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Protein influences on guayule and Hevea natural rubber sol and gel

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ABSTRACT: Guayule (*Parthenium argentatum*) is under cultivation in the southwestern United States as an alternative source of natural rubber free from proteins that cause Type I latex allergies. However, since guayule lacks the protein-polymer interactions present in Hevea latex, its physical and chemical properties may differ. The solvent-soluble (Sol) and insoluble (Gel) fractions from guayule and Hevea natural rubbers were isolated through a solubilization/centrifugation deproteinization process. Protein could be reduced or removed by centrifugation, or concentrated in the gel fraction for both Hevea and guayule rubber. Separation of the sol fraction of Hevea rubber reduced the overall protein level, in some cases to below detection limits, without impacting rubber thermo-oxidative stability. Notably, no detectable cross reactions took place between guayule protein antibodies and Hevea-based materials, nor vice-versa. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42051.

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INTRODUCTION

Guayule (*Parthenium argentatum*) is a natural rubber-producing perennial shrub under cultivation and commercial development in the southwestern USA as a source of biobased rubber, organic resins, and bioenergy feedstocks. Natural rubber from guayule is stored in bark parenchyma cells, unlike the laticifers of *Hevea brasiliensis*, so instead of tapping to extract rubber, guayule shrubs are ground in the presence of an aqueous buffer[1] or organic solvent.[2] Consequently, the non-rubber constituents present in the latex or rubber, and carried into compounding processes, differ significantly with corresponding impacts on physical and chemical properties.[3–6] For example, guayule latex has been shown to be free of Type I latex allergens and, therefore, can be used to produce products safe for people who suffer from Type I latex allergy.[7–9]

Proteins and other naturally occurring non-rubber constituents present in Hevea latex also have beneficial effects, in thermal stabilization, vulcanization acceleration, and especially strain-induced crystallization of natural rubber, all of which are readily exploited by rubber product manufacturers. An improved understanding of the differences between the non-rubber constituents present in guayule versus Hevea rubber will aid manufacturers in utilizing domestically produced natural rubber from guayule and provide insight into the mechanisms of polymer-protein interactions in natural rubber.

EXPERIMENTAL

Polymers Studied

Control natural rubber materials used were: (1) guayule natural rubber latex (Yulex Corporation, Phoenix, AZ), extracted from mature plants grown in Arizona, United States, via aqueous extraction of ground shrub,[1] (2) Centex HA latex (Centrotrade, Chesapeake, VA), collected from mature Hevea trees by tapping, washed and mechanically and chemically stabilized, and shipped as a latex preparation. Both lattices were poured out and air dried at ambient laboratory conditions under gentle air flow (fume hood) to produce bulk rubber sheets. In addition, (3) Standard Thai Rubber (STR5L) and (4) Thick Pale Crepe (TPC) bale Hevea natural rubbers were provided by Verve, Inc. (Providence, RI). Both solid bale rubbers were collected from mature Hevea trees, mechanically and chemically stabilized, then washed and coagulated into solid crepe rubber, and formed into bales for shipment.

Preparation of Gelled and De-Gelled Fractions

Degelled (Soluble) fractions of each rubber were prepared by dissolution in *n*-hexane with stirring, at room temperature, up to 72 h or as needed, as described in the following example: A 150.00 g sheet of guayule NR (Batch No. MTO#2103-02) was cut into small pieces (~0.5 cm), and placed into a 4 L Erlenmeyer flask. A total of 3.0 L *n*-hexane was added to the flask,

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and agitated until all rubber appeared solubilized, 72 h. The dissolved rubber solution was centrifuged at 27,880 × *g* for 30 min at 20°C. Following the first centrifugation, half of the rubber solution was decanted and dried at ambient temperature. The other half was centrifuged again under the same conditions, separated by decanting, and dried. The "De-gelled" fractions were labeled STR5L-S, TPC-S, Centex HA-S, and GNR-S. The swollen "Gel" (in the case of Hevea) minus visible precipitate, or small amount of viscous bottom fraction (in the case of guayule) was separated and dried. These fractions were labeled STR5L-G, TPC-G, Centex HA-G, and GNR-G.

