

EFFECT OF MOLECULAR ENVIRONMENT ON PROTEIN RUBBER PROPERTIES

NATASHA CHAUHAN,¹ JUSTIN R. BARONE^{2*}

¹CHEMICAL ENGINEERING, VIRGINIA TECH, BLACKSBURG, VA 24061

²BIOLOGICAL SYSTEMS ENGINEERING, 301D HUMAN AND AGRICULTURAL BIOSCIENCES BUILDING 1, 1230 WASHINGTON STREET SOUTHWEST; AND CENTER FOR SOFT MATTER AND BIOLOGICAL PHYSICS, DEPARTMENT OF PHYSICS, 850 WEST CAMPUS DRIVE; AND MACROMOLECULES INNOVATION INSTITUTE, VIRGINIA TECH, BLACKSBURG, VA 24061

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ABSTRACT

Many natural organisms use “protein rubbers” to store and release an imposed strain energy with high efficiency to make motion easier. Protein rubbers exist in a complicated environment surrounded by water and other molecules such as sugars, implying that amino acid composition and its environment are important in protein rubber behavior. Gelatin, the hydrolysis product of animal collagen, is hydrated or “plasticized” with water, ethylene glycol, glycerol, corn syrup, and aqueous solutions of sorbitol, glucose, and fructose. The rubber formed is “dry”, that is, is not fully immersed in liquid, and has the appearance and feel of a soft rubber band. The mechanical and thermodynamic behavior of each rubber is characterized with low strain dynamic and high strain tensile experiments with good agreement between the two. Plasticized gelatin rubbers are incompressible and follow the neo-Hookean model for rubber elasticity up to moderate extension ratios. Higher molecular weight polyols with more hydrogen bond donors and acceptors create gelatin networks with lower crosslink density. Ethylene glycol-, glycerol-, sorbitol syrup-, and fructose syrup-plasticized gelatin rubbers have similar molecular relaxation mechanisms and are the most efficient rubbers when probed in the rubbery plateau region prior to approaching the glass transition. The other plasticizers have different molecular relaxation mechanisms that detract from the efficiency of energy storage and return that is not related to network formation but perhaps the individual solvation ability of each plasticizer. [doi:10.5254/rct.23.76957]

INTRODUCTION

Certain proteins can show elastomeric or rubbery behavior, that is, hyperelasticity, entropy-dominated elasticity, and the ability to be highly swollen with solvent.^{1–3} In nature, protein rubbers behave like elastic bands capable of storing an imposed strain energy and returning a large portion of that stored energy immediately upon release (elastic recoil) to make motion easier.⁴ In some cases, protein rubbers can return >90% of the imposed strain energy with the balance lost as heat, showing they can be of very high efficiency.^{5,6} Fleas use a “catapult” or power amplifying mechanism to slowly load a resilin protein rubber and then quickly release it to jump many times their height. Other insects like dragonflies use resilin in their wings to make flight more efficient by taking the kinetic energy at the end of one wing stroke and returning it through elastic recoil to the next.^{7,8} Scallops swim by rapidly closing their shell and compressing an abductin protein rubber. The shell then opens through elastic recoil and the ejection of water out (jet propulsion) propels the scallop.^{9,10} Ungulates have an elastin protein rubber neck ligament that is stretched when the animal lowers its head and then makes it easier to raise the head through elastic recoil.^{11,12} The heart beats and moves blood into an elastin and collagen artery where the pressure stretches (expands) the artery. Elastic recoil from the elastin and collagen helps push blood more efficiently through the circulatory system. There are many other biological examples that rely on stretching a protein rubber and returning energy through elastic recoil, such as tendon collagen for running and jellyfish collagen mesoglea for swimming. Humans have even adapted protein rubber behavior for food use, such as gelatin in desserts and candy (“gummi” is the German word for rubber) and wheat gluten in baked products.^{3,13}

*Corresponding author. Email: jbarone@vt.edu

Natural protein rubbers across species have commonalities in amino acid composition that contribute to the rubber elasticity.¹⁴ The proteins have regular repeat sequences that are rich in G (abductin¹⁵), G–P (elastin,^{16,17} resilin,¹⁸ collagen,¹⁹ flagelliform silk), or G–P–Q (gluten, dragline silk), where G is glycine, P is proline, and Q is glutamine, and contribute to the disordered polymer structure required to exhibit rubbery behavior.^{20,21} G is the smallest amino acid and promotes a flexible polymer chain able to form disordered coils, while P and Q are bulky amino acids capable of preventing close packing of polymer chains and maintaining disorder.²² Crosslinks can be through a variety of mechanisms (some are still disputed, but Rauscher and Pomes give a good review²³). Some protein rubbers rely on chemical crosslinks such as disulfide bonds in glutenin, lysine derivatives (i.e., desmosines) in elastin, and tyrosines in resilin. Others rely on physical crosslinks such as triple helices in collagen and β -sheets in flagelliform and dragline silks.

Protein rubbers in the natural state exist swollen and surrounded by water and other dissolved solutes. Solvents other than pure water have been used to try to understand protein rubber thermodynamics. In some cases, it is simply to prevent the loss of water during experimentation. Elastin immersed in various alcohol solutions maintains constant sample volume (pure water causes deswelling), and it is shown that elastin has entropy-dominated rubber elasticity with no internal energy change, $\delta U/\delta T \approx 0$.^{12,24} A similar result is also found with dimethyl sulfoxide (DMSO).²⁵ In other cases, the solvent is meant to try to understand the effect of the protein amino acid composition (hydrophobic vs. hydrophilic chain portions) on thermodynamics. This could also help in understanding other phenomena, since protein rubbers exist next to sugars and other proteins (i.e., resilin/chitin²⁶ and elastin/collagen/glycosaminoglycans, GAG²⁷). Simply changing the solution pH for immersed elastin results in a swelling variation with higher pH giving higher swelling.²⁸ The changing protein ionization state allows for more elastin water absorption and volume increase with a concurrent decrease in modulus. The glass transition temperature, T_g , does not change for elastin plasticized (i.e., not fully immersed) in water or ethylene glycol as a function of plasticizer concentration.²⁹ The authors conclude that this is evidence of no specific plasticizer–elastin interaction. Elastin swollen in mixtures of extracellular matrix polymers such as chondroitin sulfate (i.e., GAG) dehydrate elastin until it has glassy behavior.¹⁴ Elastin immersed in varying length glycols at the same polymer volume fraction (v_p , or same swelling) results in shifting of loss tangent ($\tan \delta = G''/G'$) peaks to higher temperature with increasing glycol length, showing that polymer chain relaxations can be shifted to higher temperature.³⁰ Experiments on elastin plasticized (not immersed) in various solvents show that small polar solvents just dehydrate, or indirectly interact with, elastin, changing molecular relaxation rates but not mechanisms. However, DMSO and high concentrations of ethylene glycol do change the elastin viscoelastic behavior, which may change relaxation rates and mechanisms.³¹

