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Production of Natural Rubber Latex Gloves with Low Extractable Protein Content: Some Practical Recommendations

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With the reported incidence of Type 1 hypersensitivity associated with the presence of residual water-soluble proteins in latex products, there is an urgent need to produce latex gloves with minimum extractable proteins. The current practice of wet gel leaching for a short period of time is inadequate in reducing the extractable proteins. This study shows that an additional leaching operation after drying is necessary. The most effective means is the use of a re-centrifuged prevulcanised latex with a combination of wet gel leaching and dry-film leaching or direct water spraying. Under the most favourable conditions, an extractable protein content of approximately 0.03 mg/g can be achieved. Physical properties of the low extractable protein gloves are found to meet the requirements of ASTM D-3578-91 *for natural rubber examination gloves. Their allergic response, as tested clinically on latex-sensitive patients by a skin-prick test, is found to be highly negative.*

Natural rubber (NR) latex is predominantly used in the production of surgical and examination gloves. This is attributed to the superior processing behaviour of natural rubber latex and high physical strength of gloves made from natural rubber latex. The production of latex gloves involves a process of coagulant dipping, in a continuous chain dipping line. During the coagulant dipping operation, clean porcelain formers are first dipped into a coagulant solution, usually calcium nitrate and then into a natural rubber latex compound. The next stage involves the bead rolling of the gloves by rotating brushes after a short drying. This is followed by leaching of the wet gel on the former in hot water. Corn-starch is then applied to the outer surface of the formers *via* a slurry dip before the gloves are subjected to a drying and vulcanisation process in an oven. Subsequently, the dried gloves are manually stripped from the porcelain formers, tested and packed.

Malaysia is currently the leading manufacturer of latex medical gloves, exporting some 11.7 billion pieces in 1992 with a total value of RM1.36 billion. Latex glove manufacturing is therefore an important sector of Malaysian natural rubber industry. However, the incidence of latex allergy associated with water-extractable protein in gloves, of a Type I hyper-sensitivity which can be potentially fatal, has posed a serious challenge to the latex glove manufacturers[1,2]. This has resulted in various proposed actions by Food and Drug Administration (FDA) of U.S.A. such as the mandatory requirement of labelling all NR medical devices and the extension of biocompatability tests required currently for surgical gloves to examination gloves as well[3,4]. The American Society of Testing and Materials (ASTM) has formed a task group to study test methods for measuring extractable protein in latex medical devices. Indeed, a regulation for latex medical device may be introduced as

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early as 1995 by the FDA of U.S.A. There is therefore an urgent need to manufacture gloves of low extractable protein content to meet these future requirements. Several reasons have been given for the desire to produce latex medical gloves with low protein contents. These are as follows:

- Prevention of gloves of excessively high protein contents from getting into the market and thus sensitising further individuals
- Gloves of low protein contents are likely to give low allergic response[5]
- To ensure that medical devices are safe for the users.

Several methods have been suggested to effectively reduce the water extractable proteins from NR latex gloves[6]. These include:

- Use of low-protein latex
- Proper leaching of latex products during production
- Chlorination of latex products.

This paper examines the use of a low protein latex in the form of re-centrifuged prevulcanised NR latex and suitable leaching operations required to produce latex gloves of low protein contents. In addition, the allergic response of some resulting latex gloves is investigated by a skin-prick test on latex-sensitive patients[7,8].

MEASURES TO REDUCE EXTRACTABLE PROTEIN IN LATEX PRODUCTS

Currently, intensified effort made at the Rubber Research Institute of Malaysia (RRIM) emphasises on finding practical measures to reduce to the minimum the extractable protein contents in latex examination gloves. Such measures include chlorination, the optimisation of the leaching operation, and the use of a re-centrifuged prevulcanised latex or a low protein latex (LOPROL) in the production of latex examination gloves.

LEACHING PROCESS

Leaching is the process of removal of *hydrophillic materials from latex dipped* products by washing them in water[9]. It is an essential process in the production of latex dipped products. The removal of excess calcium nitrate and water-soluble non-rubbers such as proteins and added compounding ingredients results in improvement of physical properties such as tensile strength and film clarity, prevention of surface 'blooms' and reduction in water absorption of latex dipped products. The effectiveness of the leaching process is critical in the determination of the overall quality of gloves produced.