Total Protein Content

The total protein content was determined by ASTM 5712-10 assay. This is a chemical assay based on the Lowry assay that quantifies all proteins present in a sample. Briefly, samples were extracted in a buffer of 50 m*M* phosphate pH 7.4 at a ratio of 5 mL buffer per gram rubber. Extractions were carried out at room temperature for 2 h with agitation. The sample was removed and the extract centrifuged at 500 × *g* for 15 min to pelletize particulates. The cleared extracts were then used in the assay. Protein content was determined by interpolation from a standard curve. The Limit of Detection (LOD) for the ASTM D5712 assay was 2.2 μg/mL and the Limit of Quantification (LOQ) was 11.0 μg/gm.

Levels of natural rubber latex (NRL) antigenic proteins were determined from the same cleared extracts using the ASTM D6499-12 enzyme-linked immunosorbent assays (ELISA) Inhibition Assay. This assay uses antibodies developed against the full complement of proteins found in raw liquid latex. Following serial dilution on a 96 well ELISA plate, an equal volume of diluted rabbit anti-latex polyclonal antibody was added and incubated for 2 h. One half volume of sample from each well was transferred to the corresponding well of a plate coated with Hevea NRL and blocked with non-fat dry milk and incubated for 2 h. The plates were washed and a solution of Goat anti-Rabbit IgG conjugated with the enzyme HorseRadish Peroxidase was added and incubated for 1 h. Plates were washed and a 100-µL solution of substrate *o*-phenylenediamine (OPD) added to each well and color allowed to develop. The reaction was stopped by the addition of 50 µL of 2*M* H_2SO_4. Protein values were determined by interpolation from a standard curve. The LOD of the assay was 0.03 μg/mL.

The presence of guayule latex proteins was determined by a Guayule ELISA Inhibition Assay. This immunoassay is performed in the same fashion as the ASTM D6499 except that the standard antigen used is GNR and the primary antibody used is Rabbit anti-guayule total protein. The antibodies were developed against the crude unprocessed liquid extract of total guayule plant material (guayule homogenate[1]). Following serial dilution of a GNR standard and samples, an equal volume of diluted rabbit anti-GNR polyclonal antibody was added and incubated for 2 h at 37°C. One half volume of sample from each well was transferred to the corresponding well of a plate coated with GNR and blocked with non-fat dry milk and incubated for 2 h at 37°C. The plates were washed and a solution of Goat anti-Rabbit IgG conjugated with the enzyme HorseRadish Peroxidase was added and incubated for 1 h. Plates were washed and a 100-µL solution of the substrate, OPD added to each well and color allowed to develop. The reaction was stopped by the addition of 50 µL of 2*M* H_2SO_4. Protein values were determined by interpolation from a standard curve. The LOD of the assay was 0.008 µg/mL.

ELISA tests were performed using plates coated with the respective Hevea and guayule antibodies against purified protein samples to validate specific antibody recognition of Hevea and guayule proteins.

Physical Property Determinations

The molecular masses of the polymers were determined by Gel Permeation Chromatography. Approximately 3 mg of dried rubber sample was solubilized in 3 mL HPLC grade tetrahydrofuran (THF) overnight with gentle shaking (Multi-Purpose Rotator, Thermo Scientific). The rubber solution was syringe-filtered through a 1.6 µm glass microfiber GF/A filter (Whatman GE Healthcare) then injected into a Hewlett-Packard 1100 series HPLC (1.0 mL/min flowrate, 50 µL injection volume, THF continuous phase) and size exclusion separated by two Agilent PL gel 10 µm Mixed-B columns in series (35°C) coupled to (1) multi-angle laser light scattering (DAWN Heleos-II, Wyatt Technology, Santa Barbara, CA), (2) refractive index (Agilent 1260 Infinity, $dn/dc = 0.129$), and (3) UV (HP 1100 series @ 254 nm) detectors.

