J nat. Rubb Res, **10**(1), 46-62

Latex Allergy Studies: Extraction of Natural Rubber Latex Proteins with Reference to Film Thickness, Latex D.R.C. and Protein Migration Behaviour

H.Y. YEANG*, E SUNDERASAN* AND HAFSAH MOHD. GHAZALY*

Water-soluble proteins in wet natural rubber latex films co-migrated with the evaporation stream to the surface when the film was dried at 100°C and moisture allowed to evaporate from one surface. When evaporation occurred from both surfaces of the latex film simultaneously, the evaporative pull from both directions appeared to annul each other resulting in little protein migration to either surface. Since unmigrated proteins are not readily extracted, the problem of allergenic proteins in latex films relates essentially to the proteins that migrate to the surface.

Wet-gel leaching of thin latex films (0.15 mm thick when dry) and thicker films (0.25 – 0.35 mm) to remove soluble proteins was investigated by gel-leaching the films for 1 – 3 min with distilled water at room temperature after which they were completely dried at 100°C. Soluble proteins were reduced in thin films that were gel-leached. However, gel-leaching was counter-productive for the thicker films where extractable proteins increased. The amount of proteins extractable from latex films was also influenced by the d.r.c. of the latex used to prepare the films. Films prepared from 40% d.r.c. latex had significantly higher extractable proteins than films of similar thickness that were prepared from 60% d.r.c. latex.

Protein removal by wet-gel leaching was found not to be very effective because much of these proteins had not yet migrated to the surface when the film was heated briefly to attain the wet-gel state. When the wet-gel film was completely dried by prolonged heating after the leaching step, more proteins migrated to the surface. In the process of even brief (3 min or less) dry-film leaching, on the other hand, most of the soluble proteins had evidently migrated to the film surface at the time of leaching and their removal was hence much more effective.

Type I sensitivity to latex proteins that occurs in a small proportion of persons exposed to latex or latex products has been reported extensively in recent years (see reviews by Tomazic[1] and Harmann[2]). This situation has spurred extensive research on methods to reduce residual extractable proteins in latex products during their manufacture[3-8] and as a result, various practices have been adopted to reduce extractable proteins in latex products. Notwithstanding these efforts, further solutions to the problem require a basic understanding of how latex proteins behave under conditions that are encountered during latex product manufacture. Such information would enable appropriate strategies to be devised in a systematic manner to overcome the problem. Of particular relevance here is the charac-

*Rubber Research Institute of Malaysia, P O Box 10150, 50908 Kuala Lumpur, Malaysia

terisation of the way soluble proteins migrate to the surface of the latex film[9] from where they are easily leached out.

This paper presents the results from recent investigations and discusses the relevance of protein migration, thickness of latex films and latex d.r.c. to the practical aspects of reducing extractable proteins by leaching with water. In these studies, the films were prepared either by dipping a coagulant-coated shaping former (a sheet of glass) into latex to produce *dipped films,* or by pouring a thin layer of latex into a flat-bottomed plate to produce *cast films.* In the latex industry, most latex goods are manufactured by the dipping process and dipped films are therefore relevant models. On the other hand, cast films have the advantage in that coagulants are not used and, thus, one experimental variable can be eliminated in the characterisation of protein behaviour in latex films.

MATERIALS AND METHODS

Latex films were prepared from REVULTEX MR prevulcanised latex concentrate (Revertex Malaysia Sdn Bhd.) which was of 60% dry rubber content (d.r.c.). In experiments where 40% and 60% d.r.c. latices were compared, the former were prepared by dilution of the latter with 0.5% ammoniated water.

Cast latex films were prepared by pouring 12 ml of prevulcanised latex on to a flat-bottomed dish of 175 mm diameter and heating in an oven at 100°C for 30 min. *Dipped* latex films were prepared by pre-heating glass plates in an oven set at 100°C and dipping the hot plates into a coagulant of 7% to 10% aqueous solution of calcium nitrate. Following this, the glass plates were dried in the oven and then dipped into prevulcanised latex concentrate for 5 s to 40 s (depending on film thickness desired) and then heated to dryness in an oven at 100°C for 20 min to 30 min.

Where required, latex films were leached in distilled water with mild agitation at room temperature.

To assay for extractable proteins, the dried latex films were stripped off the dish or glass plate and extractable proteins were analysed by extracting out the proteins with water from the film surfaces as previously described[10]. Extractable proteins were purified and concentrated by precipitation with trichloroacetic acid and phosphotungstic acid following which, they were determined using the modified Lowry micro-assay[11]. Readings are expressed as extracted protein per cm^2 of the latex film. Except where stated otherwise, proteins were extracted from the surface of the film exposed to the air when drying (*i.e.* the surface away from the latex casting or dipping plate) since most of the extractable proteins (>95%) are located on that surface[10]. This differential distribution of extractable proteins in latex films was confirmed repeatedly in the course of the experiments (data not presented).

To detect the presence of soluble proteins on the latex film surface, the films were stained with 0.1% Naphthalene Black in 50% ethanol and 7% acetic acid for 5 min followed by destaining for 5 min with a mixture of 10% ethanol and 7% acetic acid as reported previously[12]. Uptake of stain on the cut edges of latex films (cross-section) was examined under the bright field of an Olympus SZH Zoom stereo microscope.

Other experimental details are given in the text.

RESULTS

Effect of Moisture Evaporation on Protein Migration in Latex Films

A study was carried out to determine if the migration of soluble proteins to the surface of the latex film, an observation reported pre-

viously[7,9], was linked to the evaporation of moisture from the wet film as it dried.

Pieces of silk fabric were dipped in prevulcanised latex concentrate and were allowed to dry in the oven (100°C), either hung freely or laid down on a glass plate *(Figure 1)*. In the former, moisture evaporated from both surfaces of the film whereas in the latter, evaporation occurred only from the surface exposed to air in the oven. The latex films were stained with Naphthalene Black, which has previously been shown to selectively stain soluble proteins (as opposed to the mainly insoluble proteins of the rubber particle surface) in latex films[9]. Where evaporation took place from one surface, soluble proteins accumulated at that surface, as shown by the intense stain uptake *(Figure 2)*. The other surface of this film (that which had been resting on the glass plate) was poorly stained, indicating a paucity of soluble proteins. Where evaporation was allowed to take place from both surfaces, weak stain uptake was observed on both surfaces, indicating low levels of soluble proteins.

