm x 7 cm and weighing about 2-3 grams each were cut from the gloves/ films. Each piece was then cut into 16 pieces. The pieces were weighed and extracted with 50 ml water in a 10 cm diameter container at room temperature for 48 hours with occasional agitation. The extract was centrifuged in a REMI Laboratory centrifuge R8C at 3500 rpm for 30 minutes to remove any insoluble matter.

Protein purification and concentration of the extract

Protein extracts were purified and concentrated by precipitation with trichloroacetic acid (TCA) and phosphotungstic acid (PTA).

To 6 ml. of the protein extract, 1 ml. of 35% (W/v) TCA was added and mixed. Then 1 ml. of 40% PTA (w/v) was added, mixed and allowed to stand for 20 minutes. The precipitated protein was then recovered by centrifuging the mixture in a

Remi Centrifuge and decanting all of the supernatant. The precipitate was then redissolved in 2.6 ml. of 0.1 M sodium hydroxide for at least 20 minutes.

3. Protein estimation

Protein samples were assayed by the micro-Lowry procedure. The reagents were prepared as follows.

Reagent A	6% Sodium carbonate in 0.2M NaoH
Reagent B	1.5% Copper Sulphate in 3% sodium citrate
Reagent C	(working reagent prepared on the day of estimation) 50 ml. of Reagent A mixed with 1 ml. of Reagent B.
Reagent D	Folin-Ciocalteu Reagent diluted 3 parts Folin + 1 part water.

The test reaction was carried out directly in the centrifuge tube. Added 0.3 ml.of Reagent C to the 2.6 ml. of test sample already present in the centifuge tube. Allowed to mix well and stand for 10 minutes.

Then added 0.1 ml of Reagent D and mixed well the contents in a cyclo mixer. The mixture was allowed to stand for 30 minutes and the absorbance was read on the Spectrophotometer (Shimadzu U.V.240) at 750 nm. The concentration of protein was read off against a standard protein used for calibration (bovine serum albumin 0-120 mg/ml).

III. RESULTS AND DISCUSSION

A. Extractable Protein Content in Commercial Gloves

To study the level of extractable protein content in commercial gloves manufactured in India, gloves samples were procured, tested for soluble protein content & the results are given in Table 2

Table 2

Extractable Protein Content in Commercial Gloves

Extractable Protein content(mg/kg)	No.of samples
0 - 49	1
50 - 99	2
100 - 399	19
400 - 699	22
Above 700	13
	57

Out of the 57 samples tested, only three samples were having Extractable Protein content below 100 mg/kg. Majority of the samples were having Extractable Protein Content in the range 100 - 699 mg/kg and 13 samples were having Extractable Protein Content more than 700 mg/kg. This result shows that our glove manufacturers have to take special care to reduce the Extractable Protein Content.

Effects of Wet gel leaching/Dry Film washing

The effect of wet gel leaching at different temperatures for various periods for prevulcanised and post vulcanised films are given in figures 1 and 2 respectively. Fig. 3 represents the effects of dry film washing at different temperature and for various periods of time for post vulcanised films. It is seen that the extractable protein content can be greatly reduced by treatment with water, even at room temperature. The extraction of the soluble protein is rapid initially, but the rate of extraction decreases quickly and levels off after about 5 to 10 minutes. Slow leaching of the proteins can continue for several hours but further reduction in extractable protein content is small.

The reduction of the extractable protein content can be improved by leaching at an elevated temperature.

The effects of wet gel leaching and dry film washing were found to be comparable in reducing the extractable protein content of post vulcanised latex films.

Effects of using water/methanol mixture was found to be comparable with that of leaching/washing with water.

Effect of chlorination

Chlorination was found to be effective in reducing the extractable protein content for both centrifuged and doubly centrifuged latices (Table 3). It is seen that the extractable protein is reduced by 50-60% by this process.

Table 3

Effect of Chlorination on Extractable Protein Content

Sample details	Concentration of chlorine solution	Extractable Protein content (mg/kg)
Film 1-Centrifuged latex	Control	310
	0.1%	257
	0.3%	160
Film 2-Doubly Centrifuged Latex	Control	103.5
	0.1%	52
	0.3%	40

IV. Conclusion

Wet gel leaching, dry film washing and chlorination are found to be effective in reducing the extractable protein levels in dipped latex films.