Bulk viscosity was determined using the Advanced Polymer Analyzer (APA 2000, Alpha Technologies, Akron, OH) per ASTM D6204B. The APA 2000 measures flow properties (processability) of rubber by placing a sheet of rubber between two pressurized, heated dies, subjecting one die to dynamic oscillation at a specified time, temperature, frequency and strain while the torque response to the applied dynamic strain is measured on the opposite die. Following a break-in step, the torque response is measured under test conditions and converted into dynamic modulus (kPa) or dynamic viscosity (MPa-sec). The % gel was determined by a method based on ASTM D3616-95, using 50 mL perforated stainless steel containers with overnight (20–24 h) solubilization in toluene in the dark at ambient temperature. Exactly 25.0 mL of solution was pipetted into preweighed aluminum dishes and gently heated until dry to determine the sol fraction; the percent gel was determined by difference.

To assess thermal stability, the Plasticity Retention Index (PRI) was measured per ASTM D3194-04. The Plasticity (P_o) of raw natural rubber is the median final thickness of a rubber specimen placed between heated platens of a parallel plate plastometer, following application of a 100*N* compressive force for a specified time. When measured before and after heat treatment (140°C, 30 min), the relative ratio of the plasticity values (expressed as a percentage) gives an indication of the oxidation resistance of the rubber.

Chemical Analyses

The nitrogen content of rubber fractions was determined in duplicate for ~ 150 mg rubber sample by a Leco TruSpec® CN Analyzer (Leco Corp., St. Joseph, MI) per manufacturer's

Table I. Protein Content in Natural Rubber, per ASTM D5712-10

Sample	Total protein, per ASTM D5712-10		Hevea antigenic protein per ASTM D 6499-12		Guayule antigenic protein per guayule-specific ELISA	
	Assay conc. (μg/mL)	Total protein (μg/g)	Assay conc. (μg/mL)	Antigenic protein (μg/g)	Assay conc. (μg/mL)	Antigenic protein (μg/g)
STR5L	–	<5	0.20	0.5	–	<0.1
STR5L-S	–	<6	0.09	0.2	–	<0.1
STR5L-G	–	<17	0.23	1.8	–	<0.2
TPC	–	<5	1.89	4.6	–	<0.1
TPC-S	–	<2	–	<0.03	–	<0.03
TPC-G	5	11	1.47	3.4	–	<0.1
GNR	–	<11	–	<0.2	0.11	0.54
GNR-S	–	<11	–	<0.2	–	<0.04
GNR-G	–	<11	–	<0.2	0.02	0.08
Centex HA	522	507	211.39	205.2	–	<0.0
Centex HA-S	–	<7	0.43	1.4	–	<0.1
Centex HA-G	8.00	12	21.55	32.0	–	<0.0

"–" = below detection, 2.2 μg/mL for D5712, 0.03 μg/mL for D6499, 0.008 μg/mL for guayule

instructions using fresh lab-prepared hexane soluble (Sol), insoluble (Gel), and control fractions.

The low molecular mass extractable content was evaluated by GPC and also by extraction with acetone using an Accelerated Solvent Extractor 200 (Dionex, Sunnyvale, CA). For ASE, approximately 300 mg of rubber sheet was cut into small pieces and placed into 11 mL cells partitioned with Ottawa sand. Extraction with acetone was performed at room temperature, 1500 psi, 3 × 20 min cycles, with all extracts collected into preweighed vials. Extracts were Turbovap dried under nitrogen gas and quantified gravimetrically.

All tests were performed in triplicate unless otherwise indicated. All solvents were reagent grade (Fisher Scientific, Waltham MA) unless otherwise indicated.