The trends in protein migration observed by staining were verified by quantitative protein assays *(Table 1)*. It was evident that only soluble proteins that were located at the surface of the film (and which were hence readily extractable) were stained. In the latex film dried on the glass plate, the amount of proteins extracted from the free surface was very much higher than that extracted from the surface in contact with glass. The ratio of proteins extracted from the two surfaces was 99:1. Where the film was suspended in air, roughly equal amounts of proteins were extracted from the two surfaces. It was significant that when the extracted proteins were combined for both surfaces, very small amounts were found to have been extracted from the suspended film. Only about 1 ug

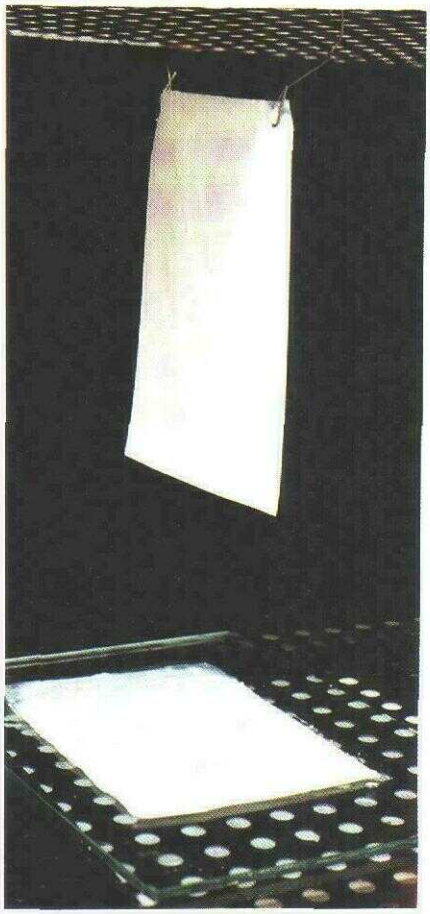
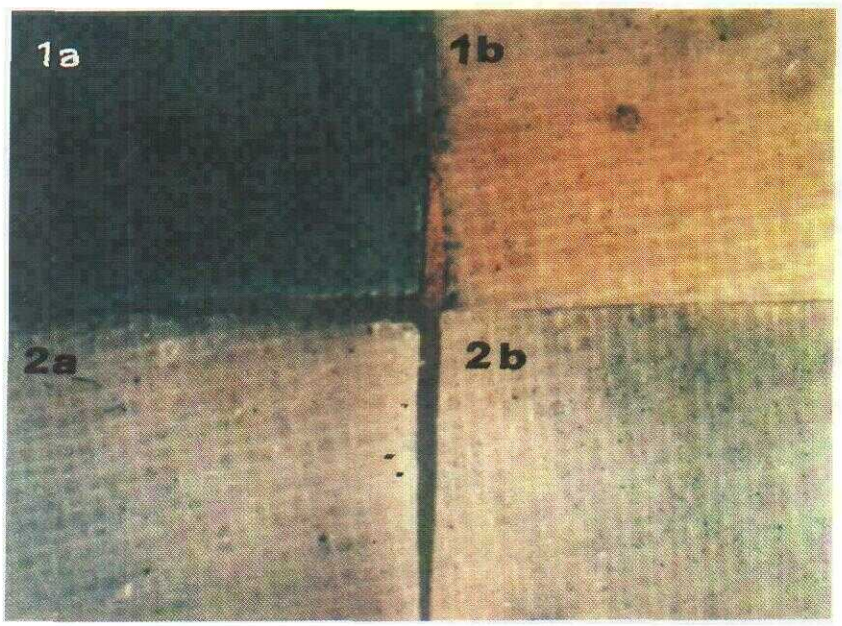


Figure 1. Silk fabric dipped in latex and oven-dried while laid on a sheet of glass (to enable evaporation from one surface of the film) and suspended in the air (to enable evaporation from both surfaces of the film).

TABLE 1. PROTEIN EXTRACTION FROM LATEX FILMS THAT WERE DRIED WHILE LAID ON GLASS (EVAPORATION FROM ONE SURFACE) OR SUSPENDED IN THE AIR (EVAPORATION FROM BOTH SURFACES)

Treatment	Film surface	*Extracted* proteins ($\mu g\ cm^{-2}$)	*Ratio of* proteins extracted from the two surfaces	*Total proteins* (μg) extracted from 1 cm square of film (Two surfaces combined)
Film laid on glass	On glass	37.68	99.2	38.06
	Exposed to air	0.38		
Film suspended in air	Exposed to air	0.57	1.4	0.99
	Exposed to air	0.42		

Values are the means of 2 readings.



1a: Film laid on glass, surface exposed to air.
1b: Film laid on glass, surface resting on glass.
2a and 2b: Two surfaces of a film suspended in the air.

Figure 2. Latex-dipped silk fabric dried while laid on a sheet of glass or suspended in the air. Segments of the films were stained in Naphthalene Black to reveal the presence of soluble proteins.

protein was extracted from a 1 cm square sample as compared with 38 μg for the film dried on glass. It thus appeared that most of the soluble proteins in the film did not migrate to the surface when moisture was allowed to evaporate from both surfaces of the film simultaneously. The unmigrated protein remained 'locked' in the sub-surface and did not diffuse out readily.

Effect of Film Thickness on Wet-gel Leaching of Proteins

Wet-gel leaching is a common practice in the manufacture of latex goods to remove unwanted water-soluble substances from the finished product. These are mainly native non-rubber latex constituents as well as excess coagulant and latex compounding ingredients that have been added. Wet-gel leaching was evaluated for its effectiveness as a means of removing extractable latex proteins.

Thin latex films (0.15 mm) were prepared by dipping in 40% d.r.c. latex for 10 – 30 s, according to the experiment. Latex concentrate of 60% d.r.c. was used for the moderately thick (0.25 mm) and thick (0.35 mm) films by dipping for 5 s and 40 s, respectively. The wet films were heated briefly (45 s, 60 s and 90 s, respectively for thin, moderately thick and thick films) in the oven at 100°C to attain the wet-gel state and were then leached in water for 1 or 3 min at room temperature. After the leaching step, the films were dried completely in the oven at 100°C for 30 min.

Compared with unleached controls, 24% of the soluble proteins was removed from thin films of 0.15 mm that were wet-gel leached for 1 min, the proteins removed increasing to 37% after leaching for 3 min *(Figure 3)*. However, wet-gel leaching did not bring about a similar decrease in the extractable proteins from the thicker films of 0.25 and 0.35 mm. On the contrary, extractable proteins increased by about 18% after wet-gel leaching for 1 min. Statistical analysis of the protein data indicated a significant interaction between the effect of leaching and film thickness ($P<0.05$). These results show that the effect of brief wet-gel leaching on extractable proteins is strongly influenced by the thickness of the latex film; the effect could be either positive or negative, depending on the film thickness *(Figure 3)*. An unexpected observation from this study is that unleached thin films (0.15 mm) give higher extractable proteins than unleached thicker films of 0.25 mm ($P<0.05$).