Gelatin is a commercially available protein rubber formed from the hydrolysis of animal collagen. In its hydrolyzed form, the large amount of native collagen triple helix is lost. Instead, the hydrolyzed collagen in gelatin is largely disordered with occasional triple helices acting as physical crosslinks, which gives gelatin a molecular structure similar to a crosslinked rubber.^{3,32} Triple helices are lost at about 37°C but reform again upon cooling, making it a very easy protein rubber to process and form into useful biological elastomers.³³ Gelatin has been shown to exhibit rubber elasticity when properly crosslinked or plasticized.^{34–38} It is hypothesized that the protein rubber's molecular environment can affect its performance. In this study, gelatin, a protein rubber, is plasticized with liquids and sugar solutions of varying molecular weight and hydrogen bonding potential. The gelatin is not fully immersed in the plasticizer, but the plasticizer is added in amounts where it remains completely contained by the gelatin rubber. The plasticizer acts to reduce the gelatin rubbery modulus.³⁹ The rubber elasticity is characterized including modulus, loss tangent, and resilience.

EXPERIMENTAL

MATERIALS

Type B gelatin from bovine skin (225 Bloom, G9382 from Sigma Aldrich) was dissolved and incubated or “bloomed” in de-ionized (DI) water at a 1:1 wt:wt ratio for 30 minutes at 65°C with occasional mixing. After 30 min, the plasticizer was added and mixed into the hydrated gelatin at a molar ratio of 2271:1 mol:mol plasticizer:gelatin. To make a “dry” system, that is, one that was not fully immersed in liquid but rather was plasticized by the liquid and behaved as a manipulatable solid, actual gummy bears were used as inspiration. The molar ratios of plasticizer:gelatin were similar to those used in commercial gummy candy. Plasticizers were de-ionized water (18.02 g/mol), glycerol (92.09 g/mol, BDH1172 from VWR, Radnor, PA), ethylene glycol (62.07 g/mol, 102466 from Sigma-Aldrich, St. Louis, MO), and light corn syrup (234.22 g/mol from Kroger, Cincinnati, OH). Corn syrup, that is, “glucose syrup,” was the hydrolysis product of corn starch, had 23 wt% water as measured with thermogravimetric analysis, and was about 3:1 mol:mol H₂O:glucose (the balance was oligosaccharides), which gave the 234.22 g/mol molecular weight (3 H₂O + 1 glucose). Plasticizers of pure sugar syrup were also made at 3:1 mol:mol H₂O:sugar using D-(+)-glucose (180.16 g/mol, G7021 from Sigma Aldrich), D-(–)-fructose (180.16 g/mol, F0127 from Sigma Aldrich), and D-sorbitol (182.17 g/mol, S1876 from Sigma Aldrich). The 3:1 molar ratio sugar syrups were heated until they formed clear solutions, which occurred at 50°C for sorbitol, 74°C for fructose, and 85°C for glucose. Upon cooling, sorbitol and fructose syrups remained stable for months, but the glucose syrup precipitated after a few days, so the syrups were used right after preparation, that is, within the stable time frame. The molecular weight used in the 2271:1 mol:mol plasticizer:gelatin calculation for the sugar syrup plasticizers was the molecular weight of each sugar plus three water molecules. The molar ratio was varied for corn syrup to 1927:1 mol:mol plasticizer:gelatin to note any effects. Gelatin–plasticizer mixing was performed lightly enough to minimize air bubbles, and any air bubbles generated were removed under vacuum. The hot gelatin–liquid mixture was then poured into an 18 cm × 18 cm silicone mold. The sample remained at room temperature for 30 min, after which it was packed tightly in plastic and refrigerated for another 24 hours, in order to avoid water loss over time. No mass change was found after refrigeration. The sample was removed from the refrigerator after 24 hours and allowed to reach room temperature prior to cutting samples for testing. 25 mm disks were cut with a round steel cutter and ASTM D412-D dog bones were cut with a steel die. Rheological and tensile testing proceeded immediately after cutting.

METHODS

Rheology. — This was testing at very low deformation well within the linear viscoelastic limit. Strain-controlled oscillatory shear experiments were performed using 25 mm sandblasted stainless-steel parallel plates on a Discovery Hybrid Rheometer (DHR-3, TA Instruments).⁴⁰ A normal force of about 1–2 N (2038–4076 Pa) was used to hold the samples in place and prevent slip. First, strain sweeps were performed to find the linear viscoelastic region. It was determined that an applied strain of $\gamma = 0.1\%$ was in the linear viscoelastic region for all samples. Frequency sweeps were performed from $\omega = 0.1$ to 100 rad/s. The gap height between the plates varied based on sample thickness, which was about 3 mm depending on sample density and how much could be poured evenly into the mold before gelatin curing began, and applied normal force, which did not cause sample bulging, mass loss, or otherwise affect the modulus measurements. Four samples of each formulation were tested, and the elastic (storage) modulus, G' , loss modulus, G'' , and loss tangent, $\tan \delta = G''/G'$, reported as an average \pm standard error. Rheological and tensile testing occurred at room temperature and humidity, which were 21°C and 35%, respectively.