RESULTS AND DISCUSSION

Dissolution of rubber in *n*-hexane followed by centrifugation, fractionation, and drying resulted in soluble (S) and gel (G) materials for which total protein, Hevea antigenic protein and guayule antigenic protein levels were quantified (Table I). The total protein (Table I) was below detection limits for all S materials tested, including those from Hevea natural rubber. There was no detectable protein for guayule: control, S, or G samples. While very low protein levels are expected for guayule rubber[7,10,11] due to the nature of latex collection, Hevea rubber requires thorough processing to effectively reduce latex proteins, apparently the case for the STR5L materials tested, within the detection limit of 2.2 μg/mL. It is noteworthy that the dissolution and decanting process used resulted in low protein Hevea gel. The remaining proteins are removed in the precipitate, rendered unextractable, or are below detection limits. The Centex HA control material showed the highest level of total protein of the tested series, 507 μg/g rubber. NR latex undergoes washing in preparation to reduce the serum protein content. However, upon storage, changes in rubber particle-bound proteins, including protein hydrolysis into more serum-soluble form, can occur. It should be noted that while high in this series, 507 μg/g is significantly lower than other reports for Hevea latex using the same method.[4,7,12] Based on the total protein detected in the HA-G materials, the fractionation process used can be considered at least 97% effective for protein removal.

The enzyme-linked immunosorbent assay (ELISA) provides much more sensitive detection of antigenic protein (0.03 μg/mL). Results for the Hevea and guayule (D6499) ELISAs versus standard and control proteins validated that that the D6499 assay recognized only Hevea proteins and the guayule assay only recognized guayule proteins, in both cases quantifying protein levels at or near expected levels (Table II). Furthermore, neither assay recognized negative control (non-fat dry milk) proteins.

No Hevea antigenic protein was detected by the ASTM D 6499-12 ELISA test (Table I) for any of the guayule-based rubber materials, as expected.[7,9] Hevea antigenic proteins were detected for all Hevea control and gel materials. Again, Centex HA had the highest level of detected protein, followed by TPC, then STR5L. Antigenic proteins were detected in higher concentrations in gel for STR5L-G but not the other Hevea gels. Remarkably, for the TPC-S material, the process used reduced Hevea antigenic protein to below detection limits (Table I). Nevertheless, the solubilization and centrifugation process used here reduced the measureable antigenic proteins at least as effectively as treatments based on surfactant/proteolytic enzyme/centrifugation/urea.[13]

While guayule natural rubber is expected to have very low levels of protein, it is well known that guayule rubber particles bind enzymatic or structural proteins on their surfaces, so they cannot be considered 100% protein free.[14] Preparation of rabbit anti-guayule latex polyclonal antibody allowed ELISA-based

Table II. Validation of Antibody Protein Detection by ASTM D6499 ELISA

	Assay antibody - Hevea		Assay antibody - Guayule	
Test sample	Theoretical	Value obtained	Theoretical	Value obtained
Hevea 2.0	2	2.38	b.d.	b.d.
Hevea control	10.17 ± 0.81	9.99	b.d.	b.d.
NFDM	b.d.	b.d.	b.d.	b.d.
Guayule 0.5	b.d.	b.d.	0.5	0.54
Guayule 2.0	b.d.	b.d.	2	2.53

Sample Hevea 2.0 represents Hevea StAG at conc of 2.0 μg/mL), Hevea Control is an internal process control (has been assayed 50 times with average 10.17+/−0.81 μg/mL), NFDM is a non-fat dry milk dilution buffer with only milk protein (negative control), Guayule 0.5 and Guayule 2.0 are isolated guayule proteins at concentrations of 0.5 and 2.0 μg/mL.

detection of guayule rubber proteins at a detection limit of 0.008 μg/mL (Table I). Immunogenic protein was detected and quantified for both control and GNR-G materials, but was below detection limits for the GNR-S, analogous to Hevea results. Similar to Hevea dried latex, the highest level of antigenic protein was found in the control material, not in the gel. Importantly, no guayule immunogenic protein was detected for any of the Hevea rubber or latex samples, demonstrating a clear lack of cross-reactivity of Hevea for guayule and vice-versa. Note that a previous study[8] demonstrated lack of cross reactivity to antibodies raised against guayule latex; in this report, the antibodies were raised against guayule homogenate. In neither case were immunogenic proteins detected.

