Surfactant Treatment Reduces Both Allergen Content and Cure Efficiency of *Hevea* Latex

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Of the major allergens in latex produced by *Hevea brasiliensis* Muell. Arg., Euphorbiaceae (NR latex), at least two are bound to the surface of the rubber particle (Kurup and Fink 2001): hevein b 1 (Hev b 1), a 14.6-kDa protein associated with large rubber particles, and Hev b 3, a 22.3-kDa protein associated with small rubber particles. A washing operation is effective (Fig. 1) in reducing the total protein level of NR latex films to below that of films produced from the latex of guayule (*Parthenium argentatum* Gray, Asteraceae) (GR latex) (Schloman et al. 1996). Although washed latex has a relatively low protein content, its membrane-bound allergens remain.

Surfactants such as Triton X-100 have already been used to strip proteins bound to the surfaces of rubber particles prior to assay (Siler and Cornish 1995). Would surfactant treatment alone be an effective way of producing bulk samples of deproteinized latex? If so, how would such deproteinized latex function as a source of hypoallergenic dipped films?

**METHODOLOGY**

**NR Latex**

Low-ammonia NR latex (62% solids) was obtained from Guthrie Latex, Inc., Tucson, Arizona.

**Control Procedures**

To prevent protein cross-contamination, several precautions were taken. Equipment and samples were handled with 100% nitrile gloves. Separate coagulant solutions, dusting powder, and leaching baths were used for each latex or latex recipe. All glass and plastic ware coming into contact either with a latex was washed in a 1% w/v aqueous detergent solution, then immersed in 10% v/v bleach for a minimum of 2 hr. All other equipment, including oven interiors and bench tops, were washed with detergent or covered with aluminum foil, where appropriate. Dipped films were stored in sealable polyethylene bags.

**Washed Latex**

To remove serum proteins, a 20% latex stock was prepared from 176 g of NR latex and 370 g of 0.33% aqueous ammonia. A 50-mL polypropylene centrifuge tube was charged with 29.3 g of the 20% latex stock. The diluted latex was centrifuged at 4,000 × g for 8 min. A 12-ga stainless steel needle was used to aspirate off the lower layer (19.4 g), which was then replaced by an equal weight of 0.33% aqueous ammonia. The mixture was gently agitated, centrifuged as before, and the stage 2 lower layer (21.1 g) removed by aspiration. This process was repeated twice to yield 20.3 g of a stage 3 lower layer and 20.2 g of a stage 4 lower layer. The latex layer (21.0 g, 49% solids) was transferred to a glass bottle and stored at 4°C.

In a scale-up run, a 500-mL polycarbonate centrifuge bottle was charged with 371 g of the 20% latex stock and 33 g of 1% (w/w) aqueous ammonium alginate.

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The bottle was sealed and gently agitated to mix the contents. The mixture, 0.1% in alginate, was centrifuged at $4,000 \times g$ for 8 min. A 12-ga stainless steel needle was used to aspirate off the lower layer (245 g), which was then replaced by an equal weight of 0.33% aqueous ammonia containing sufficient ammonium alginate to adjust the final alginate content of the diluted latex serum to 0.1%. As before, the mixture was gently agitated, centrifuged, and the stage 2 lower layer (242 g) removed by aspiration. This process was repeated to yield 224 g of stage 3 lower layer. Finally, the alginate content was adjusted to 0.05%. Recovery of the stage 4 lower layer after centrifugation at $4,000 \times g$ for 12 min was 244 g. The latex layer (156 g, 45% solids) was transferred to a glass bottle and stored at 4°C.

**Surfactant-Treated Latex**

To remove both serum and particle-bond proteins, 29.3 g of washed latex (45% solids), 40.0 g of 10% aqueous Triton X-100, and 400.0 g of 0.33% aqueous ammonia were charged to a 600-mL beaker and stirred for 60 min by means of a propeller-type agitator. To this was added 31.1 g of 1% ammonium alginate. A total of 322.8 g of the mixture was decanted into a 500-mL separatory funnel and allowed to stand overnight. The lower layer (276.9 g) was drawn off and replaced by 19.8 g of 0.33% aqueous ammonia to produce a dry rubber content of 20%. Sufficient 1% ammonium alginate (1.8 g) was added to adjust the final alginate content of the diluted latex serum to 0.1%. After gentle agitation, the mixture was again allowed to stand overnight. The stage 2 lower layer (33.3 g) was drawn off. This process was repeated to yield 37.7 g of stage 3 lower layer. Finally, the alginate content was adjusted to 0.05%. Recovery of the stage 4 lower layer after centrifugation at $4,000 \times g$ for 12 min was 45.8 g. The latex layer (21.3 g, 49% solids) was transferred to a glass bottle and stored at 4°C.

