CHARACTERIZATION AND PERFORMANCE TESTING OF GUAYULE LATEX

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ABSTRACT

Physical and biological properties were determined for a preliminary production run of low-ammonia latex from guayule (GR latex). GR latex, and dipped films produced from compounded GR latex, contain none of the *Hevea* allergens known to elicit Type I systemic allergic reactions. The polymer phase of GR latex has a lower bulk viscosity and a higher resin content than that of NR. In addition, GR latex serum and rubber lack the activating components present in NR latex. As a result, GR latex cures more slowly and reaches a lower state of cure. The aged stress–strain properties of cured GR films are nonetheless similar to those of NR films.

INTRODUCTION

Products made from the latex of *Hevea brasiliensis* Muell. Arg. (NR latex) are responsible for causing immediate hypersensitivity reactions, including Type I systemic allergic reactions, in susceptible individuals.[1] Current estimates derived from analysis of blood donor sera indicate that up to 6.5% of the U.S. population may be hypersensitive at present.[2] Attempts to identify latex allergens have implicated proteins with sizes ranging from 2 to 100 kD and include both soluble and rubber particle-bound proteins.[3-5] Extracts of finished goods such as latex gloves contain these same allergens.[6] The magnitude of the allergic response can depend on the extent to which the proteins have been denatured during processing.[3] Direct contact with rubber goods is not necessary to elicit an allergic response. For example, latex proteins bind to the cornstarch used as a glove lubricant, allowing airborne transport of the allergens when gloves are put on or removed.[7] Extractable latex allergens have also been isolated from respirable particles, possibly tire fragments, in urban air samples.[8]

Producing a hypoallergenic NR latex requires the extensive removal of both rubber particle-bound and soluble latex proteins.[5] However, latex allergenicity is species-specific. The latex of guayule (*Parthenium argentatum* Gray) (GR latex) does not elicit an allergic response in individuals with severe hypersensitivity to NR latex.[9-11]

We report here the physical and biological properties of a preliminary production run of low-ammonia GR latex. The performance of the GR latex in the preparation of dipped films was compared to those of a conventional NR latex and a low-protein NR latex. Approaches to optimizing the physical characteristics of GR latex films are also described.

EXPERIMENTAL

LATEX

GR latex. —Mature, field-grown guayule shrub was harvested during February–April, 1994, at the Maricopa Agricultural Center of the University of Arizona. The shrub was sprayed with water, baled, and covered in plastic before transport to the Bioresources Research Facility of the University of Arizona at Tucson. The shrub was passed through a hammermill with a 6.5 mm screen in an aqueous medium (1 : 5 w/v) containing 0.1% w/w

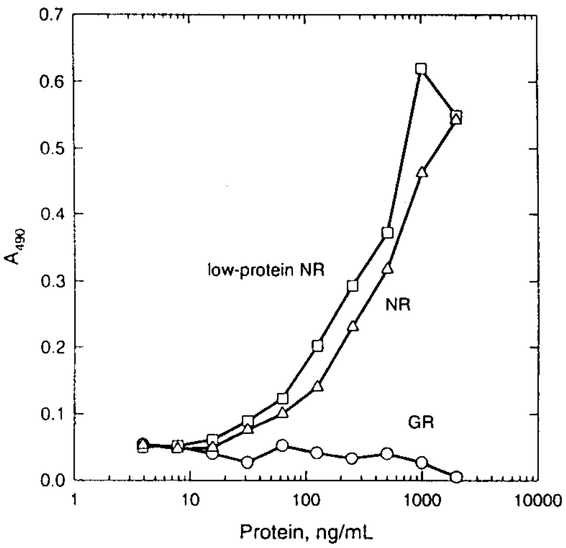

FIG. 1. — LEAP assay of latexes; absorbance at 490 nm (A_{490}) as a function of latex protein concentration.

sodium sulfite and 1% w/w polyvinylpolypyrrolidone, then filtered. Sufficient aqueous ammonia was added to the resulting homogenate to provide an ammonia content of 0.2%. Preliminary clarification was obtained with a single pass through a Westphalia Model KA 05-00-105 centrifuge fitted with a chamber-type bowl. Final purification was carried out using the centrifuge with three cycles of separation, subsequent 5-fold dilution of the light phase with 0.2% aqueous ammonia, and reseparation. Purified latex (57 L total) was diluted with 0.1% w/v ammonium alginate (Kelco Superloid) 1 : 2.5 v/v in a polyethylene tank. After phase separation was complete, the lower, nonlatex phase was drained from the tank and the latex phase rediluted as before. Two more cycles were carried out using 0.05% w/v ammonium alginate. The final product (40% dry rubber content) was transferred to polyethylene bottles and stored at 4°C until use.