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pv.

Fig.1 - Effect of Wet Gel leaching on Extractable Protein Content of Prevulcanised films

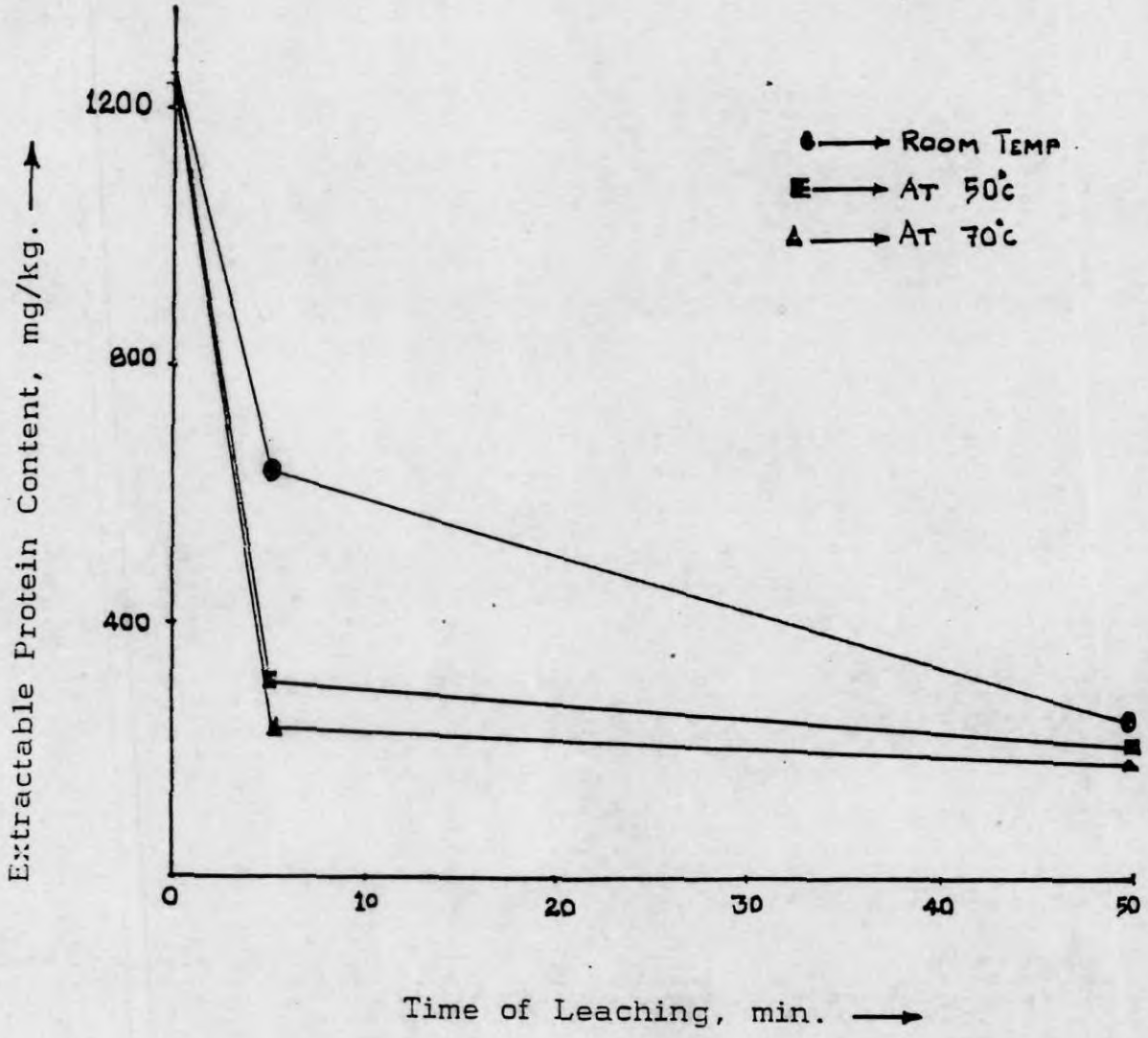

Fig. 2 - Effect of Wet Gel Leaching on Extractable Protein Content - Post Vulcanised Films

