ANALYSIS OF EXTRACTABLE PROTEINS IN RUBBER PRODUCTS

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Natural latex dipped products contain two kind of proteins.

1. Proteins that are tightly bound to the rubber particles.
2. Serum - derived soluble proteins remaining after leaching/washing procedures during manufacture.

The later gives rise to the most of the extractable proteins.

The value of extractable proteins may vary from 30 mg/kg to over 700 mg/kg in an examination glove depending on the processing conditions. Gloves made from double-centrifuged latex have shown a remarkable reduction in latex extractable proteins.

As the cause for most of the allergic problems is the water-extractable proteins, it must be possible theoretically, to prevent allergenicity by extracting the proteins from the products during manufacture.

The following describes various methods and procedures for removing and analyzing extractable proteins.

Colourmetric methods of estimating extractable proteins levels

1.	Bradford method	-	Dye-binding (U)
2.	Lowry method	-	reduction of tyrosine (*etc.*) peptide bonds(D)
3.	Ninhydrin method	-	hydrolyse to amino acids

U - Requires undialysed extracts
D - Requires dialysed extracts

RRISL follows the Bradford method to analyse extractable proteins in rubber products. Work at MRPRA-UK has mainly utilized the Bradford method whereas that at the RRIM has used the Lowry method. Although the two methods give different values for extractable protein, their results correlate well. There are certain advantages and disadvantages between two methods.

Both methods are simple and rapid but the Bradford assay has an advantage in that it is faster to perform and that only a single reagent is required whereas the Lowry assay employs two reagents. It is also convenient that a standardized reagent for Bradford assay is commercially available.

The sensitivity of a protein assay is dependent upon the protein being assayed and the Lowry assay is superior to Bradford assay in this regard *e.g.* the photometric absorbance reading in Lowry assay approximately twice that of Bradford assay. Also Lowry assay gives a markedly less variable response to different proteins.

The Lowry assay is again advantageous when the high stability of the assay mixture is concerned.

However, RRISL follows the Bradford assay since the facilities such as a dialysis unit required for the Lowry assay is not available at the RRISL.

It has been shown that a very high proportion of the extractable proteins is removed early in the first wash and that further washes extract only small-but easily measurable amounts. This suggests that the readily removable protein is situated at the surface of the product and that the proteins subsequently extractable is diffusing out from the interior of the product. The question of whether protein continues to diffuse towards the surface in dry, washed, products eg during storage, is yet to be resolved.

The results of extractions have shown that a high proportion of the extractable protein can be removed very quickly and easily and there is therefore no excuse for manufacturers to supply products containing high proportions of extractable proteins. Any such products on the market serve only to damage the reputation of all latex products and to put future growth in jeopardy.

The extraction of proteins by wet-gel leaching over a limited range of times and temperatures has shown a positive effect of both increasing temperatures and increasing times. It has also been found, however, that there is a significant amount of extractable proteins remaining in the product. This suggests that wet-gel alone-except perhaps under extreme conditions - may not be sufficient to reduce protein levels to insignificant amount. There is a trend that harder waters extract less proteins.

Various chemicals are known to react with proteins to cause precipitation, crosslinking *etc. e.g.* 4.5% trichloroacetic acid. Products which are normally chlorinated *e.g.* catheters, teats/soothers generally exhibit very low levels of extractable protein. The chlorination process, of course, uses an aqueous solution of chlorine and represents an extra wash or leach. This method is practiced effectively in glove factories and the recommended procedure is given below.

For the process, gloves must be dipped in a solution containing free chlorine. Chlorine can be made available either by dissolving chlorine gas directly in water or by reacting a solution of hypochlorite with HCl acid. The concentration of free chlorine normally used is between 0.05% to 0.10%. For thicker surfaces, a higher concentration can be used where the colour is not significant.

A 0.10% chlorine solution can be prepared as follows:

	Parts by weight
Sodium hypochlorite (10%)	1.0
Concentrated Hydrochloric acid	0.3
Water	98.7

The proportion of gloves to water ideally should be between 1:10 to 1:40. After chlorination, the solution should be drained off and the gloves washed with water. The gloves are then dipped in a solution of ammonia (1%) to neutralise any residual acid. This process should be rapid in order to avoid the darkening effect of ammonia on gloves. It is again washed with water. The pH of water (about 6.8 -7.0) is checked to ensure adequate neutralization.