For Hevea HA latex, the highest concentration of protein was in the control material. While it might be expected to find protein concentrated in gels, protein-polymer linkages in some Hevea gels might render the protein less extractable, therefore undetected. Nitrogen analysis of the bulk rubber samples (Table III), frequently used as an estimate of protein in natural rubber, confirmed that this is the case, not only for Hevea but also guayule rubber. In every case, the gel fraction contained the highest level of nitrogen and the sol fraction the lowest. Note Hevea materials before fractionation (controls) all have about the same amount of nitrogen. Based on chemical and immunogenic tests, protein in the latex rubber is more extractable (vs. bales), therefore more detectable. For many fractions, the protein is tightly bound to the polymer and thereby rendered unextractable.

Molecular masses and their distributions, determined by gel permeation chromatography (Table IV), indicate high molecular masses, 1–2 million g/mol (M_w), and 1–1.4 million g/mol (M_n) for all materials evaluated. The control materials' M_w differed significantly, TPC > STR5L > GNR (±3 SD). The guayule natural rubber sample had lower M_w, ∼ 1.2 million g/mol, compared to the Hevea samples, and a broader distribution, $M_w/M_n = 2.16$. Molecular mass differences between the original samples can be attributed to multiple factors, including species (clone), season, harvesting and postharvest treatments. Separation of the rubber into soluble (S) fractions resulted in no differences in measured molecular mass or distribution (vs. controls), because only the THF soluble fraction is accessible to the GPC and measured. It is worth noting that fractionated S materials were not biased to low M_w chains for Hevea, so the branch points that are proposed to compose the NR gel[15] were, apparently, not disrupted. In addition, since at least some polymer was solubilized from the G materials, they cannot be considered permanently gelled rubber. This is not surprising since rubber gel is a dynamic phenomenon, highly dependent upon solvent, time, and temperature.[16] Nevertheless, the amount of rubber detected by the refractive index concentration detector (Table IV) showed the expected relative solubility. For example, % soluble rubber for the TPC-G, TPC, and TPC-S were 27.6%, 51.5%, and 71.9%, respectively.

When the % gel was determined by ASTM D3616 (overnight in toluene), the G fractions ranged from 60–80% gel (Table V), and the S fractions ranged from 3–15% gel. Comparison of the calculated solubility (in THF) using the GPC compared to the ASTM (toluene) gravimetric procedure highlights the relative nature of gel in natural rubber. Solubilization of very high molecular weight polymers is a strong function of solvent, stirring, temperature, and time, especially so for natural rubbers.

The fractionation/purification process used produced relatively insoluble G fractions and relatively soluble S fractions (8.4, 15.1% gel) for the Hevea rubber materials, comparable to that of commercial deproteinized HA latex (16.2%).[17] In the case of guayule rubber, % gel in the control sample was low (11%), in agreement with other reports[6,18] and similar to that of Hevea S fractions (8.4% and 20.8%, respectively, for TPC-S and STR5L-S). Guayule GNR-S has even lower gel (3.8%). The quantity of GNR-G material recovered was too low to be tested.

The thermal-oxidative stability of Hevea natural rubber[19] has been attributed to the presence of non-rubber constituents

Table III. Nitrogen Content of Rubber Fractions

	% Nitrogen		
	Gel	Control	Sol
TPC	1.66	0.493	0.155
STR5L	0.838	0.412	0.043
Centex HA	0.582	0.423	0.076
GNR	0.076	0.071	0.038

Determined in triplicate by Leco TruSpec® CN Analyzer, per manufacturer's instructions.