NR latexes. —Low-ammonia NR latex (62% dry rubber content) was provided by Guthrie Latex, Inc., Tucson, AZ. To prepare low-protein NR latex, 11 L of a high-ammonia latex was diluted 1 : 4.1 v/v with 0.1% w/v ammonium alginate. As was done with GR latex, the resulting nonlatex phase was removed and the latex phase rediluted as before. Two more cycles were carried out using 0.05% w/v ammonium alginate and sufficient aqueous ammonia to adjust the ammonia content to 0.2%. The final product (18 L with 40% dry rubber content) was transferred to polyethylene bottles and stored at 4°C until use.

CONTROL PROCEDURES

To prevent protein cross-contamination, several precautions were taken during compounding, dipping, and curing operations. Personnel wore 100% nitrile gloves. Separate coagulant solutions, dusting powder, and leaching baths were used for each latex recipe. All glassware was washed in a 1% w/v aqueous detergent solution, then immersed in 10% v/v bleach for a minimum of 2 hours. When latexes were changed, all other equipment, including oven interiors and bench tops, were washed with bleach solution or covered with aluminum foil, where appropriate. Cured films were stored in sealable polyethylene bags.

FILM PREPARATION

Compounding and prevulcanization. —Raw latex was filtered through a 200-mesh cloth into a 1000 mL tall-form beaker. With the exception of antioxidant, compound components were added with the aid of a mechanical stirrer fitted with a propeller-type agitator. The

TABLE I

LATEX PROTEIN CONCENTRATIONS

Latex	Protein concentration		
	Latex,[a] mg protein/g	Cast film, mg protein/g	Dipped film, mg protein/g
NR	11.8	2.11	0.36
Low-protein NR	6.7	1.24	0.022
GR	2.3	0.81	0.16

[a] Dry solids basis.

beaker was placed in a water bath and its contents heated to 65°C over a 2 hour period. The compound was then maintained at 65°C for a minimum of 1 hour. Antioxidant was added at the conclusion of prevulcanization.

Dipped films. —Borosilicate glass culture tubes, 35 × 195 mm, (Bellco Biotechnology) were preheated in an oven maintained at 65°C. Tubes were dipped in 18% w/w alcoholic $Ca(NO_3)_2 \cdot 4H_2O$ coagulant, then air dried for 1 minute with manual rotation to assure even distribution. The coagulant-coated tubes were immersed for 20 seconds in prevulcanized latex compound maintained at ambient temperature, then air dried with rotation for 2 minutes. Between dips, the compound was gently agitated. A two-stage leach followed: 2 minutes in 3000 mL of water maintained at 60°C and 2 minutes in 3000 mL of water maintained at 70°C. Both leaching baths were changed after 6 tubes had been dipped. After air drying for 30 seconds, the upper edge of each film was rolled down to form a bead. The coated tubes were then dried in a forced-air oven maintained at 104°C. Samples for protein determinations were dried for 20 minutes. The exposed surfaces of the dried films were brushed with cornstarch (USP absorbable dusting powder). Finally, the films were pulled from the tubes in such a way that the films were turned inside-out. Film thicknesses (expressed as the means of six film samples) were 0.19 ± 0.00 mm, 0.22 ± 0.01 mm, and 0.21 ± 0.01 mm for the NR, low-protein NR, and GR films, respectively.

Cast films. —Approximately 15 mL of prevulcanized latex compound was placed on a 25 cm square sheet of polyester film taped to a rigid backing plate. A film knife with a 0.3 mm gap was drawn over the latex and down the polyester sheet. The resulting film was

TABLE II

BULK RUBBER CHARACTERIZATION

Origin	Mooney viscosity ML 1 + 4 (100°C)	Resin content, %
NR latex	100.4–109.0	1.9–2.3
Low-protein NR latex	101.7	1.7
GR latex[a]	49.9–69.2	8.2–9.9
GR unfractionated bulk[b]	34.5–65.5	4.0
GR fractionated bulk[c]	99.6	2.2[d]

[a] For the testing reported here: Mooney viscosity = 69.2, resin content = 8.2%.

[b] USDA cultivar composite data from Reference 13.