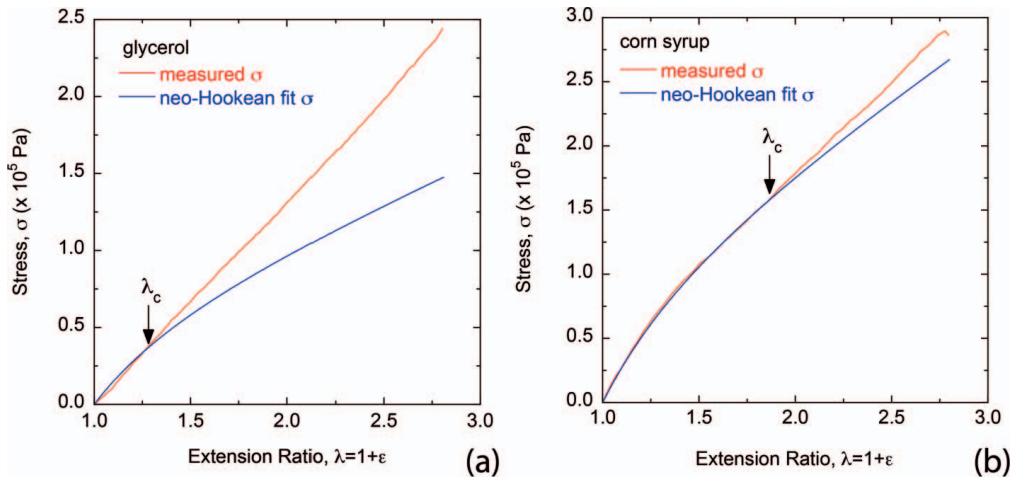


FIG. 1. — Example engineering stress–extension ratio (σ – λ) curves comparing the measured stress to the neo-Hookean model fit (Eq. 1) for (a) glycerol- and (b) corn syrup–plasticized gelatin rubber. Extension ratio λ_c defines the critical extension where the measured σ – λ data deviate from the neo-Hookean model.

Tensile Testing. — This was testing at very high deformation beyond the linear viscoelastic limit. ASTM D412 Die D tensile specimens were uniaxially deformed to an applied strain of $\varepsilon = 200\%$ at a crosshead speed or rate of loading of $r = 8.33$ mm/s (500 mm/min) for six loading/unloading cycles on a Texture Analyzer TA.HDPlus (Texture Technologies Corp., Hamilton, MA, USA) equipped with a 5-kg load cell and self-tightening crosshatched grips. Engineering or nominal stress, σ , and strain, ε , are found from the original sample cross-sectional area and the crosshead displacement relative to the sample gage length, respectively. Six samples were tested for each formulation, and results reported as an average \pm standard error. Tensile or Young’s modulus, E , was calculated by linear regression analysis of the low strain Hookean, $\sigma = E\varepsilon$, region of the engineering stress–strain plots. Modulus was also found by fitting the neo-Hookean model

$$\sigma = G \left(\lambda - \frac{1}{\lambda^2} \right) \quad (1)$$

using $E = 3G$ and $\lambda = 1 + \varepsilon$ (extension ratio) with great agreement (Figure 1).⁴¹ The rubber efficiency or “resilience, R ” (imposed strain energy stored per loading/unloading cycle) was calculated from Eq. 2, where A_l and A_u were the areas under the loading and unloading stress–strain curves, respectively.

$$R(\%) = 100 \times \frac{A_u}{A_l} \quad (2)$$

Therefore, the energy lost (hysteresis, %) is $100 - (A_u/A_l)$. Residual strain, x , or “set” is the residual deformation left on the rubber sample at the end of a loading and unloading cycle and is defined in Figure 2. The set is not permanent and relaxes out over long periods of time but remains within the time frame of the experiment.

Plasticizer Viscosity. — The plasticizer viscosity was found in a simple shear concentric cylinder geometry. A cup (diameter, $D = 30.32$ mm) and bob (or cone, $D = 27.98$ mm and length, $L = 42.00$ mm) fixture was used on the DHR3 rheometer. The fixture allowed for 16.5 ml of sample to be tested, and samples were tested in triplicate with results reported as an average \pm standard error. Shear rate was varied from $\dot{\gamma} = 1.24$ – 1240 s^{-1} . Water viscosity was not measured, and its reported

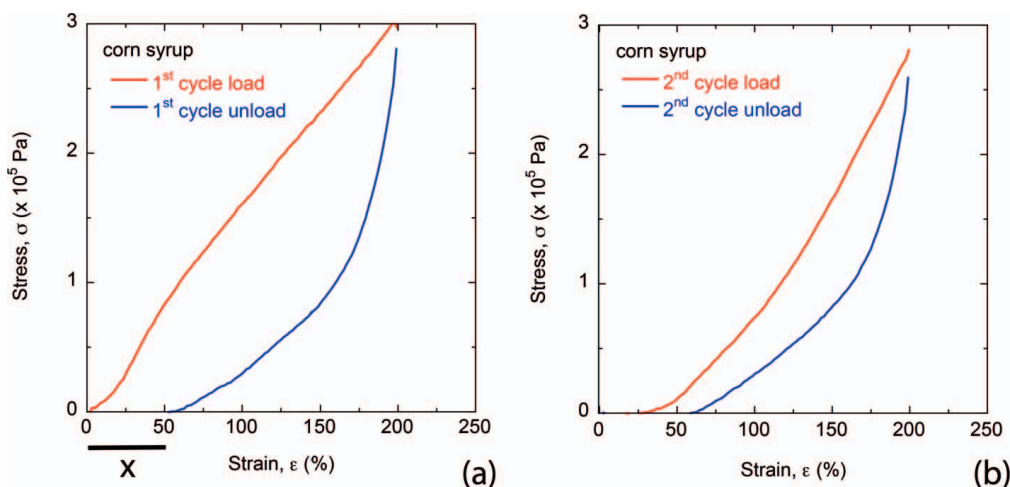


FIG. 2. — Example engineering stress–strain (σ – ϵ) curves for corn syrup–plasticized gelatin rubbers (a) first load–unload cycle and (b) second load–unload cycle. There is rubber network damage after the first cycle defined by the residual strain or “set,” x . There is a small toe region on the first load that defines the difference between the zero stress points on the subsequent cycles and is excluded in the residual strain, x , calculation.

value at 21°C was used.⁴² The measured viscosities of glycerol and ethylene glycol were very close to literature values. All plasticizers were Newtonian liquids over the shear rate range studied.