Table IV. Natural Rubber Molecular Masses and Distributions

Sample	$M_w \times 10^{-6}$ (avg)	$M_w \times 10^{-4}$ (stdev)	$M_n \times 10^{-6}$ (avg)	M_w/M_n (avg)	M_w/M_n (stdev)	% Soluble (avg)	% Soluble (stdev)
STR5L	1.867	2.34	1.209	1.55	0.019	54.1	1.50
STR5L-S	1.956	2.93	1.354	1.45	0.020	66.0	0.43
STR5L-G	1.880	6.73	1.294	1.45	0.003	47.0	4.51
TPC	2.045	2.33	1.305	1.57	0.060	51.5	7.45
TPC-S	1.970	0.32	1.388	1.42	0.016	71.9	4.79
TPC-G	1.766	9.82	1.071	1.65	0.030	27.6	3.92
GNR	1.200	2.84	0.557	2.16	0.091	51.8	3.59
GNR-S	1.124	6.15	0.485	2.34	0.236	59.0	6.92

Weight average molecular weights, and polydispersity indices determined by gel permeation chromatography of NR samples after solubilization in THF overnight followed by filtration (0.6 μ*M*). Data reported represent the average of three determinations.

including proteins[20] and/or amino acids.[21] Hevea natural rubber shows much better thermal stability than that from guayule, as seen here (Table VI) and elsewhere,[22,23] and the difference is usually attributed to the much lower protein levels in guayule rubber compared to Hevea rubber. However, neither the reduction in the amount of protein present in Hevea rubber S fractions nor the concentration of protein in G fractions impacted PRI in this study (Table VI). Moreover, the thermal stability of TPC-S, the Hevea rubber fraction without detectable total or antigenic protein, is excellent (PRI = 87.1). These results contrast a previous report[20] of protein reduction by proteolysis reducing thermal stability. While it is possible that protein degradation products present in the rubber might escape antibody detection in D 6499, the reported total protein D 5712 detects even very small peptides. Our results suggest that the non-rubber constituents responsible for rubber thermal stabilization are hexane soluble (nonpolypeptide in structure) or are tightly associated with Hevea rubber in a nonextractable form.

Rubber bulk viscosity is a strong function of polymer molecular weight. However, in natural rubber, it reflects the contributions of soluble rubber (S), insoluble rubber (G), and non-rubber constituents, unlike GPC which can only characterize soluble rubber. Bulk rubber rheological parameters (Table VII) clearly illustrate the practical significance of S versus G fractions in Hevea natural rubber. Removal of the soluble (S) fraction from control Hevea materials increased the dynamic shear viscosity (η^*, 100°C, 100% strain), by as much as 100%, to highly unprocessable levels. The Hevea S materials were lower in viscosity (15–20%) than Hevea controls, but still higher in bulk viscosity than guayule (by 25%), due to the lower molecular weights of the guayule rubber but also to the higher extractables content (Table V), specifically coextracted resins, naturally occurring low molecular weight nonpolar compounds that are miscible with rubber and have a plasticizing effect in guayule rubber. Note, all measures of bulk viscosity (P_o, η^*, and G' at two frequencies) ranked control materials STR5L > TPC > GNR, despite TPC having the highest measured M_w, and attributed here to its higher gel content (STR5L control 69.4%). Once extracted, Hevea S fractions were nearly identical in molecular masses and bulk properties. However, for Hevea gel fractions, TPC-G showed 14% higher bulk viscosity and 30% higher gel compared to STR5L. The antigenic protein level was nine times higher for TPC-G indicating that differences in the bulk properties of these Hevea rubbers can be attributed to protein-polymer interactions. Guayule S versus control fractions showed the same bulk rheological properties, due to insignificant gel reduction in the solution process.

Table V. Gel and Extractables Content of Natural Rubber

Sample	Gel content		Acetone extractables	
	% By weight	Stdev	% By weight	Stdev
STR5L	69.4	0.3	1.29	0.13
STR5L-S	20.8	1.0	1.36	0.10
STR5L-G	60.9	0.2	1.12	0.02
TPC	58.5	0.4	1.12	0.10
TPC-S	8.4	0.4	1.00	0.07
TPC-G	79.5	0.6	0.85	0.05
GNR	10.8	0.2	5.61	0.11
GNR-S	3.8	1.5	6.35	0.25
GNR-G[a]	–	–	–	–

Testing performed on: 0.40 ± 0.01 g samples by ASTM D3616-95.
[a] Insufficient GNR-G material recovered.

