

Extraction of antigenic protein from latex films and gloves

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Abstract

Reliable methods by which to measure latex proteins are necessary if manufacturers hope to reduce the levels of protein allergens in latex medical devices. Colorimetric protein assays are plagued by problems with interfering substances and lack of sensitivity. Immunological methods have the advantage of increased sensitivity and specificity for latex proteins. In this study, the extraction of antigenic latex proteins from latex films and products was investigated by using the latex ELISA for antigenic latex proteins (LEAP®). The following extraction parameters were evaluated with respect to the levels of antigenic latex proteins in the resulting extracts: pH, volume and time of extraction. The extraction of latex proteins from films is enhanced at higher pH levels (>7.0). Extraction of latex proteins from latex gloves, however did not show the same pH dependency. This study yields data which is useful for establishing latex protein extraction parameters prior to protein testing of end products with the ELISA method. In addition, a better understanding of protein extraction from latex films and products should be useful to individuals involved with manufacturing leaching processes.

Introduction

With increased awareness of latex protein allergy, procedures for testing extractable latex proteins have been developed.¹⁻³ The latex ELISA for antigenic proteins (LEAP®) has proven to be a very useful tool for quantifying the level of antigenic protein in latex extracts.⁴ The extraction of proteins for this type of testing is normally carried out in buffered solutions in order to increase protein stability. This study extends an earlier study which investigated extraction parameters using total protein analysis methods (Bradford and Lowry) with detection limits of approximately 4µg/ml.⁵ By using the LEAP assay (detection limit of approximately 5ng/ml) to measure antigenic protein levels in the extracts, various extraction parameters were evaluated. With the lower detection limits of the LEAP assay, analysis can be performed on latex gloves containing lower levels of extractable protein.

Materials and methods

Preparation and extraction of latex films and gloves

Latex films were produced by spreading ammoniated latex evenly onto a clean glass plate, and allowing it to air dry overnight. In some experiments, the films were oven dried for one hour at 70°C after air drying overnight. Both, latex films and commercially obtained gloves were weighed, cut into small pieces (2-5cm²) and extracted with various test solutions in polypropylene containers using constant mixing. To avoid skewing the results, latex beads around the glove opening were removed from the glove before weighing and extraction.

LEAP (Latex ELISA for antigenic proteins)

The LEAP assay was performed as previously described.⁴ In brief, latex reference protein and samples containing latex proteins were absorbed onto the inner surface of 96 well polystyrene ELISA plates (Corning #25805-96) by incubating the plates four hours at 37°C. Plates were then washed one time with phosphate buffered saline (PBS) at pH 7.2 containing 0.1% Tween 20 (TPBS), after which the unreacted sites were blocked with 5% bovine serum albumen (BSA) in TPBS for one hour. The plates were washed, incubated overnight at 37°C with rabbit anti-latex protein antiserum, washed three times and then reacted with peroxidase-labelled goat anti-rabbit IgG (Sigma #A-0545) for 1 hour at 37°C. After carefully washing the plate for the last time, a coloured reaction was produced by using orthophenyldiamine (OPD, 1mg/ml) in H₂O containing 0.1% H₂O₂. The reaction was stopped by the addition of 50µl of 4N sulphuric acid and the coloured product was quantitated by reading the absorbance at 490nm using an MR 5000 ELISA plate reader (Dynatech, Chantilly, VA). The concentration of latex protein in unknown samples was determined by comparing absorbance units of unknowns to the latex protein standard curve at three consecutive dilutions. The lower part of the calibrated curve was used to avoid interference which may be noted when using less dilute samples.