[c] Bale produced by Bridgestone/Firestone, Inc., at its Sacaton, Arizona, pilot plant.

[d] Includes antioxidant.

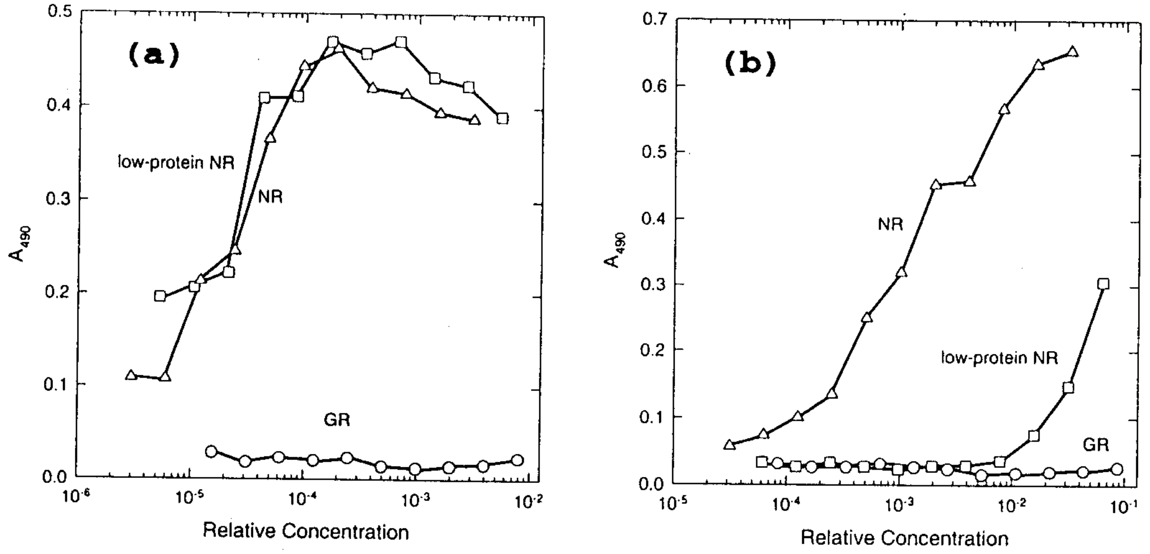

FIG. 2. — LEAP assay of film extracts; absorbance at 490 nm (A_{490}) as a function of extract concentration; **(a)**, cast film extract; **(b)**, dipped film extracts.

dried for 2 hours under a slow stream of air followed by 15 minutes in a forced-air oven maintained at 100°C. Each side of the film was dusted with cornstarch to reduce tack. Film thicknesses were approximately 0.3–0.4 mm.

PROTEIN CHARACTERIZATION

Latex protein content. —Protein concentrations in liquid latex were determined by solubilizing the proteins with Triton X-100, removing interfering substances by precipitation of the proteins with trichloroacetic acid (TCA) and deoxycholate (DOC), and assaying with the disodium salt of 2,2′-bicinchoninic acid (BCA) (Micro BCA Protein Assay kit, Pierce Chemical Co., Rockford, IL) as described in Siler and Cornish.[12] Latex films were cut into 1 cm square pieces and extracted with phosphate-buffered saline (PBS), pH 7.5 (25 mL/7 g of film), at 37°C for 2 hours, then at 4°C overnight. The extract was removed and passed through a 0.22 μm filter prior to protein precipitation with TCA and DOC and assay with BCA.

Enzyme-linked immunosorbent assay (LEAP assay). —Latex proteins were analyzed using the Guthrie LEAP assay (Latex ELISA for Antigenic Proteins, Guthrie Research Institute, Sayre, PA). The assay, based on an antiserum from rabbits immunized against proteins extracted from films of ammoniated latex, was performed as described by the manufacturer with slight modification as described in Siler and Cornish.[5] The amount of antigen present in a given protein solution is directly proportional to the concentration of the oxidation product (UV max 490 nm) produced by the action of enzyme-conjugated horseradish peroxidase on *o*-phenylenediamine.

PHYSICAL TESTING

Bulk viscosity and resin content. —Samples of GR latex, low-protein NR latex, and NR latex were coagulated by the addition of 10% v/v acetic acid. The crude rubber samples were rinsed in water, then dried for 18 hours under vacuum at 40°C. Preparation was completed by passing the samples once through a 2-roll mill and redrying for 18 hours at 50°C. Mooney Viscosities [ML 1 + 4 (100°C)] were determined in accordance with ASTM method D 1646-94. Resin contents were determined by exhaustive acetone extraction of sheeted rubber samples in a Soxhlet apparatus.