RESULTS AND DISCUSSION

There is no measurable volume change during the experiments, showing no loss of plasticizer. The storage modulus is about an order of magnitude higher than the loss modulus, showing that these are highly elastic networks like crosslinked rubber (Figure 3). Water results in the stiffest gelatin network, and fructose the softest network (Figure 3a). Water-, ethylene glycol-, and glycerol-plasticized gelatin rubbers have relatively flat G' over all ω and are squarely in the rubbery plateau region. However, the sugar syrup–plasticized rubbers have a gradual increase of the storage modulus, G' , and rapid increase of the loss modulus, G'' , at high frequencies where $\tan \delta$ is getting closer to 1. This shows that these rubbers are approaching the rubber to glass transition at high frequencies. Viscoelastic loss is frequency-dependent for each plasticized rubber (Figure 3c). Fructose-plasticized gelatin exhibits the least and corn syrup–plasticized gelatin the most loss at low frequency, but ethylene glycol- and glycerol-plasticized gelatin exhibit the least loss at moderate to high frequency.

In high strain tension, all rubbers have an initial modulus decrease upon the first loading/unloading cycle, followed by attainment of relatively constant modulus at higher cycles (Figure 4a). The initial modulus decrease is small for all the rubbers except corn syrup–plasticized gelatin, which has a very large modulus decrease after the first cycle. Cycles 4 and 5 for water- and cycle 5 for ethylene glycol–plasticized gelatin represent only one sample because these rubbers tend to break at high strain after several cycles. Water- and fructose-plasticized gelatin again have the highest and lowest modulus, respectively. The modulus trend is generally the same for the tensile data as for the shear data, and the same trend has been observed for gellan gum plasticized with the same sugars and tested in compression.⁴³ The plasticized gelatins truly behave like rubbers with hyperelasticity. Although the experiments proceeded to a predetermined strain of $\epsilon = 200\%$, this is much higher than gelatin films studied for food packaging applications,^{44–47} previous gelatin

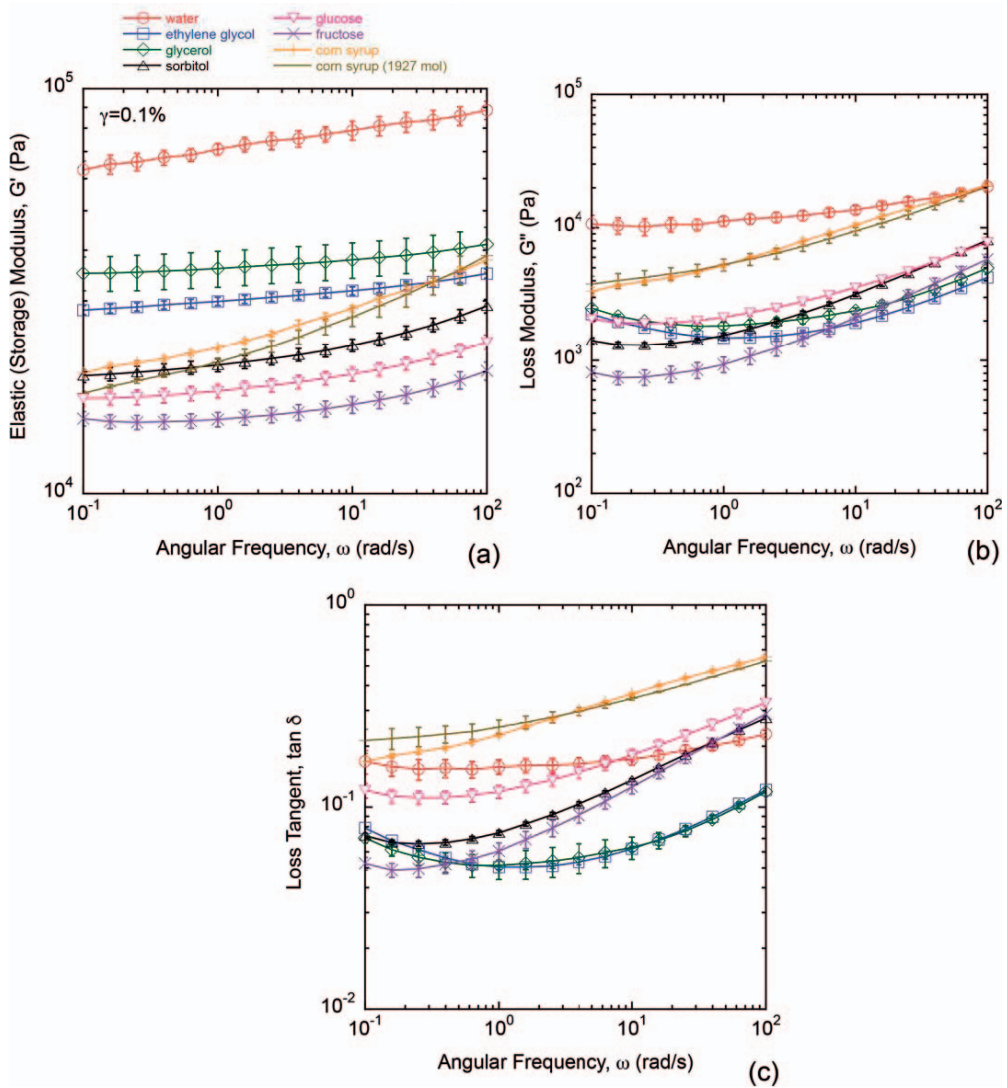


FIG. 3. — Low strain dynamic shear rheology for plasticized gelatin. (a) Storage, G' , and (b) loss, G'' , modulus and (c) loss tangent, $\tan \delta = G''/G'$. Data obtained at an applied strain of $\gamma = 0.1\%$ and 21°C .