Results/Discussions

Extraction buffer comparisons

To probe potentially important parameters influencing extractability of antigenic proteins from latex, air-dried

Table

Film and glove extraction with various buffers. (Films and gloves were extracted for two hours at 37°C.)

	pH	Molarity	LEAP, µg/g ^a								
			Films						Gloves		
			Air-dried			Oven-dried ^b					
			Lot I	Lot II	Lot III	Lot I	Lot II	Lot III	Brand A	Brand B	Brand C
Distilled water	not buffered	not buffered	110	1748	2621	301	367	213	0.7	150	225
Acetate buffer	5.5	0.1M	370	522	59	607	449	477	1.2	209	555
Phosphate buffered saline	7.2	0.01M PO ₄ - 0.15M NaCl	825	1591	1692	673	832	379	1.8	172	229
Carbonate buffer	9.6	0.05M	6080	12045	21176	5591	6966	13608	0.9	204	1110

a. Results are given as µg of antigenic protein per g of rubber.

b. Oven dried films were heated for 1 hour at 70°C.

latex films were made from ammoniated latex. The films were extracted for two hours at 37°C using various solutions chosen for protein compatibility. Antigenic latex protein levels in the extracts were quantitated using the LEAP assay. Levels of extractable protein are given in the Table. This data indicates that high pH buffers (phosphate buffered saline, pH 7.2 and carbonate buffer, pH 9.6) extracted the highest levels of antigenic protein, while the low pH solution (acetate buffer, pH 5.5) was less efficient at extracting antigenic proteins from air-dried latex films. The distilled water extract could not be used to draw conclusions on pH, since the pH would depend on the latex film properties. Films that had been heated at 70°C showed lower levels of extractable protein in almost every case. As with the air dried films, increasing the pH of the leaching solution increased the levels of extractable protein.

The manufacturing of latex gloves involves leaching steps. Leaching results in lower levels of extractable latex proteins in the final products. To determine what effects various solutions have on the extraction of latex proteins from end products, three different brands of gloves were extracted using the same solutions. As shown in the Table, lower levels of proteins were extracted from the gloves than from films. This was not unexpected, since gloves are leached during manufacturing. The amount of extractable protein, however, varied significantly between the brands tested. Interestingly, the glove experiments do not show a distinct advantage in using a higher pH (>7) buffers. This is, however, consistent with studies by others which show no significant difference between dilute base, dilute acid or water rinsing of exam gloves.⁵ The extraction difference between air-dried films and gloves, regarding pH, is most likely due to solubility characteristics of the latex proteins. This difference is significant for manufacturers. For films, the difference between extraction levels using water vs

carbonate buffer (pH 9.6), suggest that high pH buffered solutions will greatly increase the efficiency of in-process leaching and therefore should result in lower protein levels in the end products. Although extended leaching times and low temperatures (compared to industrial glove processing) were used in the experimental conditions to ensure maximal extractability, high pH leaching appears to be most effective if carried out prior to oven drying steps.

Effects of extraction volume

The previous experiments were performed in order to define the optimal protein leaching conditions that may be used in manufacturing processes. Determining the extractability of proteins from end products is very important in light of reports of patients with extreme latex protein sensitivity. To mimic physiological conditions, phosphate buffered saline at 37°C was used. Volume and time parameters were investigated using three glove brands, known to have different levels of extractable proteins. To test the effect of volume, each glove brand was extracted overnight at 37°C in PBS, using increasing volumes of extraction buffer. Separate gloves were used for each data point, therefore the experiment was repeated twice to assess glove-to-glove variability. As seen in Figure 1, extractable protein levels were most consistent with the two glove brands containing the highest protein levels. The relative standard deviation between the various extraction volumes for glove brands A, B and C was 133% (0.1–6.4µg/g), 34% (16–41µg/g) and 35% (250–800µg/g), respectively. Although brand A showed the greatest relative standard deviation, it also contained the lowest levels of extractable antigenic latex proteins. Considering variability, total extractable protein was independent of the volume used for extraction.

Figure 1

Varying extraction volume. Three separate glove types were extracted overnight at 37°C using PBS. Separate gloves were used for each data point. The experimental protocol was carried out twice. The LEAP assay was used to quantitate levels of extracted proteins in terms of µg of antigenic protein per gram of rubber.