Effect of Latex D.R.C. on Wet-Gel Leaching of Proteins

While film thickness influenced protein removal by wet-gel leaching, it was possible that the effects reported above could be due primarily to:

(a) the different dry rubber content (d.r.c.) of the latex concentrates used to prepare the thin and thick films,

or

(b) the presence of greater amounts of coagulant in thick films.

These possibilities were therefore investigated.

To test the first possibility, 60% d.r.c. latex was used to prepare both thick films (using 10% coagulant on the dipping glass plates) and thin films (with 3.5% coagulant). In addition, thin films were prepared using 40% d.r.c. latex (with 7% coagulant).

Comparing *Treatments A* and *B* in *Figure 4* that were respectively, thick and thin films prepared from 60% d.r.c. latex, it was evident that the increase in extractable proteins after wet-gel leaching occurred only with the thick films and not the thin films despite the fact that both were prepared from 60% d.r.c. Comparing

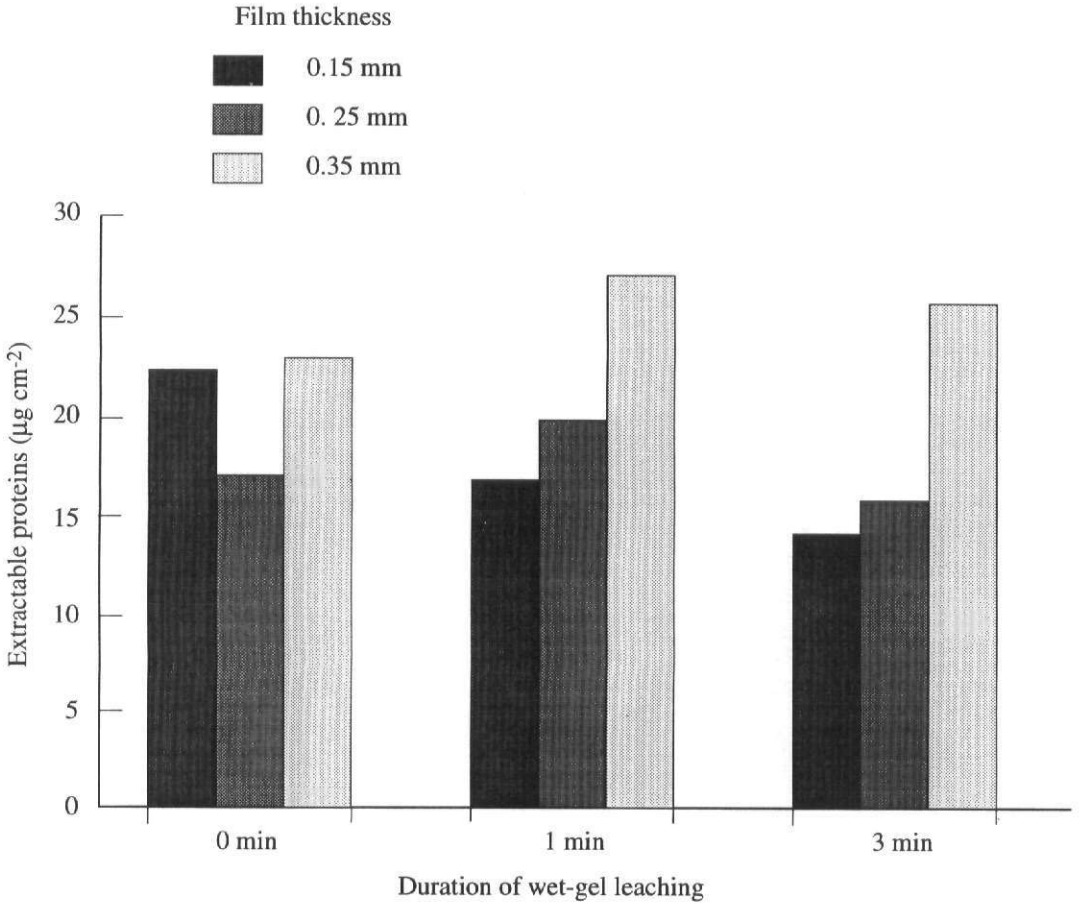
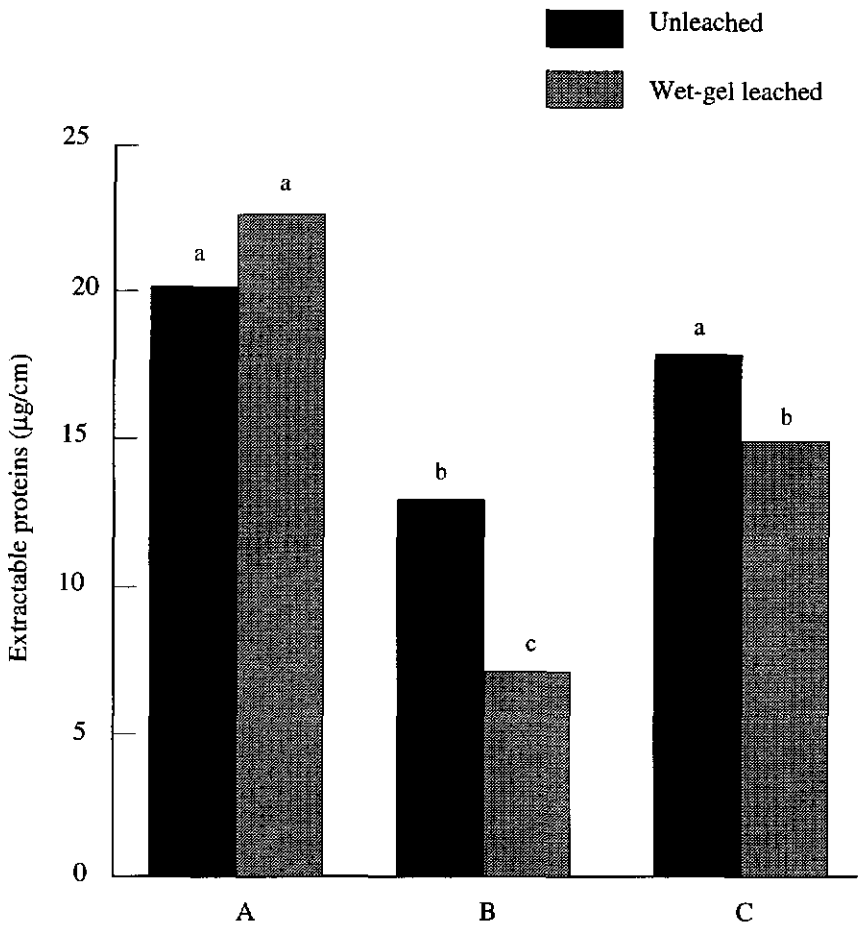


Figure 3. Effect of thickness of dipped latex films on the change in extractable proteins following wet-gel leaching for 1 and 3 min. Readings are for the film surface exposed to air when drying. Each value is the mean of 6 readings. The effects of leaching duration (D), *film thickness* (T) *and* D × T *interaction are significant at* $P<0.05$.