There are basically two methods of leaching *viz.* wet gel leaching and dry-film leaching. The wet gel leaching involves the washing of the 'wet gel' i.e. gelled deposit on former, prior to drying and vulcanisation. Wet gel leaching is usually carried out on-line. In contrast, dry-film leaching consists of the washing of the dried, vulcanised latex product after removal from the former and is an 'off-line' process. Where complete removal of hydrophillic materials is required, dry-film leaching for an extended period of 16 – 48 h, depending on the type of products made, is the recommended practice.

In the production of latex examination gloves, wet gel leaching is often carried out for a period of several minutes, usually 1–10 min in a continuous chain dipping line, the actual leaching time is very much dependent upon the design of the dipping unit. Leaching is by far the simplest and practical method to reduce the extractable protein in latex gloves. However, the need to incorporate the dry-film leaching operation in addition to wet gel

leaching has largely been ignored in the current process of production of latex examination gloves. This is rather unfortunate as it has been previously established that a substantial amount of water-soluble protein is generated upon drying and vulcanisation of dipped products[10] and that proteins are drawn towards the surface away from the former during this stage, giving rise to asymmetry of extractable protein distribution[11,12]. Any form of leaching or washing, including the slurry dip, after drying is therefore expected to further remove the extractable proteins.

Effect of Slurry Dip after Drying on Extractable Protein Contents of Latex Gloves

The slurry dip of corn-starch is often being carried out prior to the drying and vulcanisation stage during the production of latex gloves. This has been found to be inadequate in reducing the extractable protein content in the latex gloves. In fact, it has been observed that a slurry dip immediately after drying gives much more effective reduction of the extractable protein content in latex gloves. A comparison of extractable protein values between slurry dips before and after drying clearly demonstrates this *(Table 1)*. The slurry dip has been carried out for a period of 10 s and under agitation. However, it should be borne in mind that while a substantial amount of extractable protein of latex gloves can be effectively removed when the slurry dip is performed after drying, there is a danger of build-up of proteins with time in the slurry tank during the continuous production of gloves. A soluble protein level of as high as 1 mg/ml has been noted in corn-starch slurry which has been topped up daily for a period of several weeks. In such cases there is a possibility of re-deposition of the soluble proteins onto the gloves, particularly when the concentration has reached a certain high level. Therefore while it is advantageous to slurry dip after drying, the protein level in the tank should not be allowed to build-up.

Combination of Wet Gel Leaching and Dry-film Leaching

In order to effectively remove the extractable protein during the production of latex examination glove from a prevulcanised latex, it has been found that a combination of wet-gel leaching and drying film leaching is most desirable *(Table 2)*. In addition, the dry-film leaching can be carried out either 'on-line' or 'off-line'. The 'off-line' leaching treatment appears to be more effective, as both surfaces of the gloves are involved *(Figure 1)*.

TABLE 1. EFFECT OF SLURRY DIP ON EXTRACTABLE PROTEIN CONTENTS OF NR LATEX GLOVES MADE FROM RE-CENTRIFUGED PREVULCANISED LATEX

Wet gel leaching time (min)	Extractable proteins (mg/g)[a]		% Reduction
	Dip before drying	Dip after drying	
0	0.438	0.280	36
1	0.396	0.077	81
2	0.367	0.074	80
3	0.323	0.041	87
5	0.277	0.040	86

[a]The extractable protein contents quoted throughout this paper are measured using the modified Lowry microassay and calibrated against Bovine Serum Albumin (BSA)[13].

TABLE 2. EFFECT OF DRY-FILM LEACHING ON EXTRACTABLE PROTEINS OF GLOVES MADE FROM A PREVULCANISED NR LATEX

Leaching time (min)	Extractable proteins (mg/g)[a]	
	Without wet gel leaching	With wet gel leaching 50°C/5 min
0	0.729	0.061
1	0.394	0.044
2	0.143	0.036
3	0.201	0.042
5	0.145	0.038
10	0.128	0.037