rubber characterizations,^{34,35,48} and gelatin from sources other than bovine or porcine,^{49,50} which exhibit ultimate strain limits of 25–100%. The exception is glutaraldehyde crosslinked gelatin, which has an elastic limit $>400\%$ at low crosslink density.³⁸ Glycerol- and fructose-plasticized gelatin result in rubbers of similar high efficiency (or resilience) with ethylene glycol-plasticized gelatin close behind (Figure 4b). The corn syrup-plasticized gelatin rubbers have the lowest efficiency. Since breaking at higher cycles is an issue, both water- and ethylene glycol-plasticized gelatin data at cycles higher than two represent only one sample. The efficiency increases with the number of cycles because the area under the loading σ – ε curve dramatically decreases after the first cycle and the area is relatively constant for all subsequent cycles. Each rubber exhibits a residual strain, x , after deformation to $\varepsilon = 200\%$, with corn syrup-plasticized gelatin having the most and fructose-plasticized gelatin the least (Figure 4c).

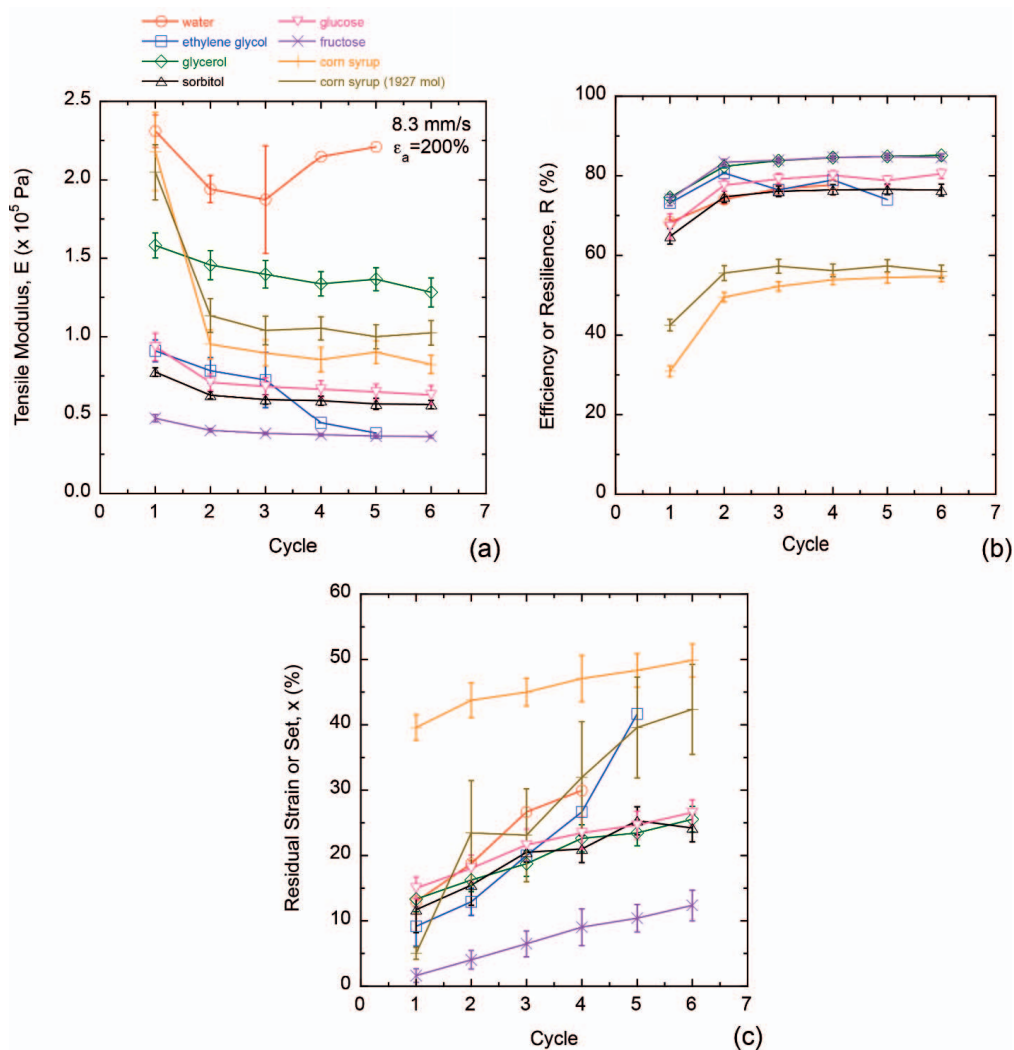


FIG. 4. — High strain tensile properties for plasticized gelatin. (a) Tensile modulus, E , (b) efficiency or resilience, R , and (c) residual strain or “set,” x . Data obtained at a rate of loading of $r = 8.33 \text{ mm/s}$ applied to a strain of $\epsilon = 200\%$ at 21°C .

The shear elastic modulus, G' , is about one-third of the tensile modulus, E (Figure 5). In tension the rate of loading is $r = 8.33 \text{ mm/s}$ and the sample gage length is $L_g = 33.3 \text{ mm}$, which can be converted to a radial frequency of $\omega = 2\pi \times r/L_g = 1.57 \text{ rad/s}$, and G' is used at this frequency to make the best comparison with E from tension. For an incompressible material, Poisson's ratio is $\nu = 0.5$, yielding $E = 2(1 + \nu)G' = 3G'$, showing that the plasticized gelatin samples behave like incompressible rubbers. All of the data on the first loading cycle except the corn syrup–plasticized gelatins follows the trend. On the second loading cycle, all of the rubbers follow the trend (which continues for each subsequent cycle, since the properties on the second cycle are about those at higher cycles). The high tensile modulus exhibited by corn syrup–plasticized gelatin is repeatable and appears in all of the samples, even at different corn syrup molar concentrations. The mixture of sugars in corn syrup must create a network that breaks massively after the first cycle. The stress–extension ratio (σ – λ) curves appear similar to those for typical protein rubbers like resilin and