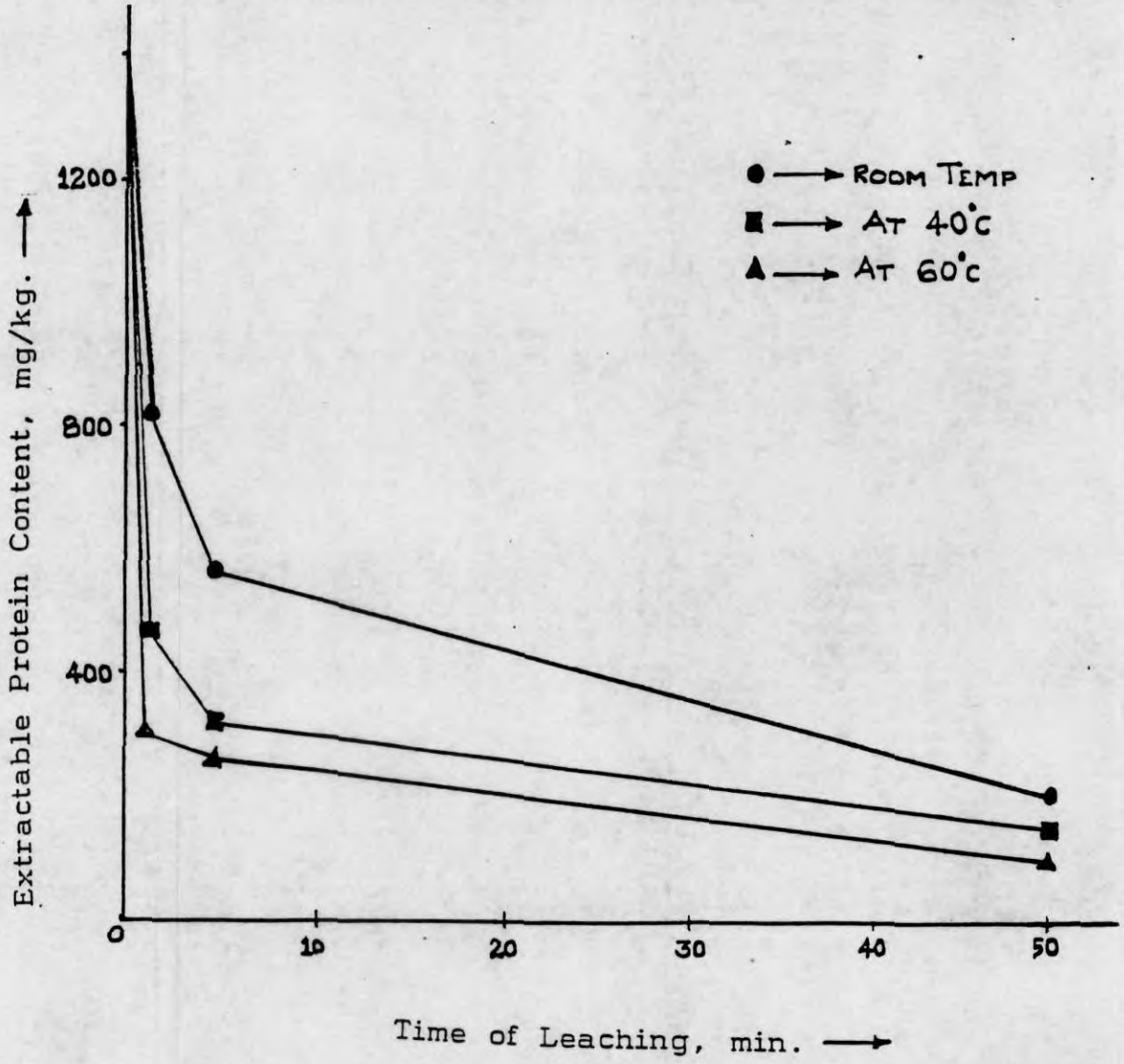

Fig. 3 - Effect of Dry Film Washing on Extractable Protein Content - Post Vulcanised Films