Treatments B and *C*, a decrease in extractable proteins was observed in both the thin films where one film had been prepared from 60% d.r.c. latex and the other from 40% d.r.c. latex. It was clear from the results, therefore, that latex d.r.c. had no direct bearing on the positive or negative response to wet-gel leaching, and that thin films benefited from wet-gel leaching whereas this treatment was counter-productive for thick films.

Since more coagulant was used to prepare the thicker films, it was possible that the increased amount of coagulant was responsible for the increase in extractable proteins upon wet-gel leaching. In an investigation, the wet-gel leaching experiment that had been carried out on dipped films was repeated with cast latex films for which no coagulants were used. The results showed that thick films prepared in the complete absence of coagulant showed the



Latex d.r.c.	60%	40%
Film thickness	Thick (0.31 mm)	Thin (0.15 mm)

Figure 4. Effect of film thickness and latex d.r.c. on the change in extractable proteins following wet-gel leaching for 1 min. Readings are for the film surface exposed to air when drying. Each value is the means of 6 readings. Values bearing the same letter are not significantly different at P<0.05.

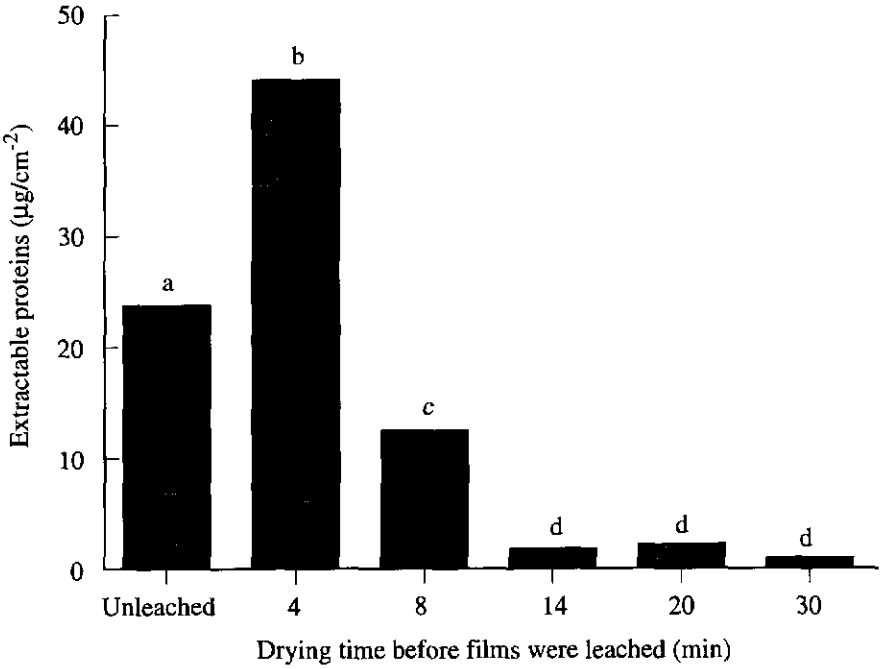


Figure 5. Extractable proteins in latex cast films (0.33 mm thick) that were dried at 100°C for varying periods after casting, and then leached for 1 min in water. Values are the means of 3 readings. Readings are for the film surface exposed to air when drying. Values bearing the same letter are not significantly different at P<0.05.

same characteristic of increased extractable proteins when wet-gel leached *(Figure 5)*. In fact, the increase was more marked in cast films prepared without the use of coagulants than in dipped films.

Another noteworthy observation from this investigation showed that thin films prepared from 60% d.r.c. latex had significantly ($P<0.01$) lower extractable proteins than films of similar thickness, but prepared from 40% d.r.c. latex (*Figure 4, B vs C*). A similar trend was also evident from the results in *Figure 3*. The discrepancy was further enhanced after wet-gel leaching when films prepared from the higher concentration latex was only 47% that of films prepared from the lower concentration latex *(Figure 4)*. Since a stronger coagulant had to be used with the lower d.r.c. latex to prepare the dipped film, there might have been an interaction between coagulant and the d.r.c.; perhaps the higher coagulant concentration entrapped more serum from the lower d.r.c. latex. To check this possibility, an experiment was carried out using cast films where coagulants were not used. Films of similar thickness (0.2 mm) were cast using 60% d.r.c. latex and using the same latex diluted to 40% d.r.c. To compensate for the lower rubber (and serum) content of the 40% latex, the volume that was poured into the casting dish was increased by 50%. The two films were identical in thickness when oven-dried, but films prepared from 60% d.r.c. latex were found to yield, on average, 11.0 μg cm^{-2} extractable proteins which was only 61% of the extractable

proteins from films prepared from 40% d.r.c. latex (18.2 μg cm^{-2}). The results obtained with dipped films were thus confirmed with cast films (8 replicates per treatment, $P<0.05$).

Protein Migration in Relation to Wet-gel and Dry-film Leaching of Proteins

It was thought possible that the effect of leaching with water might vary depending on the residual moisture content in the film. An investigation was therefore carried out to determine the effectiveness of protein removal by leaching the latex film as it progressed from the wet-gel state to the dry-film state with increasing duration of drying.

Films of approximately 0.33 mm were cast from prevulcanised latex of 60% d.r.c. and placed in the oven at 100°C. After varying periods of heating ranging from 4 to 30 min, the films were leached in water for 1 min at room temperature and then replaced in the oven to dry completely. The dry-film samples were then extracted and assayed for proteins. Moisture in the latex concentrate was initially 40%, but the residual moisture in the film dropped to 2% by the 14th minute *(Figure 6)*. The leaching of films that had been in the oven for 14 min or longer was therefore dry film leaching whereas before 14 min, the films were leached while they were in varying degrees of gelation. Wet-gel leaching (after the film was dried 4 min) did not result in any decrease in extractable proteins as compared with unwashed control films. On the contrary, the extractable proteins were significantly increased ($P<0.05$) as already noted above *(Figure 5)*. In contrast, films that were leached when completely or almost completely dry had very little extractable proteins, indicating that soluble proteins were readily removed from