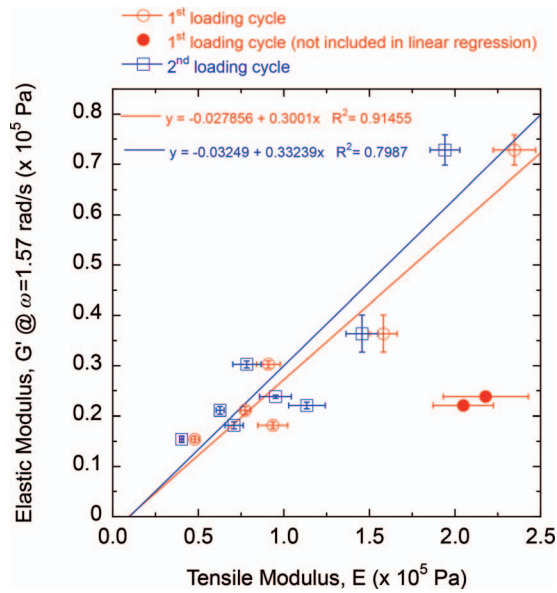


FIG. 5. — The shear modulus, G' , and tensile modulus, E , follow $E=3G'$ for incompressible materials with Poisson's ratio, $\nu=0.5$, similar to typical rubbers.

elastin (Figure 1 gives examples).¹⁴ For resilin and elastin, the deviation of the actual σ - λ curve from the neo-Hookean model occurs at a critical extension ratio of about $\lambda_c = 1.6$ ($e = 60\%$ strain). For most plasticized gelatin rubbers, λ_c is slightly lower than for resilin and elastin (Figure 6, data for first loading curve shown). Extension ratio λ_c is higher for corn syrup-plasticized gelatin than for other plasticized gelatin rubbers and even resilin and elastin, which is interesting because it has the lowest efficiency and highest loss tangent. The trend remains the same for all subsequent cycles for all samples, i.e., $\lambda_c = 1.29 \pm 0.02$ for the 3rd fructose loading cycle, except for the corn syrup-plasticized ones. It goes down significantly for the corn syrup-plasticized gelatin rubbers with $\lambda_c = 1.50 \pm 0.04$ for the third corn syrup loading cycle, but that is still high for all protein rubbers.

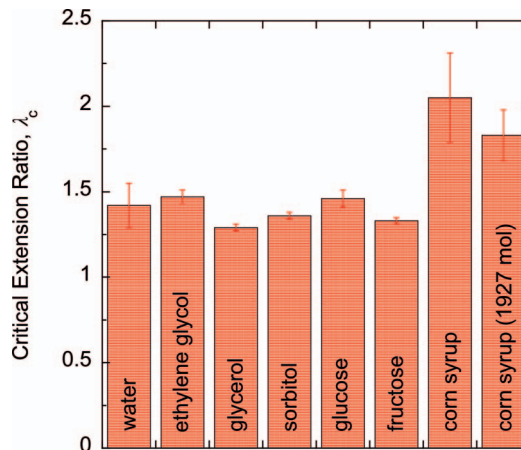


FIG. 6. — Critical extension ratio, λ_c (defined in Figure 1), is the point the measured σ - λ curve deviates from the neo-Hookean model for rubber elasticity.

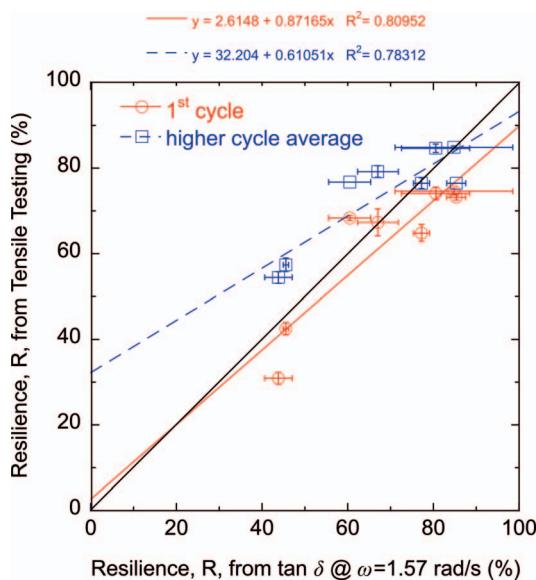


FIG. 7. — Resilience, R , from first cycle tensile data (red) and from an average of the subsequent higher cycles (blue) versus the resilience found at a similar frequency in low strain shear. The black line is a 1:1 correlation.

The efficiencies at low and high strain correlate well (Figure 7). The loss tangent, $\tan \delta$, is converted to an efficiency or resilience, R , through

$$R(\%) = \frac{100}{\exp(\pi \times \tan \delta)} \quad (3)$$

where $\tan \delta$ is found at an applied frequency of $\omega = 1.57 \text{ rad/s}$.⁵ There is a good 1:1 correlation when using the first cycle tensile data. However, the higher cycle data give a good linear regression correlation coefficient, $r^2 = 0.77$, but do not give a 1:1 correlation. This is probably because there is structure loss at subsequent cycles. Overall the efficiencies compare well to typical protein rubbers but are lower than the highest, such as resilin and abductin at $R > 90\%$.^{5,51} (pure crosslinked natural rubber has $R \sim 80\%$ at room temperature⁵²).

The elastic modulus, G' , decreases with increasing plasticizer Newtonian viscosity, η_0 (Figure 8). Rubber elasticity theory relates the elastic modulus, G' , to the molecular weight between crosslinks, M_c , through

$$G' = \frac{\rho RT}{M_c} \quad (4)$$

where ρ is the rubber density, R is the gas constant, and T is absolute temperature, all of which are constants making G' solely dependent on M_c (Table I). Rubber density ρ is found from a rule of mixtures law that considers all of the gelatin rubber components.^{40,53} The plasticizer viscosity increases with increasing molecular weight, which also increases the number of hydrogen bonding donor and acceptor sites (Figure 9).⁵⁴ This seems to suggest that higher molecular weight plasticizers separate gelatin molecules more and/or interrupt triple helix formation to increase the molecular weight between crosslinks, M_c (Figure 10, which is drawn with a higher molecular weight between crosslinks *and* triple helix loss from plasticizer solvation when the plasticizer molecular weight is higher, as shown in Figure 10b). Given the stronger correlation with hydrogen