TABLE III

LATEX DIPPING COMPOUND FORMULATIONS

	Weight, parts	
	Dry	Wet
40% Latex[a]	100.0	250.0
10% Potassium hydroxide solution	0.5	5.0
33% Surfactant[b]	2.0	6.0
68% Sulfur dispersion[c]	0.8–1.2	1.18–1.77
60% Zinc oxide dispersion	0.50–0.75	0.83–1.25
Activated dithiocarbamate solution[d]	—	1.50–2.25
Water (premix with accelerator)	—	3.0–4.5
65% Antioxidant emulsion[e]	1.0	1.54

[a] Sufficient water added to NR latex to provide 40% total solids content.

[b] 1 : 1 Darvan SMO + Darvan WAQ (Vanderbilt).

[c] Bostex 410 (Akron Dispersions).

[d] Setsit 104 (Vanderbilt).

[e] Agerite Superlite (Vanderbilt).

Stress–strain properties. —Tensile properties of cured films were determined in accordance with ASTM method D 412-92, Test Method A. Accelerated aging was carried out for 166 hours in a forced-air oven maintained at 70 ± 2°C.

RESULTS AND DISCUSSION

LATEX CHARACTERIZATION

Proteins purified from crude NR latex and from low-protein NR latex reacted strongly in the LEAP assay, whereas GR latex gave no reaction (Figure 1). This indicates that antibodies raised against proteins from NR latex recognized protein antigens in both NR preparations, but not in GR latex. Washing NR latex reduces protein levels by about 80% (Table I), but the proteins that remain are also antigens. These results are in agreement with preliminary medical tests where individuals with severe hypersensitivity to NR latex were skin tested with latexes from different sources. All test subjects reacted strongly to NR latex, whereas none responded to GR latex.[9–11]

TABLE IV

CURED FILM STRESS–STRAIN PROPERTIES[a]

	Unaged			Aged	
Latex	500% Modulus, MPa	Tensile strength, MPa	Ultimate elongation, %	Tensile strength, MPa	Ultimate elongation, %
NR	5.1 ± 0.6	21 ± 1	720 ± 10	21 ± 2	670 ± 40
Low-protein NR	3.3 ± 0.8	23 ± 4	880 ± 80	22	840
GR	1.4 ± 0.1	—	—	19 ± 2	850 ± 30

[a] Prevulcanization 60 min at 65°C, oven dry 20 min at 104°C. Data are the means of 6 measurements, with the exception of a single measurement for aged low-protein NR film.

The *cis*-1,4-polyisoprene in GR and NR latexes was isolated for comparison with unfractionated GR from whole guayule shrub and with GR from a representative bale produced at the Sacaton, Arizona, pilot processing facility operated by Bridgestone/Firestone, Inc. Production of commercially-acceptable bulk GR requires fractionation of crude shrub polymer to increase the bulk viscosity and to reduce the level of resin (nonrubber extractables).[13] As summarized in Table II, GR latex contains polymer with a bulk viscosity similar to that of unfractionated, whole-shrub rubber. The lower resin content of the unfractionated bulk GR is an artifact of the process used to coagulate the polymer from a rubber-resin miscella. Compared to the NR latexes, GR latex contains a lower-viscosity polymer having a higher resin content. In an earlier investigation of GR latex, Jones reported relatively high resin contents which exhibited some dependence on shrub age at harvest.[14]

Guayule latex produced by the method described here initially contained less than 1% total solids, so that further concentration was necessary. Freshly-separated, dilute latex was first concentrated to about 20% solids using a Westphalia centrifuge. Further concentration to 40% solids was effected by creaming with ammonium alginate. GR latexes with total solids as high as 50% have been obtained with other separation equipment.14 For purposes of comparative physical testing, the NR latex was diluted to 40% dry rubber content prior to compounding.

LATEX COMPOUND PERFORMANCE

Leaching dipped films prior to oven drying reduced extractable protein levels by about 80% (Table I). These results are similar to those reported for several commercial examination and surgical gloves.[15] LEAP assay of the extracts prepared from cured NR and low-protein NR films showed that extractable immunogenic proteins, while reduced in concentration by leaching, persist nonetheless (Figure 2). Clearly, even products made from purified NR latex would not be suitable for those individuals already allergic, although their use might well reduce the incidence of new cases of latex allergy. No cross-reactive proteins were detected in dipped guayule films.