The gloves should then be tumble dried at low temperature (50 °C - 60 °C). The gloves should be cooled and aired before packing.

Sample Analysis

Extraction

1 g or more, of latex article in large pieces to reduce edge effect is extracted with 8 ml 0.85% NaCl (physiological saline) for 2-3 h with occasional shaking (temperature 35 °C). The resulting extract is centrifuged to remove powder and portions (equivalent to 30-100 mg of article) assayed in duplicate.

Protein assay

Bradford method : This dye binding method is well known and can be obtained as a kit from Bio-Rad. This kit is not used at the RRISL. But RRISL makes it's own reagent. The dye solution (Sigma Brilliant Blue G) or sigma 0770 is mixed with AR ethanol and phosphoric acid and stirred ½h to help dissolve the dye. The absorbance at 550 nm should be measured and should be >10.5 This wave length is the isosbestic point where the cationic and anionic dye-species have the same light absorption and so the absorption is a measure of the total dye concentration.

Assay: 0.4 ml of 0.85% NaCl solution and 3.6 ml of dye are mixed and the spectrophotomer is set to zero absorbance on this solution at 595 nm. 0.4 ml of

extract and 3.6 ml of dye are mixed and the time noted; the absorbance (A) is then read 10 min after this time. The readings can be converted to protein concentration using a calibration curve prepared with a standard bovin albumin serum which gives A 595 nm about 0.3 at a concentration of 20/kg per 0.4 l under our conditions.

Levels of proteins

The amount of water extractable proteins (EP) from latex film is very much lower than the actual amount of proteins present in the rubber. The EP content of clonal HA latex film is only about 0.04 mg/g rubber whereas the total protein content of HA latex concentrate is about 20-26 mg/g rubber. Compounding the latex could increase the amount of EP to about 0.14 mg/g rubber. Heating the compounded latex could increase the level of EP further to about 0.34 mg/g rubber.

The amount of EP decreases significantly on chlorination *e.g.*

Sample	EP mg/g (Lowry assay)
Control	0.859
0.01% Cl	0.023
0.05% Cl	0.011
0.30% Cl	0.021

The concentration of chlorine has an insignificant effect on EP content.

The table below shows the effect of double centrifuging, leaching and chlorination on the EP of gloves made of different prevulcanized latices (Source: Paper No.6 of Latex protein workshop held in Malaysia in 1993).

Treatment	EP mg/g	
	HA PV	DC PV
WL	0.481	0.229
DL	0.100	0.110
WDL	0.097	0.081
WLC	0.016	0.007
DLC	0.008	0.007

WL - Wet gel leaching in water, 70 °C/5 min
DL - Dry film leaching in water about 1 hour
WDL - Wet gel leaching.followed by dry film leaching
WLC - Wet gel leaching.followed by chlorination
DLC - Dry film leaching followed by chlorination

HA PV - Single - centrifuged high ammonia prevulcanized latex
DC PV - Double centrifuged high ammonia prevulcanized latex

Extractable protein contents of a total sample size of 84 examination gloves have been analysed at RRIM using Lowry assay and this value was found to vary from 0.019 mg/g to 1.816 mg/g. These include some differently leached and enzyme - treated samples, in addition to normal and chlorinated gloves. The range of the gloves analysed at RRISL using Bedford assay varies from 0.011 mg/g to 0.085 mg/g.

Finally, it should be mentioned that, in response to the problem of allergic proteins in dipped goods, factors controlling the level of extractable proteins have been studied by several workers and it has been shown that wet gel leaching, chlorination, and the vulcanization system can affect protein levels in dipped latex products. There is no doubt that the allergens causing anaphylactic reactions in sensitive persons are latex proteins remaining in the articles after manufacture and there is general agreement that it is desirable to lower the protein content as far as practically possible, although it must be stressed that it is not yet known whether there is a 'safe' level.

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