Table VI. Plasticity and Plasticity Retention Index, per ASTM D 3194-04

Sample	P_o	P_a	PRI	Antigenic proteins (μg/g)
STR5L	75.5	64.9	86.0	0.5
STR5L-S	53.9	51.6	95.7	0.2
TPC	59.8	50.2	83.9	4.6
TPC-S	49.5	43.1	87.1	<0.03
GNR	35.0	4.0	11.4	<0.2
GNR-S	39.0	5.5	14.1	<0.2

Table VII. Bulk Rubber Rheological properties, per ASTM D 6204B

Sample	G' (kPa) 0.1 Hz	G' (kPa) 1.0 Hz	η^* (MPa-s) 0.1 Hz	η^* (MPa-s) 1.0 Hz	tan D 0.1 Hz	tan D 1.0 Hz
STR5L	26.9	35.5	73.10	8.53	1.38	1.13
STR5L-S	17.7	21.1	55.95	6.33	1.72	1.60
STR5L-G	40.7	51.6	102.2	12.0	1.22	1.06
TPC	22.8	31.3	64.17	7.87	1.46	1.23
TPC-S	17.1	21.3	54.22	6.33	1.73	1.58
TPC-G	42.0	57.4	116.2	13.5	1.42	1.10
GNR	11.8	12.8	41.23	4.43	1.96	1.93
GNR-S	11.2	12.1	39.54	4.35	1.99	2.04

Conditioned at: 100°C 2.8% Strain 0.5 Hz 8 min.
Tested at: 100°C, 100% Strain, 0.1 Hz, 1.0 Hz, 8 min, per ASTM 6204 B.

CONCLUSIONS

One of the primary differences between Hevea and guayule natural rubbers is the amount and nature of the naturally occurring proteins associated with the latex and rubber. To gain a better understanding of the nature of the proteins and their interactions with the polymer, a solvent-based fractionation and separation protocol was used for guayule and Hevea rubbers, with chemical and physical characterization. Our results support the evidence that latex-derived guayule rubber has a very low level of protein that does not cross-react with Hevea antibodies. Guayule antibodies, likewise, do not cross-react with Hevea proteins.

For Hevea materials, the fractionation process described here can be considered at least 97% effective for protein removal, based on the total protein detected. In some, but not all, cases Hevea Sol rubber was free of detectable antigenic protein. However, nitrogen analyses suggests concentrated protein, especially in gel rubber, is bound to the polymer such that it has been rendered unextractable. For example, the TPC gel had the highest level of nitrogen, yet little detectable protein (by chemical or immunological methods) following extraction. Very little gel was detected in guayule rubber; based on the process used, insufficient GNR-G material was recovered for physical property characterization.

Similar thermal stability for Hevea materials differing in protein content and gel level was found. This indicates the nature of thermal stabilization is not protein-based and is not the gelling component. Other non-rubber constituents, probably amino acids from protein degradation, are responsible for thermal stabilization of Hevea rubber. Bulk viscosities were independent of molecular weight, due to the presence of strong protein-polymer linkages in Hevea and to plasticizing non-rubber constituents in guayule.

One of the questions we sought to answer was whether a "degelled," "de-proteinized" Hevea rubber polyisoprene is equivalent to guayule polyisoprene. The solubilization and centrifugation processes used here produced two S materials of similar % gel to the guayule control. However, the materials were still quite different from guayule in terms of molecular weights (sol), bulk properties, and detectable antigenic proteins. Hevea "sol" was not the same as guayule natural rubber. The excellent thermal stability combined with zero detectable protein content of these low-gel, solvent-soluble fractions of Hevea NR provided evidence that the thermal stabilizing species is associated with solvent-soluble rubber. Our data support the Tanaka model[24,25] of protein-polymer linkages in Hevea that are not readily disrupted by organic solvents. It also suggests guayule rubber likewise exhibits protein-polymer linkages but from its own naturally occurring species-specific proteins.