[a]With post-drying slurry dip, 10 s

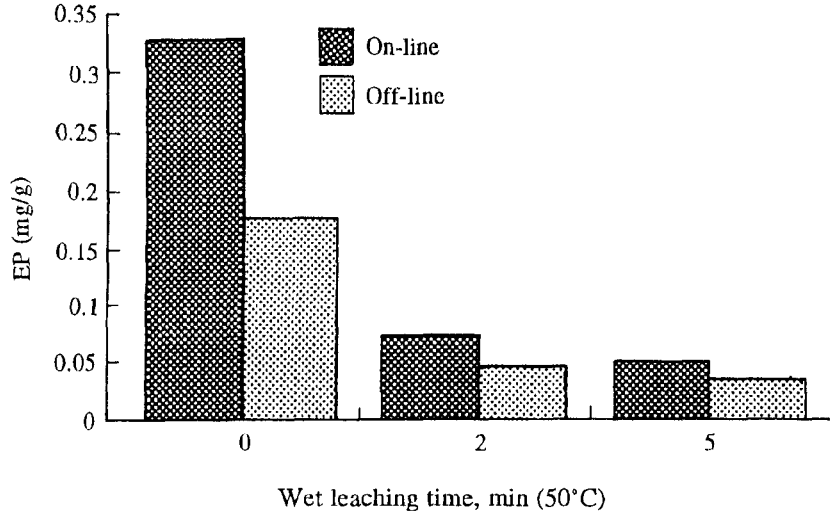

Figure 1. Comparison between on-line and off-line dry-film leaching (50°C/30 s) on EP.

Combination of Wet Gel Leaching and Direct Water Spraying after Drying

The application of a direct water spray to wash off the water-soluble proteins from the dried gloves is an alternative method to an on-line dry-film leaching for the production of NR latex gloves of low extractable proteins from a prevulcanised latex. It is observed that a spraying time of about 30 s could reduce the EP of gloves to below 0.1 mg/g for a normal prevulcanised NR latex *(Table 3)*.

Use of Re-centrifuged Prevulcanised NR Latex

A substantial amount of water-soluble protein can be generated during the compounding of latex and upon heating of a NR latex compound[9]. As such, by a process of re-centrifugation of a diluted (with water) prevulcanised NR latex, it should be possible to remove a large amount of water-soluble protein in the prevulcanised NR latex. Indeed, a re-centrifuged prevulcanised NR latex is

TABLE 3. EFFECT OF ON-LINE SPRAYING IN ADDITION TO WET GEL LEACHING

Time of spraying(s)	Extractable proteins (mg/g)	
	PV 1	PV 2
0	0.189	0.159
10	0.095	0.105
30	0.085	0.070
60	0.070	0.075
120	0.060	0.056

Normal high-ammonia prevulcanised NR latex (PV);
Wet gel leaching for 2 min at 50°C; post-drying slurry dip, 10 s

TABLE 4. EXTRACTABLE PROTEIN CONTENTS OF NORMAL AND RE-CENTRIFUGED PREVULCANISED NR LATICES

Treatment	Extractable proteins (mg/g)	
	Normal PV	Recentrifuged PV
Unleached	1.484	0.432
Wet leaching + dry powdering	0.552	0.262
Wet leaching + slurry dip (after drying, 10 s)	0.133	0.068

Wet gel leaching: For 2 min at 50°C
PV: Prevulcanised NR latex

TABLE 5. EXTRACTABLE PROTEIN CONTENTS OF LATEX GLOVES MADE FROM A RE-CENTRIFUGED PREVULCANISED LATEX (WET GEL AND DRY-FILM LEACHING)

Time of dry-film leaching (min)	Extractable proteins (mg/g)
0	0.069
0.5	0.034
1	0.035
2	0.026
3	0.026

Wet gel leaching: For 2 min at 50°C; with post-drying slurry dip, 10 s

TABLE 6. EXTRACTABLE PROTEIN CONTENTS OF LATEX GLOVES MADE FROM A RE-CENTRIFUGED PREVULCANISED LATEX
(Wet gel leaching and water spraying)

Time (s)	Extractable proteins (mg/g)
0	0.062
10	0.029
30	0.029
60	0.029
120	0.024

Wet gel leaching for 2 min at 50°C; with post-drying slurry dip, 10 s

found to contain a significantly lower extractable protein content when compared to a normal prevulcanised latex *(Table 4)*.