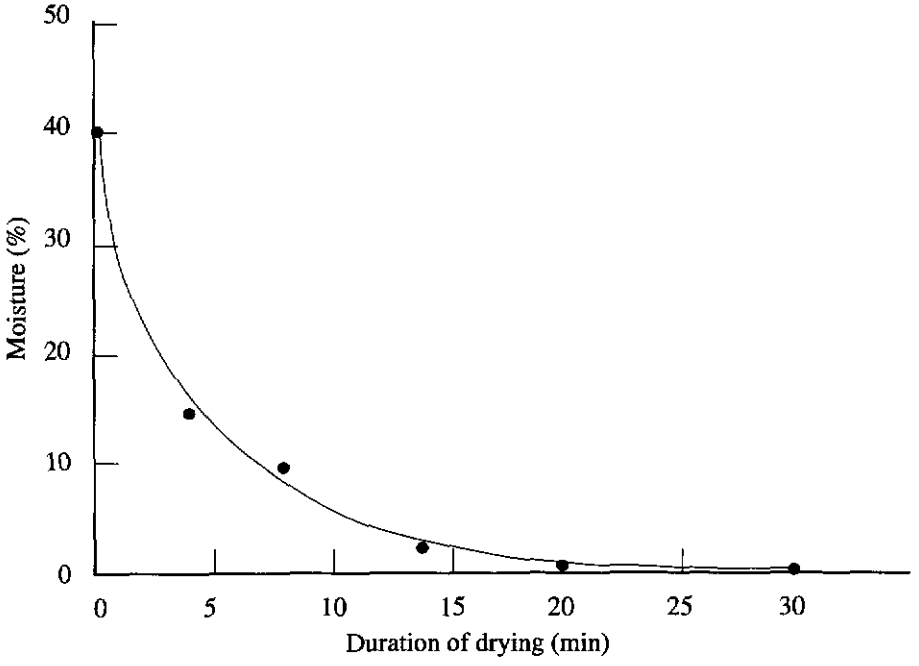


Figure 6. Loss of moisture (transition from wet-gel state to dry film state) in latex films dried in the oven at 100°C. Values are the means of two readings.

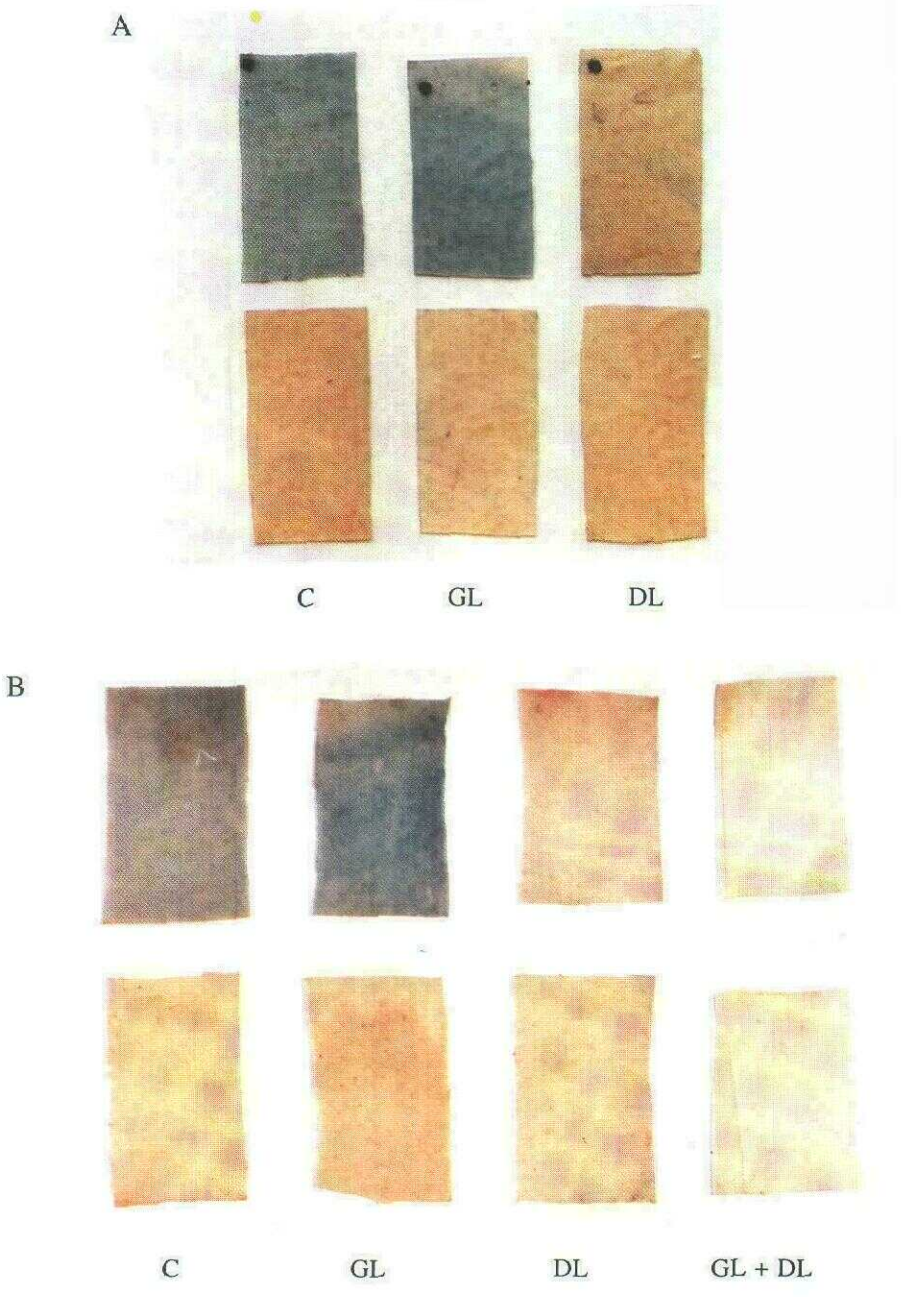


Figure 7. Staining of cast latex films (A) and dipped latex films (B) with Naphthalene Black to reveal the presence of soluble proteins. The samples on the top row are surfaces that were exposed to the air when the films were dried in the oven. Samples on the bottom row are surfaces that were adjacent to the casting or dipping plates when the films were dried. C = Unleached control; GL = Wet-gel leached; DL = Dry-film leached.

dried films by simple washing with water *(Figure 5)*.