Table III summarizes the compositions of the dipping compounds. An initial side-by-side comparison of the three latexes was made using a recipe incorporating 0.8 phr sulfur, 0.5 phr zinc oxide, and 1.5 phr (wet) accelerator. The stress–strain properties of the cured films are summarized in Table IV. Figure 3 illustrates representative stress–strain curves for each film type. The GR latex film had a modulus substantially lower than that of the two NR latex

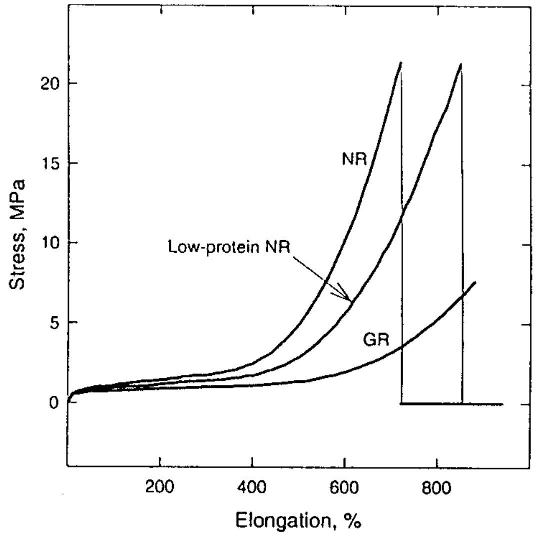

FIG. 3. — Stress–strain performance of unaged cured films.

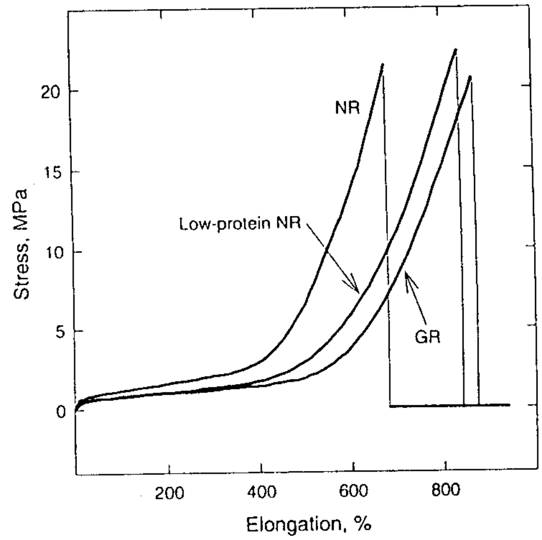

FIG. 4. — Stress–strain performance of cured films aged 166 h at 70°C.

films. By way of comparison, ASTM method D 3578-91 specifies a minimum unaged tensile strength of 21 MPa at a minimum ultimate elongation of 700% for rubber examination gloves.

The low GR film modulus was the result of incomplete curing. When the films were aged for approximately 7 days at 70°C, the tensile strength of the GR film (19 MPa) was comparable to that of the NR films (21–22 MPa). Figure 4 illustrates representative stress–strain curves for each film type after aging. ASTM method D 3578-91 specifies a minimum aged tensile strength of 16 MPa at a minimum ultimate elongation of 500% for rubber examination gloves. These observations are consistent with prior work[14] indicating that, in comparison to NR latex, GR latex produced slower-curing, lower-modulus films. Winkler and coworkers[16] reported that bulk GR exhibited a slower cure rate and reached a lower state of cure. These properties have been ascribed to the absence of nitrogenous activators of the type found in NR and to the presence of resin which acts as a diluent and plasticizer.[17]

As one approach to increasing both the rate and state of cure, the effects of increased sulfur, zinc oxide, and accelerator on cured film properties were evaluated. The conditions of prevulcanization and oven drying were adjusted until the films could be elongated to break (Table V). Increased zinc oxide and accelerator produced the best performance (tensile strength 16 MPa at 870% elongation) with 2 hours prevulcanization and 20 minutes oven

TABLE V

EFFECT OF CURE SYSTEM AND CURE CONDITIONS ON STRESS–STRAIN PROPERTIES[a]

Sulfur, phr	ZnO, phr	Accel.[b], phr (wet)	Prevulc., min at 65°C	Oven dry, min at 140°C	500% Modulus, MPa	Tensile strength, MPa	Ultimate elongation, %
0.8	0.50	1.50	60	20	1.4 ± 0.1	—	—
0.8	0.75	2.25	60	60	1.3 ± 0.1	—	—
0.8	0.75	2.25	120	20	2.0 ± 0.3	16 ± 1	870 ± 30
0.8	0.75	2.25	180	20	2.1 ± 0.3	12 ± 1	780 ± 50
1.2	0.75	2.25	120	20	1.9 ± 0.1	—	—
1.2	0.75	2.25	180	20	1.7 ± 0.1	14 ± 2	940 ± 50

[a] Data are the means of 6 measurements.