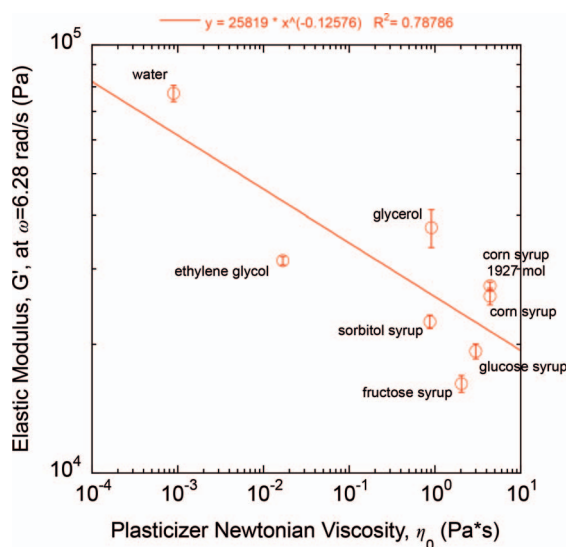


FIG. 8. — There is a good correlation between the gelatin rubber elastic modulus, G' , and the plasticizer Newtonian viscosity, η_0 .

bonding, it may be that the plasticizer solvates gelatin molecule sections that would normally form triple helices, preventing them from forming and keeping M_c high (Figure 9b).

While G' correlates with the plasticizer viscosity to give insight into the gelatin rubber molecular structure, $\tan \delta$ (and R) and G'' do not correlate with the plasticizer viscosity, indicating they originate in other gelatin rubber molecular features. Water-, ethylene glycol-, and glycerol-plasticized gelatin rubbers show an increase in loss tangent at higher frequency (ω) than the sugar syrup-plasticized rubbers (Figure 3c). Loss tangent can be shifted in frequency space using Williams-Landel-Ferry (WLF)-type shift factors, a_T (Figure 11 and Table I). All of the rubbers are in the rubbery region and approaching the rubber to glass transition depending on the frequency (Figure 11c).^{55,56} The gelatin rubbers separate into two categories: Group A where there is good shifting into a master curve for polyol- and sugar-plasticized rubbers (Figure 11a, shifted to

TABLE I
CALCULATED M_c VALUES AND WLF-TYPE SHIFT FACTORS, a_T , FOR GELATIN RUBBERS^a

Rubber	Structure	G' , Pa ^b	ρ , g/cm ³	M_c , g/mol	a_T
Water	H ₂ O	77,206 ± 3382	1.12	35,992	1 ^c
Ethylene glycol	C ₂ H ₆ O ₂	31,356 ± 738	1.13	89,418	1 ^d
Glycerol	C ₃ H ₈ O ₃	37,442 ± 3810	1.19	78,914	1 ^d
Sorbitol	C ₆ H ₁₄ O ₆	22,574 ± 811	1.10	120,670	12 ^d
Glucose	C ₆ H ₁₂ O ₆	19,259 ± 747	1.10	142,033	20 ^d
Fructose	C ₆ H ₁₂ O ₆	16,178 ± 739	1.12	170,797	10 ^d
Corn syrup	C ₆ H ₁₂ O ₆ ·3H ₂ O	27,379 ± 584	1.26	114,117	100 ^c
Corn syrup (1927 mol)	C ₆ H ₁₂ O ₆ ·3H ₂ O	25,874 ± 1161	1.25	120,046	95 ^c

^a Structures, molecular weights, and physical properties can be found at NIH PubChem.⁵⁴

^b Found at $\omega = 6.28$ rad/s.

^c Shifted relative to water (Group B).

^d Shifted relative to ethylene glycol and glycerol (Group A).

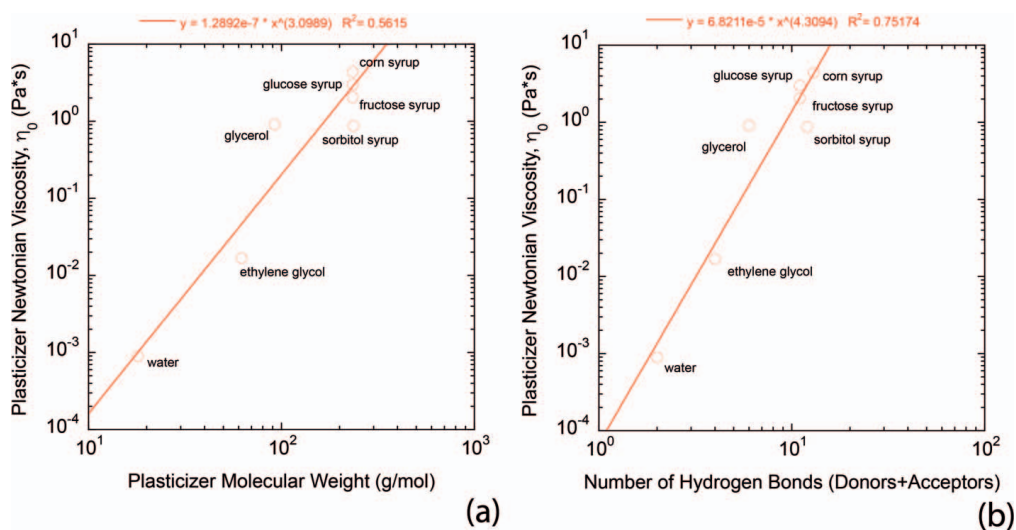


FIG. 9. — The plasticizer Newtonian viscosity correlates with the plasticizer molecular weight and number of hydrogen bonds.

ethylene glycol and glycerol references) and Group B where there is good shifting into a master curve for water- and corn syrup-plasticized rubbers (Figure 11b, shifted to water reference).