The above findings on wet-gel and dry-film leaching were confirmed by staining the films with Naphthalene Black. Films that were washed in the dry state were observed to have little stain uptake *(Figure 7)*. On the other hand, unwashed films and films that were washed in the wet-gel state showed intense stain uptake at the surface exposed to air (*i.e.* away from the casting surface). As was to be expected from the findings of previous studies[10], the film surface adjacent to the casting plate had very low proteins as indicated by the absence of staining. The uptake of Naphthalene Black stain was assessed further by examining cross-sections of the latex films under the microscope. Dry leached films were unstained in cross-section (*i.e.* devoid of soluble proteins)

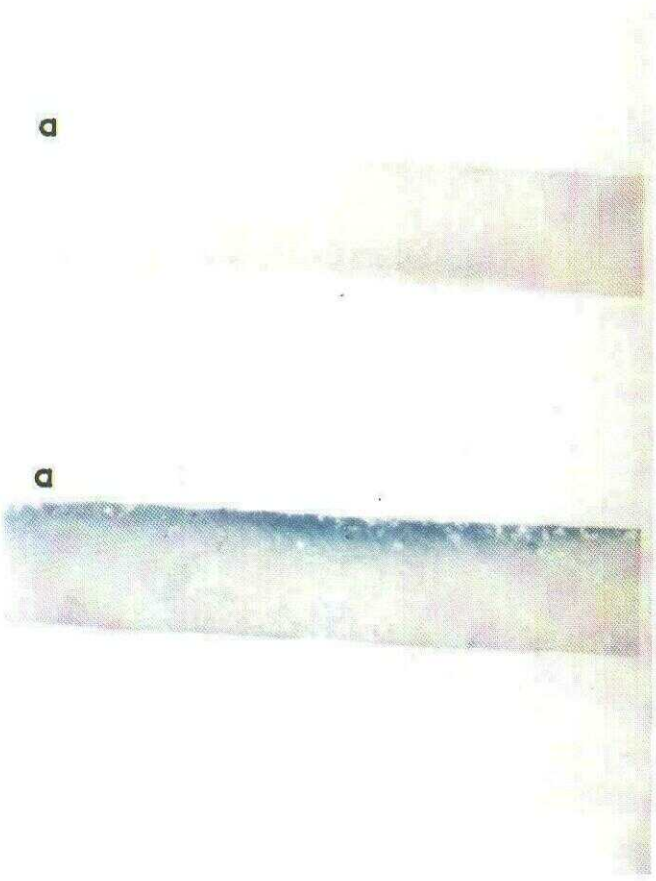


Figure 8. Cross-section of cast latex film that was dry-film leached (top) and wet-gel leached (bottom); a = Surface exposed to the air when the film was cast. Naphthalene Black staining, × 50.

whereas wet-gel leached films showed an accumulation of proteins at the surface exposed to air *(Figure 8)*.

Having assessed wet-gel leaching and dry-film leaching separately, their combined effect in reducing extractable proteins was next evaluated in dipped latex films. Films approximately 0.2 mm thick were wet-gel leached (after drying 30 s at 120°C) or dry-film leached for 1 or 3 min, or were subjected to combinations of wet-gel and dry-film leaching. The films were dried completely after the leaching step and assayed for extractable

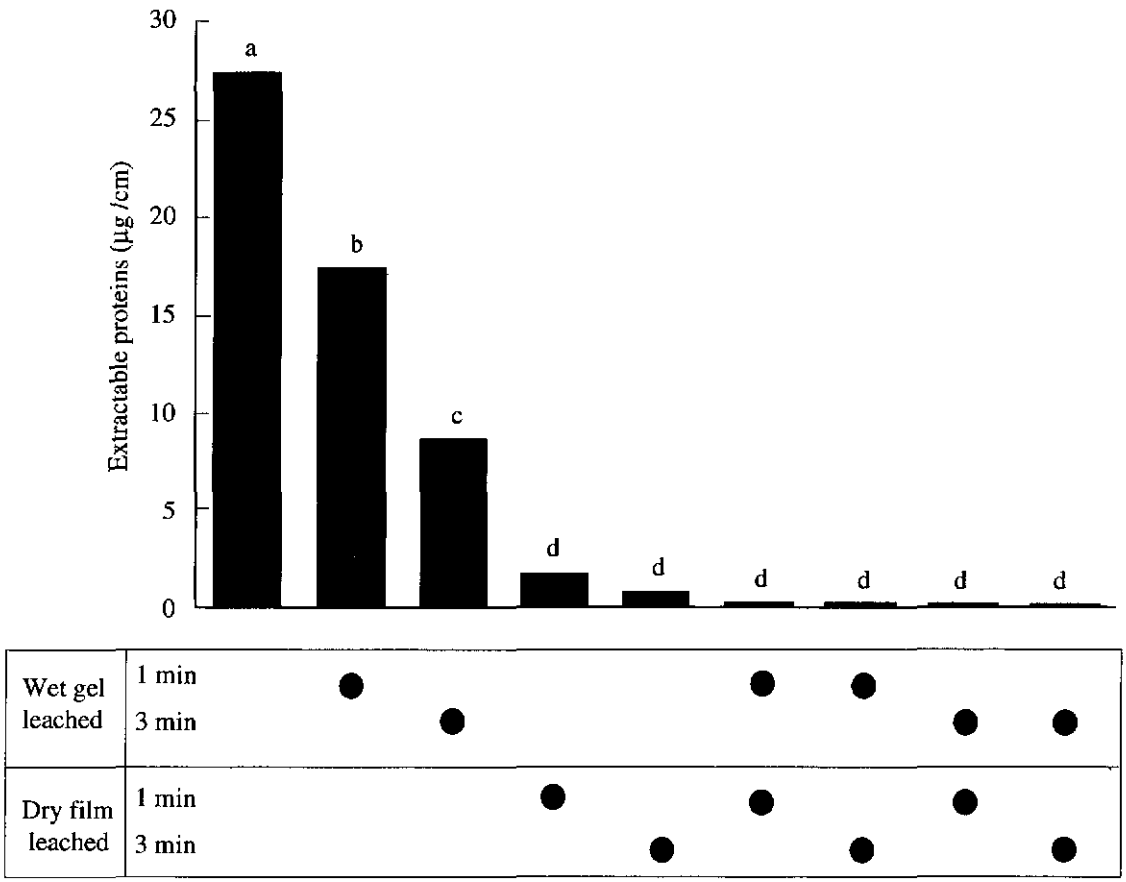


Figure 9. Extractable proteins in dipped latex films (0.2 mm) that have been subjected to wet-gel leaching, dry-film leaching and the two treatments combined. Leaching was carried out for 1 or 3 min in water. The first column is the unleached control. Values are the means of 3 readings. Readings are for the film surface exposed to air when drying. Values bearing the same letter are not significantly different at $P<0.05$.

proteins. The results are presented in *Figure 9*. Wet-gel leaching of dipped films reduced the extractable protein content of thin latex films (an effect already noted in the earlier experiment depicted in *Figure 3*). Compared with the unleached control, extractable proteins were reduced by 37% after wet-gel leaching for 1 min, and by 68% after 3 min leaching. In comparison with wet-gel leaching, dry-film leaching was far more effective in that even after only 1 min, 94% of the extractable proteins were removed and after 3 min, protein removal was 97.6%. Since dry-film leaching was already very effective, combining wet-gel leaching with dry-film leaching could only further increase the removal of extractable proteins marginally. Wet-gel leaching for 3 min followed by dry film leaching for another 3 min removed 99.7% of the proteins as compared with the unleached control film *(Figure 9)*.