In a scale-up run, 160 g of NR latex, 160 g of 10% aqueous SDS, and 1600 g of 0.33% aqueous ammonia were added to a 2-L Erlenmeyer flask and stirred for 60 min by means of a magnetic stirring bar. To this was added 166.8 g of 1% ammonium alginate. A glass tube was introduced into the flask to act as a drain during aqueous phase removal. The mixture, 0.1% in alginate, was allowed to stand overnight to effect creaming of the latex particles. The lower, aqueous phase (1032 g) was aspirated off. The recovered rubber phase (703 g) was transferred to two 500-mL polycarbonate centrifuge bottles and centrifuged at $4,000 \times g$ for 4 min. A 12-ga stainless steel needle was used to aspirate off the stage 2 lower layer (505 g). The recovered rubber phase (197 g) was diluted with 0.33% aqueous ammonia to a dry rubber content of 20%. Sufficient 1% ammonium alginate was added to adjust the final content of the diluted latex serum to 0.1%. Alginate was used for this and the final stage in order to obtain a clearer phase separation. After gentle agitation, the mixture was centrifuged at $4,000 \times g$ for 4 min. The stage 2 lower layer (306 g) was removed by aspiration. Aqueous ammonia and 1% ammonium alginate were again added to adjust the alginate content to 0.1%. As before, the mixture was gently agitated, centrifuged, and the stage 3 lower layer (442 g) removed by aspiration. Finally, the alginate content was adjusted to 0.05%. Recovery of the stage 4 aqueous phase was 290.3 g. The washed latex (175 g, 49% solids) was transferred to a glass bottle and stored at 4°C. This material was used for dipped film preparation.

**Compounding and Prevulcanization**

Latex was filtered through a 100-mesh PET cloth into a 250-mL beaker. With the exception of antioxidant, compound components (Table 1) were then added. The mixture was heated to 65°C over a 30-min period with mixing by PTFE-coated magnetic stirring bar. The compound was then maintained at 65°C for 2 hr (untreated latex and washed latex) or 4 hr (surfactant-treated latex). Antioxidant was added at the conclusion of prevulcanization.

**Table 1.** Latex dipping compound formulation.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Weight, parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>100.0</td>
</tr>
<tr>
<td>10% Potassium hydroxide solution</td>
<td>0.5</td>
</tr>
<tr>
<td>33% Surfactant(^\text{z})</td>
<td>2.0</td>
</tr>
<tr>
<td>68% Sulfur dispersion</td>
<td>0.8</td>
</tr>
<tr>
<td>60% Zinc oxide dispersion</td>
<td>0.75</td>
</tr>
<tr>
<td>Dithiocarbamate solution(^\text{y})</td>
<td>2.25</td>
</tr>
<tr>
<td>Water</td>
<td>4.5</td>
</tr>
<tr>
<td>65% Antioxidant emulsion(^\text{x})</td>
<td>1.0</td>
</tr>
</tbody>
</table>

\(^\text{z}\) 1:1 Darvan SMO + Darvan WAQ.
\(^\text{y}\) Setsit 104.
\(^\text{x}\) AgeRite Superlite.
Dipped Films
Borosilicate glass test tubes, 16 × 150 mm, were preheated in an oven maintained at 65°C. The tubes were dipped in 18% w/w alcoholic Ca(NO₃)₂·4H₂O coagulant, then air dried for 1 min with manual rotation to assure even distribution. The coagulant-coated tubes were immersed for 20 sec in prevulcanized latex compound maintained at ambient temperature, then air dried with rotation for 2 min. Between dips, the compound was gently agitated. A two-stage leach followed: 2 min in 900 mL of water maintained at 60°C and 2 min in 900 mL of water maintained at 70°C. After air drying for 30 sec, the upper edge of each film was rolled down to form a bead. The coated tubes were then dried for 20 min in a forced-air oven maintained at 104°C. The exposed surfaces of the dried films were brushed with USP/FCC calcium carbonate. Finally, the films were pulled from the tubes in such a way that the films were turned inside out.

Rubber Analysis and Physical Testing
To determine solids contents, 1-g samples of latex were coagulated by the addition of 6.0 M acetic acid. The crude rubber was rinsed in water, sliced into 1- to 2-mm wide strips, then dried in a 700-W microwave oven for 5 min at 50% power, followed by 10 min at 40% power.

Cured film crosslink densities, expressed as \( n \), the number of active network chain segments per unit volume, were determined by equilibrium swelling using the Flory-Rehner equation (Sperling 1992):

\[
n = \frac{-[\ln(1 - v_2) + v_2 + \chi v_2^2]}{V_1(\frac{1}{v_2} - 0.5v_2)}
\]

where \( v_2 \) is the volume fraction of polymer in the swollen mass, \( V_1 \) is the molar volume of the solvent, and \( \chi \) is the polymer-solvent interaction term. Test pieces were 25-mm diameter circles swollen in cyclohexane (\( \chi = 0.31 \); Queslel et al. 1988). For determination of the mass and volume of crosslinked rubber, the swollen gel was deswollen with ethanol and dried to a constant weight in a 70°C oven.

Protein Contents
Total protein (modified Lowry, ASTM D5712-95) and allergen (LEAP™ ELISA) assays were performed by the LEAP Testing Service of the Guthrie Research Institute.