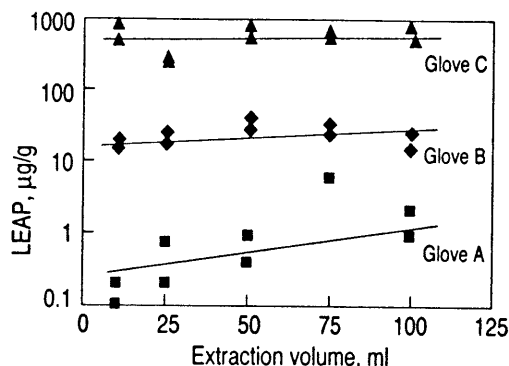
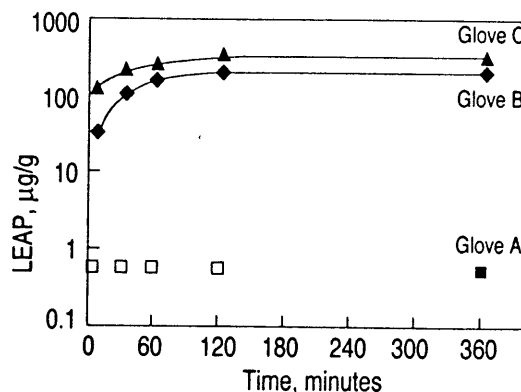


Figure 2

Varying extraction time. Three separate glove types were extracted with PBS at 37°C for various lengths of time (5, 30, 60, 120 and 360 minutes). The LEAP assay was used to quantitate levels of extracted proteins in terms of µg of antigenic protein per gram of rubber. Six grams of glove was extracted with 20ml of PBS. At each time point, 100µl of extract was removed, centrifuged and then tested by LEAP assay. Open squares indicate less than values.



Kinetics of extraction

Testing of extraction time was carried out by extracting the same three glove brands for different lengths of times at 36°C. As seen in Figure 2, the latex proteins were rapidly extracted from the gloves. A steady increase in protein level was noted in the extract up to 120 minutes, after which time little additional protein was extracted. This data is in agreement with total protein results from other studies.^{4,5}

Efficiency of extraction

Efficiency of extraction was tested by performing sequential extractions. Glove brand C, which contained the highest level of extractable latex proteins, was used for this set of experiments. The experimental protocol involved the extraction of three separate gloves for 24 hours using three different extraction volumes (10, 20 and 30ml). The initial extraction was then followed up with three sequential one hour extractions using fresh PBS for each extraction, with a water rinse between extraction steps. Figure 3 shows the measured latex protein levels in the different extractions. Calculations indicate that the first extraction step yields a large percent of the total extractable text proteins; 92, 83 and 86% for 10, 20 and 30ml extraction volumes, respectively. Conclusions from this data include: a) the initial 24 hour extraction produced comparable yields to the two hour extractions using the same glove brands (see Figures 1 and 2) and b) although a single extraction removes >80% of the protein, additional protein can be recovered in subsequent extractions. This data also indicates that gloves from brand C may vary ten fold in extractable protein levels, even within the same lot.