As in the case with cast films, the amount of proteins extractable from the surfaces of dipped films that had undergone different leaching treatments were reflected in the Naphthalene Black stain uptake *(Figure 7)*.

Effect of Drying Films after Dry-film Leaching

During manufacture, dipped latex products have to be dried again after dry-film leaching to remove the traces of water used in the leaching process before the products are packed. An experiment was carried out to determine if there was further protein migration to the surface during this second drying procedure.

Latex films, 0.25 mm thick, prepared by dipping with glass plates (4.5 cm × 6 cm) were dried and leached (on the glass plate) in 300 ml of water for 6 min. In one treatment, the glass plates were then immediately transferred to 30 ml water for protein extraction of the latex film for 3 h. In another treatment, the leached films were first oven-dried for 20 min at 100°C before protein extraction was carried out identically. As shown in *Table 2*, oven-drying the leached film significantly lowered the residual proteins that could be extracted subsequently. Thus, dry films that were leached

TABLE 2. EFFECT OF OVEN-DRYING DRY-FILM LEACHED FILMS ON THE EXTRACTABLE PROTEINS

Oven-drying	Proteins in water used in dry-film leaching (mg)	Residual extractable proteins (mg)
Without drying after dry-film leaching	1.34[a]	0.32[a]
With drying after dry-film leaching	1.28[a]	0.08[b]

Films were dry-film leached in 300 ml water for 6 min and subsequently extracted in 30 ml water for 3 h with or without oven drying in between. Readings are for the film surface exposed to air when drying. Values are the means of 4 readings per treatment. Values in the same column and bearing the same letter are not significantly different at $P<0.001$.

and immediately transferred into a fresh quantity of water for the purpose of protein assay would yield about four times as much protein as similar films that were oven-dried before being subjected to the same subsequent protein extraction. It seems that the drying step 'locks' in the otherwise extractable residual proteins.

DISCUSSION

It is well known that the excessive use of coagulant and latex compounding ingredients results in these chemicals 'blooming' to the surface of the latex film when it is dried[7,13]. This defect, which is minimised by wet-gel leaching, is caused by the unreacted chemicals having been drawn to the surface following the evaporation stream as moisture is lost from the film. It has been suggested that the same mechanism might be in operation for proteins[7,14]. This hypothesis was verified in the present investigation.

It has previously been suggested that more extractable proteins can be leached out from latex films if the latex or latex film has been heated because the proteins are converted into a soluble form at high temperature[6]. The results from the present study do not support this proposition since latex films that were dried by evaporation of moisture from both surfaces gave very low extractable proteins despite having been heated. It is inferred, therefore, that one major effect of heating is to transfer soluble proteins to the film surface from where they are easily extracted. Latex films dried at room temperature have been shown experimentally to give films with relatively low extractable proteins[6]. It is plausible, therefore, that the effect of evaporation on protein migration is significant only when the evaporative rate is sufficiently high, such as that encountered in the manufacture of dipped products (about 100°C).

When the latex film (coated on thin silk fabric) was allowed to dry from both surfaces simultaneously, two possible outcomes were anticipated. The first possibility was that the soluble proteins in the film would migrate equally in both directions and that one half of the total proteins would accumulate at each of the two surfaces. The second possibility was that the evaporative pull from both directions would annul each other and there would be little migration of the proteins to either surface. Results from the present study indicate the latter proposition to be true. There is no particular reason to suppose that protein migration might have been influenced by the presence of the silk fabric since the films, in which evaporation was allowed from one surface, behaved similarly to normal latex films prepared in the absence of the fabric. Unlike soluble proteins at the film surface that were rapidly extracted with water, unmigrated proteins that were 'locked' in the sub-surface of the film were not readily extracted. Hence, it would not be entirely accurate to view the potential allergenicity of a latex film or product on the basis of the total soluble proteins it contains since this might include soluble proteins that have not migrated to the surface and are hence not readily extractable. The problem of potentially allergenic proteins extractable from latex films relates essentially to the proteins that migrate to the surface. Accordingly, the relative amount of protein presented to someone coming in contact with the film (*e.g.* when wearing a latex glove) would be more accurately depicted as the amount of protein extracted per unit film area than as protein extracted per unit weight of film.

Leaching latex films while in the wet-gel state to reduce extractable proteins is a commonly adopted practice in latex glove manufacture[13]. Whereas the viscosity of latex is normally kept low during latex product

manufacture by diluting to 35% – 40% d.r.c.[15], wet-gel leaching of thin films (the thickness of latex examination gloves) was found in the present study to be more effective when they were prepared from 60% d.r.c. as compared with 40% d.r.c. latex. Hence, while it is more difficult to work with high viscosity latices in latex product manufacture, the d.r.c. of the latex should nevertheless be kept as high as is practicable. The reason for lower extractable proteins from films prepared from 60% d.r.c. latex could be that, for a given dry latex film thickness, wet films prepared from 40% d.r.c. latex are thicker than those from 60% d.r.c. latex. They therefore take longer to dry and hence afford more opportunity for the proteins to migrate to the surface.

Irrespective of film thickness or latex d.r.c., dry-film leaching was found to be far more effective that wet-gel leaching for the removal of soluble proteins. The more effective removal of proteins by dry-film leaching as compared to wet-gel leaching has been reported[14], but the difference is more marked in the present experiments. The results presented in *Figures 7* and *8* explain the reason for the effectiveness of dry-film leaching. Only proteins that had migrated to the surface of the film could be extracted with water rapidly. Migration of soluble proteins to the surface had not yet taken place to an appreciable extent when the latex film was in the wet-gel state. When the wet-gel leached film was completely dried by prolonged heating after the leaching step, more proteins migrated and appeared at the surface from where they were easily extractable. In the process of even brief (3 min or less) dry-film leaching, on the other hand, most of the soluble proteins would have already migrated to the film surface at the time of leaching and their removal was hence effective. To remove soluble proteins efficiently, therefore, the wet-gel leach should be supplemented with a dry-film leach (more accurately a dry-film wash since most of the proteins removed are already at the film surface. At best, wet-gel leaching alone (unless very much prolonged) is inefficient. It is in fact counter-productive with thicker films (>0.25 mm), where brief (1 to 3 min) wet-gel leaching increases rather than decreases extractable proteins. The reason for increase in extractable proteins after wet-gel leaching is not clear from the present experiments, but there has been a previous report of this phenomenon by Truscott[5] who attributed it to the hydration and swelling of the film during wet-gel leaching. Despite the reported importance of dry-film leaching in the reduction of extractable proteins[7,8,14] and allergenicity[16], this practice is relatively uncommon in latex product manufacture at the present time.