RESULTS
A 4-stage washing sequence (dilution-centrifugation-redilution) reduced the total serum allergens in commercial NR latex by 53%. By comparison, an initial treatment with Triton X-100 followed by a 4-stage washing sequence (alginate creaming-phase separation-redilution) reduced serum allergens by 93% (Fig. 2). Treatment with SDS reduced the allergen level by 92%.

Surfactant treatment was an effective means of solubilizing particle-bound proteins. LEAP™ ELISA assay indicated that a representative 40-g sample of NR latex contained 24 mg of allergenic proteins in its serum (aqueous phase). The lower, aqueous phase obtained by creaming latex after treatment with Triton X-100 contained 601 mg of allergenic proteins, the bulk of which presumably had been displaced from the surfaces of latex particles. The serum of the final latex product, obtained after the 4-stage washing sequence, contained 1.8 mg of allergenic proteins.

It appeared, then, that surfactant treatment could be useful in lowering the protein level in NR latex. The impact of such treatment on the properties of dipped films pro-

Fig. 2. Serum allergen levels in NR lattices.
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duced from such latex was therefore of particular interest.

Unwashed, washed, and surfactant-treated NR latices were compounded as reported earlier (Schloman et al. 1996). Dipped films were then prepared from each latex after prevulcanization at 65°C. Surfactant-treated latex did not give a stable film when prevulcanized for the standard period of 2 hr. Instead, a 4-hr prevulcanization was used. Figs. 3 and 4 illustrate the total extractable protein and allergen levels detected in the cured films.

Equivalent levels of extractable allergens were obtained from both the unwashed and washed NR latex films (Fig. 4). Although washing could not remove these allergens, the conditions of compounding and dipping apparently rendered them susceptible to extraction from a cured film.

Dipped film prepared from surfactant-treated latex contained high levels of both total proteins (Fig. 3) and allergens (Fig. 4). The film prepared from surfactant-treated latex yielded 1800 mg of allergen per gram of rubber, 15 times the quantity (120 mg/g) extracted from the control prepared with unwashed NR latex. This allergen level is 25 times higher on a dry solids basis than the 71 mg/g detected in the surfactant-treated latex from which the film was prepared (Fig. 2).

Vulcanization of surfactant-treated latex was very inefficient. The highest state of cure, as measured by crosslink density (Fig. 5), was obtained with unwashed NR latex. A significantly lower state of cure was obtained with washed latex. Surfactant-treated latex had the lowest crosslink density of all. The extent of vulcanization correlates well with both total latex serum proteins ($r^2 = 0.994$) and latex serum allergens ($r^2 = 0.987$). The more protein in NR latex, the better it cured.

Crosslink development with surfactant-treated latex was nonetheless complete after dipping and drying. Heat aging did not increase the crosslink density (Fig. 6), as has been reported for GR latex (Schloman et al. 1996).

**DISCUSSION**

Surfactant treatment is an effective means of solubilizing particle-bound proteins, but at the cost of reduced vulcanization efficiency. With a conventional cure recipe, the ultimate state of cure attained with deproteinized NR latex is substantially lower than that attainable with commercial low-ammonia NR latex.

Most importantly, the level of extractable allergens is substantially higher in films prepared from surfactant-treated latex. One possible explanation relates to the quality of the dipped film itself and how a continuous film is formed from the discrete rubber particles in the latex (Fig. 6). As a wet latex film dries, water-soluble proteins migrate to the surface (Yeang et al. 1995), where they concentrate (Grote et al. 2000). The rubber particles aggregate. Serum proteins then coat the surface of the rubber particles, which may already bear their own complement of bound proteins. As final coalescence to a continuous film occurs, the more polar protein phase tends to separate from the non-polar hydrocarbon of the rubber phase. The result is a dried film with

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**Fig. 3.** Total extractable proteins from dipped NR films. Means of three measurements. Error bars indicate 1 standard deviation.

**Fig. 4.** Extractable allergens from dipped NR films. Means of three measurements. Error bars indicate 1 standard deviation.
proteins concentrated at various surface features (Grote et al. 2000). This protein-enriched surface readily yields material for extraction during the various assay procedures.

In a poorly-cured film, serum proteins are free to coat the surfaces of the drying rubber particles as they slowly aggregate and coalesce into a continuous solid mass. The proteins are then readily accessible for dissolution and extraction. Interparticle crosslinking may be adversely affected as well.

A well-cured film is necessary to meet the performance specifications established for finished rubber goods such as gloves. A well-cured film has fewer physical imperfections than a film with a comparatively low crosslink density: fewer holes and tears, a lower surface area.

Additional washing would reduce residual protein levels in the surfactant-treated latices. However, these lower protein levels would necessitate even closer attention to latex cure properties. One approach to improving film quality would involve increasing crosslink density through adjustments in the cure package—the levels of sulfur, accelerator, and zinc oxide. Further investigation would be necessary to establish whether such adjustments produce crosslink densities equivalent to that obtained with conventional NR latex. Alternatively, aggressive cure systems such as those used with enzymatically-deproteinized NR latex (Nakade et al. 1997) might be useful.

REFERENCES