[b] Activated dithiocarbamate solution, Setsit 104 (Vanderbilt).

drying. Increasing the sulfur level along with zinc oxide and accelerator gave no further improvement. None of these particular recipe variations yielded properties equivalent to those of either unaged NR films or aged GR films. We recognize that, not having naturally occurring cure activators, GR latex is chemically similar to synthetic polyisoprene (IR) latex.[18]

CONCLUSIONS

GR latex, and dipped films produced from compounded GR latex, contain none of the Hevea allergens known to elicit Type I systemic allergic reactions. The GR polymer has a lower bulk viscosity and a higher resin content than NR. In addition, GR latex serum and rubber lack the activating components present in NR latex. As a result, GR latex cures more slowly and reaches a lower state of cure. The aged stress–strain properties of cured GR films are nonetheless similar to those of NR films.

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REFERENCES

[1] J. E. Slater, *J. Allergy Clin. Immunol.* **94,** 139 (1994).
[2] D. R. Ownby, H. E. Ownby, J. A. McCullough, and A. W. Shafer, *J. Allergy Clin. Immumol.* **93,** 282 (1994).
[3] D. H. Beezhold, G. L. Sussman, D. A. Kostyal, and N. S. Chang, *Clin. Exp. Immumol.* **98,** 408 (1994).
[4] A. B. Czuppon, Z. Chen, S. Rennert, T. Engelke, H. E. Meyer, M. Heber, and X. Baur, *J. Allergy Clin. Immunol.*, **92,** 690 (1993).
[5] D. J. Siler and K. Cornish, *Ind. Crops Prod.* **2,** 307 (1994).
[6] R. G. Hamilton, B. L. Charous, N. F. Adkinson, Jr., and J. W. Yunginger, *J. Lab. Clin. Med.* **123,** 594 (1994).
[7] V. J. Tomazic, E. L. Shampaine, A. Lamanna, T. J. Withrow, N. F. Adkinson, Jr., and R. G. Hamilton, *J. Allergy Clin. Immunol.* **93,** 751 (1994).
[8] P. B. Williams, M. P. Buhr, R. W. Weber, M. A. Volz, J. W. Koepke, and J. C. Selner, *J. Allergy Clin. Immunol.*, **95,** 88 (1995).
[9] D. J. Ber, K. H. Munemasa, R. Gilumilion, K. Cornish, D. E. Klein, and G. A. Settipane, *J. Allergy Clin. Immunol.* **91,** 271 (1993).
[10] P. J. Schrank, A. B. Carey, R. A. Simon, B. Ward, and K. Cornish, *J. Allergy Clin. Immunol.* **91,** 385 (1993).
[11] A. B. Carey, K. Cornish, P. Schrank, B. Ward, and R. Simon, *Ann. Allergy Asthma Immunol.* **74,** 317 (1995).
[12] D. J. Siler and K. Cornish, *Anal. Biochem.*, **229,** 278 (1995).
[13] J. P. Wagner and W. W. Schloman, Jr., in "Guayule Natural Rubber," J. W. Whitworth and E. E. Whitehead, Eds., GAMC-USDA/ARS, Tucson, AZ, 1991, ch. 11, p. 261.
[14] E. P. Jones, *Ind. Eng. Chem.* **40,** 864 (1948).
[15] V. J. Tomazic, T. J. Withrow, and R. G. Hamilton, *J. Allergy Clin. Immunol.* **96,** 635 (1995).
[16] D. S. Winkler, H. J. Schostarez, and H. L. Stephens, RUBBER CHEM. TECHNOL. **50,** 981 (1977).
[17] L. F. Ramos-De Valle, RUBBER CHEM. TECHNOL. **54,** 24 (1981).
[18] D. M. Preiss, W. M. Sawyer, and W. C. Simpson, *J. Appl. Polym. Sci.* **7,** 1803 (1963).

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