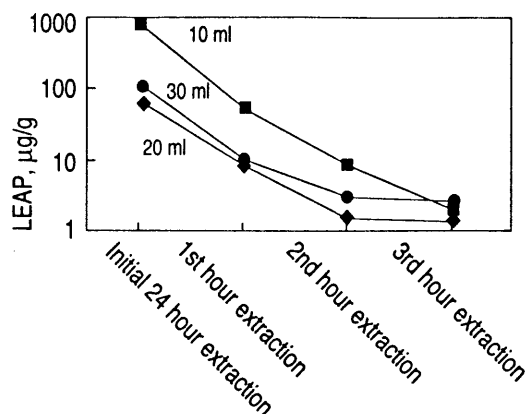
Conclusions

We have examined various parameters influencing protein extraction from both latex films and gloves. Tests conducted on extractability of antigenic protein with air-dried latex films showed maximum protein extraction with high pH solutions. However, latex glove end products did not show this same pH dependency. As observed by others,⁵ this difference may be related to the time and temperature of the drying steps used during the production of the gloves. In addition, vulcanization, presence of calcium nitrate, process leaching steps and latex protein solubility characteristics may be involved. Above all, the extraction of latex proteins from gloves occurs quickly at 37°C. Additional points regarding the extraction of proteins from gloves include: a) extracted protein level was constant when the extraction volume was varied from 1–10ml per gram of latex (Figure 1); b) two hour extraction time showed little advantage over a 24 hour extraction period (Figures 2 and 3) and c) single extraction step recovers greater than 80% of the extractable latex protein (Figure 3). Although not tested, thorough mixing is important in any extraction system.⁵ Others have shown greater than 10 fold differences in protein levels between inner and outer surfaces of the same glove,^{5,6} thus, cutting the whole glove into smaller pieces is considered an integral step in the extraction procedure.

Future work with extraction buffers prior to protein determination should focus on the stability of latex proteins in the extracts. This may be an important factor since repeat testing of extracts is often required. Finally, a standardized protein extract would be useful for inter- and intra-laboratory comparisons of latex protein testing methods.

Figure 3

Efficiency of extraction with PBS. Three separate brand C gloves were extracted with 10, 20 or 30 ml of PBS for 24 hours at 37°C. The initial 24 hour extract was removed and fresh PBS (same volume as originally used) was incubated at 37°C with the latex for one hour. The removal of extract, addition of fresh PBS and one hour incubation was repeated for a total of three times. The LEAP assay was used to quantitate levels of extracted proteins in terms of µg of antigenic protein per gram of rubber.



Acknowledgements

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Friction on ice (A. Roberts)

Laboratory measurements show that the average temperature rise produced on an ice surface by the passage of a rubber slide is limited by rubber hardness. Temperature rise measurements and observations made during sliding experiments help to explain this in terms of the rubber-ice micro contact area. The results are relevant to the grip of tyres on icy roads.

Temperature rise produced on an ice surface by a rubber slider, Roberts, A., *Journal of Natural Rubber Research*, 1993, **8**, 163-75.

Publication 1496

Prevulcanization (R. Davies/K. Gazeley)

A peroxide prevulcanization method involving t-butyl hydroperoxide (tBHP) with fructose as an activator was assessed for its suitability for commercial applications. The effects of prevulcanization temperature, concentration of tBHP, fructose/tBHP ratio and intrinsic iron content on the rate and extent of prevulcanization were investigated. Addition of the tBHP in several stages throughout the prevulcanization, instead of one aliquot at the start, improves the overall crosslinking efficiency. Peroxide prevulcanized latex can be used in conventional coagulant dipping and

heat-sensitive dipping processes to produce films with similar unaged properties to those films made from a low modulus sulphur prevulcanizate.

Peroxide prevulcanization of natural rubber latex with a t-butyl hydroperoxide, Davies, R., and Gazeley, K., *Journal of Natural Rubber Research*, 1993, **8**, 176-89.

Publication 1497

Noise control (H. Ahmadi/A. Muhr)

Structure-borne vibrations are converted to airborne noise when they excite vibration of a surface, such as a metal plate. The surface may form the path of the vibrational waves, or it may be set in resonance by passage of the waves through a structure to which it is attached. Some results are reported for damping treatments, based on unvulcanized epoxidized NR applied to a freely suspended steel beam. The technique involves resonance rather than travelling waves, but the incorporation of damping reduces both types of problem.

Use of rubber to suppress ringing of metal components, Ahmadi, H., and Muhr, A., Institute of Materials Conference 'Quieter transport with rubber. Shock, vibration and noise control for the benefit of user and environment', University of the West of England, Bristol, April 1994.

Publication 1498