The best result, in terms of reduction of the extractable protein contents of latex gloves to the minimum, can be achieved by the use of a re-centrifuged prevulcanised NR latex together with a combined protocol of wet gel and dry-film leaching *(Table 5)* or a combined protocol of wet gel leaching and direct on-line water spraying *(Table 6)*. A final low extractable protein content of 0.024 – 0.026 mg/g can be obtained.

Allergic Response of Latex Gloves made from Prevulcanised NR Latices

The allergic response of gloves made from prevulcanised NR latices has been studied by a skin-prick test performed on 10–13 latex-sensitive patients[13]. Except for gloves not subjected to wet gel leaching and have relatively higher extractable protein contents, all samples tested clinically indicate low allergic responses, particularly those obtained from the normal prevulcanised latex after a wet gel leach of 5 min and a dry-film leaching of 10 min and the re-centrifuged prevulcanised latex after a wet gel leach of 2 min and a water spray of 30 s. In fact 100% negative responses are shown by the patients tested with these two glove samples *(Table 7)*. For comparison, a commercial glove with a high extractable protein content and giving 100% positive response is included as a positive control.

Physical Properties of Latex Gloves made from Prevulcanised NR Latices with Different Extractable Protein Contents

The physical properties of latex gloves, made from prevulcanised and re-centrifuged prevulcanised latices, with different extractable protein contents have been studied. In general, the unaged and aged properties of the latex gloves are found to be satisfactory *(Table 8)*.

CONCLUSION

Our findings show that it is possible to produce NR latex gloves with low extractable protein contents and low allergic responses from a prevulcanised latex by mainly leaching. To achieve this the following applications are recommended:

- A suitable combination of wet gel and dry-film leaching operations, or
- A combination of wet gel leaching and a direct water spraying operation immediately after drying and/or
- The use of a re-centrifuged prevulcanised latex.

In each case a final post-drying slurry dip is essential.

In accordance with our findings, a wet-gel leaching of 2–5 min at 50°C coupled with a short dry-film leaching of about 30 s is sufficiently adequate for the production of gloves with low extractable proteins and low allergic response from a natural rubber prevulcanised latex. The most effective method of reduction of extractable protein contents in NR latex gloves is found to be the use of a re-centrifuged prevulcanised latex in combination of a protocol of wet gel leaching and dry-film leaching or a direct water spraying application. A guide of leaching protocol required for the production of NR latex gloves with low extractable protein contents is summarised in *Table 9*. Some modifications to these conditions may be necessary for continuous commercial production of latex gloves in view of the different designs of glove dipping units and variations in latex compound formulations and preparations.

All low protein gloves tested indicate low allergic response when tested clinically on latex hypersensitive persons.

TABLE 7. ALLERGIC RESPONSE OF LATEX GLOVES MADE FROM PREVULCANISED NR LATICES

Sample				Allergic response (skin-prick test)		
PV type	WL (min)	DL (min)	EP (mg/g)	Negative (%)	Mildly positive (%)	Clear positive (%)
Normal	–	1	0.394	69	23	8
Normal	–	10	0.128	69	23	8
Normal	5	–	0.061	90	10	–
Normal	5	2	0.036	90	10	–
Normal	5	10	0.037	100	–	–
Recentrifuged	2	–	0.069	86	7	7
Recentrifuged	2	0.5[a]	0.029	100	–	–
Control[b]	–	–	0.694	0	30	70

[a]Water spray, after drying

[b]Commercial brand of glove showing positive response

PV = Prevulcanised NR latex; WL = Wet gel leach, 50°C

DL = Dry-film leaching after drying, 50°C

EP = Extractable proteins (Modified Lowry, BSA standard)

TABLE 8. PHYSICAL PROPERTIES OF LATEX GLOVES WITH DIFFERENT EXTRACTABLE PROTEIN CONTENTS

Sample	EP(mg/g)	M300(MPa)	M500(MPa)	M700(MPa)	TS(MPa)	EB(%)
Unaged						
PV 1	0.279	1.0	2.2	10.0	27.0	900
PV 2	0.065	1.0	2.2	9.5	26.3	900
RCPV 1	0.069	1.0	2.0	8.5	24.8	900
RCPV 2	0.035	1.0	2.0	9.3	26.3	900
ASTM D3578-91 (min)					21.0	700
Aged 100°C/22h						
PV 1	–	0.9	1.6	7.4	26.0	950
PV 2	–	0.9	1.5	6.5	27.7	970
RCPV 1	–	0.9	1.5	6.7	27.3	950
RCPV 2	–	1.0	1.6	6.6	27.0	970
ASTM D3578-91 (min)	–	–	–	–	16.0	500

PV = Normal prevulcanised HA NR latex.