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REFERENCES

1. Cornish, K. Patent 5,580,942, **1996**.
2. Schloman, W. W., Jr.; Hively, R. A.; Krishen, A.; Andrews, A. M. *J. Agric. Food Chem.* **1983**, *31*, 873.
3. Schloman, W. W., Jr.; Wyzgoski, F.; McIntyre, D.; Cornish, K.; Siler, D. J. *Rubber Chem. Technol.* **2006**, *69*, 215.
4. Cornish, K.; Williams, J. L.; Hall, J. L.; McCoy, R. G., Jr. *Rubber Chem. Technol.* **2008**, *81*, 709.
5. Cornish, K. *Rubber Latex Technol.* **2011**, *1*, 78.
6. McMahan, C.; Xie, W.; Wong, R.; Cornish, K.; Wood, D.; Mattoso, L. H. C.; Malmonge, J. A.; Shintani, D. A.; Whalen, M. In *Proceedings of 176th Technical Meeting of the Rubber Division of the American Chemical Society, Inc.* Pittsburgh, PA, October 13–15, Paper #39, **2009**.

7. Cornish, K. *Rubber Sci.* **2012**, *25*, 139.
8. Hamilton, R. G.; Cornish, K. *Ind. Crops Prod.* **2010**, *31*, 197.
9. Siler, D. J.; Cornish, K.; Hamilton, R. G. *J. Allergy Clin. Immunol.* **1996**, *98*, 895.
10. Cornish, K.; McMahan, C. M.; Xie, W.; Williams, J. L.; Nguyen, K. C.; Kostyal, D. A.; Horton, K.; Marsh, D. T. In *Proceedings 9th International Latex Conference*, Charlotte, NC, July 25–26, **2006**.
11. Kostyal, D. A.; Horton, K.; Cornish, K. In *Proceedings of the 14th International Latex Conference*, Fairlawn, Ohio, July 25–26, **2011**.
12. Kostyal, D. A.; Horton, K.; Beezhold, D.; Lockwood, S.; Hamilton, R. G. *Ann. Allergy Asthma Immunol.* **2009**, *103*, 354.
13. Klinklai, W.; Saito, T.; Kawahara, S.; Tashiro, K.; Suzuki, Y.; Sakdapippanich, J. T.; Isono, Y. *J. Appl. Polym. Sci.* **2004**, *93*, 555.
14. Backhaus, R. A.; Cornish, K.; Chen, S.-F.; Huang, D.-S.; Bess, V. H. *Phytochemistry* **1991**, *30*, 2493.
15. Tanaka, Y. *Rubber Chem. Technol.* **2001**, *74*, 355.
16. Allen, P. W.; Bristow, G. M. *J. Appl. Polym. Sci.* **1963**, *7*, 603.
17. Tangpakdee, J.; Tanaka, Y. *Rubber Chem. Technol.* **1997**, *70*, 707.
18. McPherson, A. T. *Bur. Stand. J. Res.* **1932**, *8*, 751.
19. Gorton, A. D. T. *Rubber Chem. Technol.* **1970**, *43*, 1255.
20. Tuampoemsab, S.; Sakdapipanich, J. *Kautchuk Gummi Kunstsoffe* **2007**, *12*, 678.
21. Abad, L. V.; Reilewe, L. S.; Aranilla, C. T.; Aliganga, A. K.; San Diego, C. M.; dela Rosa, A. M. *Polym Deg. Stab.* **2002**, *76*, 275.
22. Bhowmick, A. K.; Rampalli, S.; Gallagher, K.; Seeger, R.; McIntyre, D. *J. Appl. Polym. Sci.* **1987**, *33*, 1125.
23. Schloman, W. W., Jr.; Mclntyre, D.; Hilton, A.; Beinorz, R. *J. Appl. Polym. Sci.* **1996**, *60*, 1015.
24. Amnuaypornsri, S.; Sakdapipanich, J.; Toki, S.; Hsiao, B. S.; Ichikawa, N.; Tanaka, Y. *Rubber Chem. Technol.* **2008**, *81*, 753.
25. Tanaka, Y.; Tarachiwin, L. *Rubber Chem. Technol.* **2009**, *82*, 283.