Notwithstanding the fact that wet-gel leaching has the function of removing other unwanted native or added non-rubber substances from the latex film besides proteins, is there any advantage in carrying out both wet-gel leaching and dry-film leaching specifically for the purpose of protein reduction? Will dry-film leaching alone suffice in as far as protein removal is concerned? The increment from 97.6% protein removal by dry-film leaching alone to 99.7% with the addition of wet-gel leaching *(Figure 9)* seems negligible (and is statistically insignificant in the experiment) when expressed as a percentage of protein extractable from the unleached control. Viewed in terms of the extractable proteins still *remaining* in the film, however, the decrease from 0.68 μg cm^{-2} to 0.08 μg cm^{-2} represents a sizeable change. The exact protein figures obtained under actual conditions of latex product manufacture would vary from case to case, but similar trends can be expected. The problem of latex proteins essentially pertains to its induction of allergic reaction. As immunological reactions can, in general, be triggered by very low concentrations of

antigens, it remains to be seen from future immunological studies if these levels of latex proteins do make a critical difference in the induction of latex allergy.

The migration of soluble proteins to the surface of the latex film as it dries has important implications in the manufacture of dipped latex products As most of the soluble proteins are concentrated on the surface of the film away from the shaping former[10], a large proportion of the extractable proteins can be extracted from the product even by washing only one surface of the film on-line (while the film is still on the shaping former). Although the extractable proteins from the surface in contact with the shaping former are left untreated by this approach, the amount of proteins extractable from this surface is very low relatively Other treatments to reduce extractable proteins (*e g.* chlorination, dipping with a non-latex coating, *etc.*) can similarly be carried out on-line, with one film surface receiving the treatment The results from an on-line treatment of the latex film might not be as effective in reducing extractable proteins when compared with a thorough off-line operation, but the advantage of the latter should be gauged by the benefit gained in the context of the allergy response Once surface proteins have been removed by dry-film leaching, further drying the washed film before storage or packing does not cause more proteins to migrate to the surface Indeed, this drying step appears to lock in whatever residual soluble proteins that still remained.

ACKNOWLEDGEMENT

We are grateful to Dr Samsidar Hamzah for her help in the microscopy of latex films and to Mohammed Napi Daud for some of the statistical analyses. The excellent technical assistance of S Renganathan, Latifah Abdullah and Hanizah Selamat is acknowledged Dr B G Audley of the Malaysian Rubber Producers' Research Association, England, is thanked for his useful comments on this work We thank Revertex Malaysia Sdn Bhd for a gift of prevulcanised latex concentrate.

Date of receipt. February 1995
Date of acceptance. May 1995

REFERENCES

1 TOMAZIC, V J , WITHROW, T J , FISHER, B R AND DILLARD, S. F. (1992) Latex-associated Allergies and Anaphylactic Reactions *Clin Immunol Immunopath* , **64(2)**, 89

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

3 SUBRAMANIAM, A (1992) Reduction of Extractable Protein Content in Latex Products. *Proc Int Latex Conf Sensitivity to Latex in Medical Devices, Baltimore, U S A* , 63

4 SUBRAMANIAM, A , YIP, E., NG, K P AND MOK, K L (1993) Extractable Protein Content of Gloves from Prevulcanised Natural Rubber Latex, *Latex Proteins and Glove Industry (Abdul Aziz S A Kadir, ed)* Rubber Research Institute of Malaysia

5 TRUSCOTT, W (1992) Manufacturing Methods Sought to Eliminate or Reduce Sensitivity to Natural Rubber Products *Proc Int Latex Conf Sensitivity to Latex in Medical Devices, Baltimore, U S A.*, 66

6 AMIR HASHIM MOHD YATIM (1993) Effect of Leaching on Extractable Protein Content, *Latex Proteins and Glove Industry (Abdul Aziz S A Kadir, ed)* Rubber Research Institute of Malaysia

7 BODYCOAT, I (1993) Manufacture of Hypoallergenic Rubber Products Paper presented at the conference on *Latex Protein Allergy The Present Position, 6 Dec 1993, Amsterdam, The Netherlands* Crain Communications Ltd , Rubber Consultants

8. NG, K.P., YIP, E. AND MOK, K.L. (1994) Production of Natural Rubber Latex Gloves with Low Extractable Protein Content: Some Practical Recommendations. Paper presented at the *Workshop on Latex Protein Allergy, 9 June 1994, Kuala Lumpur, Malaysia.*

9. SHAMSUL BAHRI, A.R., SAMSIDAR HAMZAH, HAFSAH MOHD. GHAZALY AND YEANG, H.Y. (1993) Latex Allergy Studies: Location of Soluble Proteins in Latex Examination Gloves. *J. nat. Rubb. Res.*, **8(4)**, 299.

10. YEANG, H.Y. AND FARIDAH YUSOF (1993) Latex Allergy Studies: Differential Leaching of Soluble Proteins from the Inner and Outer Surfaces of NR Latex Examination Gloves. *J. nat. Rubb. Res.*, **8(2)**, 154.

11. FARIDAH YUSOF AND YEANG, H.Y. (1992) Quantitation of Proteins from Natural Rubber Latex Gloves. *J. nat. Rubb. Res.*, **7(3)**, 206.

12. YEANG, H.Y., SUNDERASAN, E. AND SHAMSUL BAHRI, A.R. (1994) New Approaches in the Quantitation of Total Proteins from Latex Gloves. Paper presented at the *Workshop on Latex Protein Allergy, 9 June 1994, Kuala Lumpur, Malaysia.*

13. GORTON, A.D.T. (1988) Faults in Examination Gloves, *Notes on NR Examination Glove Manufacture*. (Foo, K.Y. and Chuah, P.G., eds.), 42. Rubber Research Institute of Malaysia.

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

15. WONG, N.P. (1988) Formulating Latex Compounds, *Notes on NR Examination Glove Manufacture. (Foo, K.Y. and Chuah, P.G., eds.)*, 22. Kuala Lumpur: Rubber Research Institute of Malaysia.

16. MORALES, C., BOSOMBA, A., CARREIRA, J. AND SASTRE, A. (1989) Anaphylaxis Produced by Rubber Glove Contact: Case Reports and Immunological Identification of the Antigens Involved. *Clin. Exp. Allergy*, **19**, 425.