RCPV = Re-centrifuged prevulcanised HA NR latex

EP = Extractable protein, mg/g (Modified Lowry, BSA standard)

TS = Tensile strength; EB = Elongation at break;

M300 = Modulus at 300% extension

M500 = Modulus at 500% extension

M700 = Modulus at 700% extension

TABLE 9. A GUIDE OF LEACHING PROTOCOL REQUIRED FOR LOW PROTEIN NR LATEX GLOVES

PV type	WL condition	DF condition	WS time (s)	EP (mg/g)
Normal	50°C/5'	50°C/1' (on-line)	–	0.044
Normal	50°C/5'	50°C/30 s (on-line)	–	0.051
Normal	50°C/5'	50°C/30 s (off-line)	–	0.037
Normal	50°C/2'	50°C/30 s (off-line)	–	0.048
Normal	50°C/2'	–	30	0.085
Normal	50°C/2'	–	60	0.070
Normal	50°C/2'	–	120	0.060
Re-centrifuged	50°C/2'	50°C/30 s	–	0.034
Re-centrifuged	50°C/2'	–	30	0.029

WL = wet gel leaching (50°C/5')
PV = Prevulcanised HA NR latex
DF = dry-film leaching; WS = water spraying
EP = Extractable protein (Modified Lowry, BSA standard)

In general, the physical properties of the low-protein gloves are found to meet the requirements of *ASTM D3578-91* for NR latex examination gloves.

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REFERENCES

1. LEYNADIER, F. AND DRY, J. (1991) *Clinical Review in Allergy*, **9**, 371.

2. HAMANN, C.P. (1993) Natural Rubber Latex Protein Sensitivity in Review. *Am. J. Contact Dermatitis*, **4(1)**, 4.

3. STIGI, J. Proposed Labelling Requirements. *Seminar on Current Position of the U.S. Import Requirements and Regulations for Latex Medical Devices, 23 Mar. 1994, Kuala Lumpur.*

4. LOWERY, A. Biocompatibility Tests and Submission of Bio Data 510(K). *Seminar on Current Position of the U.S. Import Requirements and Regulations for Latex Medical Devices, Mar. 1994, Kuala Lumpur.*

5. LOVELL, C.R. Relation between Protein Level and Allergic Response. *Conference on Latex Protein Allergy, 6 Dec. 1993, Amsterdam.*

6. SUBRAMANIAM, A. *Proc. Int. Latex Conference: Sensitivity to Medical Devices, Paper No.51, Nov. 5-7, 1992, Baltimore, Maryland, U.S.A.*

7. TURJANMAA, K. (1994) Contact Urticaria from Latex Gloves, *Protective Gloves for Occupational Use (Mellstrom, G.A., Wahlberg, J.E. and Maibach, H.I., eds.)*, p.241. CRC Press.

8. DREBORG, S. (1989) Skin Tests tested in Type I Allergy Testing, Position paper. *Allergy*, **44**, suppl. 10, 22-30.

9. Latex Dipping – Leaching Treatments. MRPRA Technical Information Sheet, L16, 1977.

10. HASHIM, A. Effect of Leaching on Extractable Protein Contents. *Latex Protein Wkshop, Kuala Lumpur,* June 1993.

11. DALRYMPLE, S.J. AND AUDLEY, B.G. (1992) Allergenic Proteins in Dipped Products: Factors Influencing Extractable Protein Levels. *Rubber Developments,* **45(2/3),** 51.

12. YEANG, H.Y., SUNDERASAN, E., BAKRI, A.R.S., GHAZALY, H. AND HAMZAH, S. Studies in the Behaviour and Characterisation of Latex Glove Proteins. *Proc. Int. Rubb. Conf., Kuala Lumpur, June 1993.*

13. FARIDAH YUSOF AND YEANG, H.Y. (1992) Quantification of Proteins from Natural Rubber Latex Gloves. *J. nat. Rubb. Res.,* **17(3),** 206.