Plasticized protein rubbers that shift into a master curve have the same molecular relaxation process but different relaxation rates.^{30,31} Therefore, Group A plasticizers create gelatin rubbers with one molecular relaxation process and Group B plasticizers create gelatin rubbers with a different molecular relaxation process. Ethylene glycol-, glycerol-, and fructose syrup-plasticized gelatin rubbers (Group A) completely shift into a master curve with sorbitol syrup-plasticized gelatin rubber close to the master curve (the lower ω data are mostly inside the error bars, Figure 11a). Glucose syrup-plasticized gelatin rubber only shifts at higher frequency, with the lower frequency data lying above the master curve. Glucose syrup-plasticized gelatin rubber is plotted with Group A gelatin rubbers because it has a similar loss tangent shape and some shifting overlap and it approaches the glass transition similarly. This would suggest that Group A plasticizers are capable of plasticizing gelatin similarly if used at the correct molar amount except for glucose syrup.

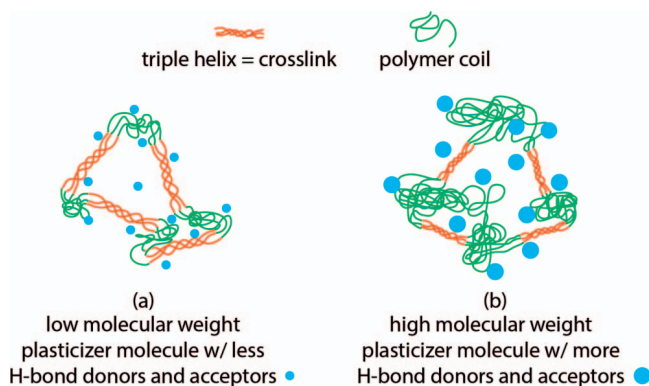


FIG. 10. — Schematic showing gelatin rubber structure with (a) low and (b) high molecular weight between crosslinks, M_c , represented by the length of polymer coils between crosslinks in green.

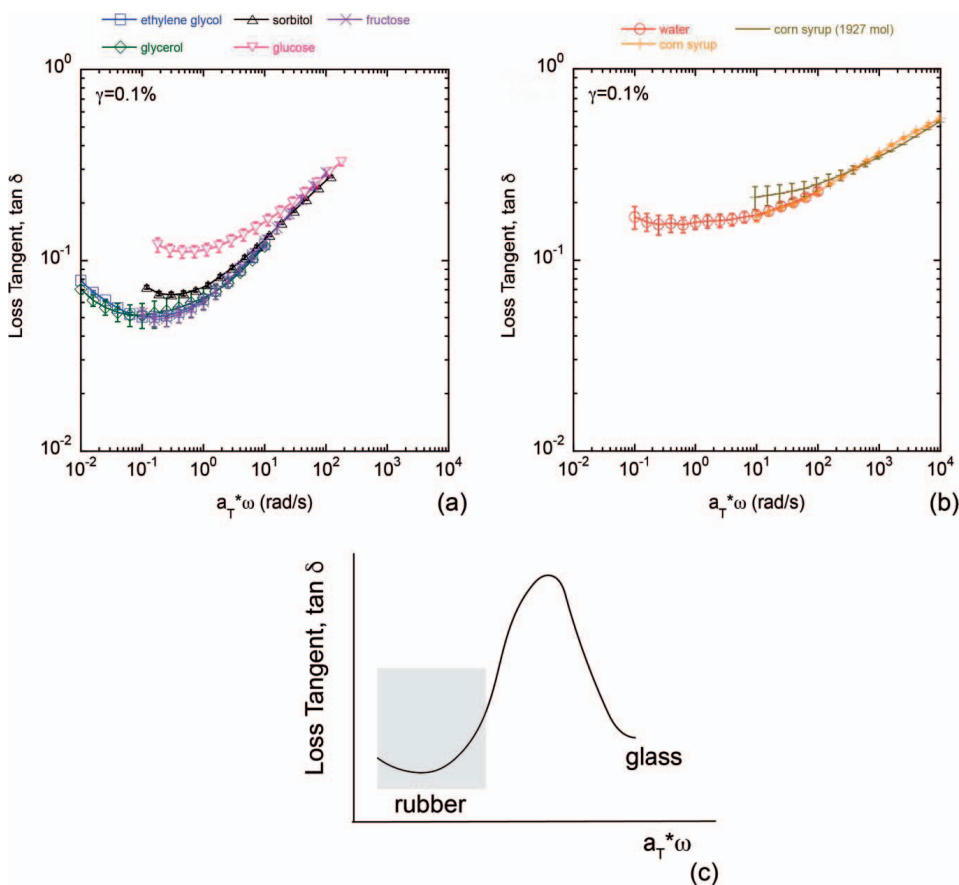


FIG. 11. — WLF-like shifting of (a) polyol- and sugar-plasticized gelatin rubbers shifted relative to ethylene glycol and glycerol (Group A), (b) water- and corn syrup-plasticized gelatin rubbers shifted relative to water (Group B). (c) Schematic of the rubber to glass transition. The gelatin rubbers described here exist in the shaded area.

Ethylene glycol-, glycerol-, sorbitol syrup-, and fructose syrup-plasticized gelatin rubbers all have the same viscoelastic loss, or efficiency, when compared at equal plasticization/hydration and constant frequency. It would be the same as plasticizing the rubbers to equal extents such that the rubbers would show the same loss tangent at constant frequency. The exception is glucose syrup at low frequency, which has a different relaxation mechanism such that it experiences more viscoelastic loss. The master curve insinuates that the viscoelastic response is dominated by gelatin, which has been observed previously.^{29,30,57}

Water- and corn syrup-plasticized gelatin rubbers (Group B) also shift onto a master curve, showing similar relaxation mechanisms to each other (Figure 11b). The low frequency corn syrup 1927:1 mol:mol plasticizer:gelatin rubber lies slightly above the master curve but close to the experimental error, so there might be a different relaxation mechanism here, like glucose. However, Group B water- and corn syrup-plasticized gelatin rubbers approach the rubber to glass transition differently than the Group A rubbers and have a different loss tangent curve shape. The difference is interesting because water and corn syrup delay the Group B glass transition, but in the rubbery region there is more viscoelastic loss (higher $\tan \delta$) than the Group A plasticizers. Corn syrup does have some water in it, but so do the prepared sugar syrups, so it is not the presence of water. Group B

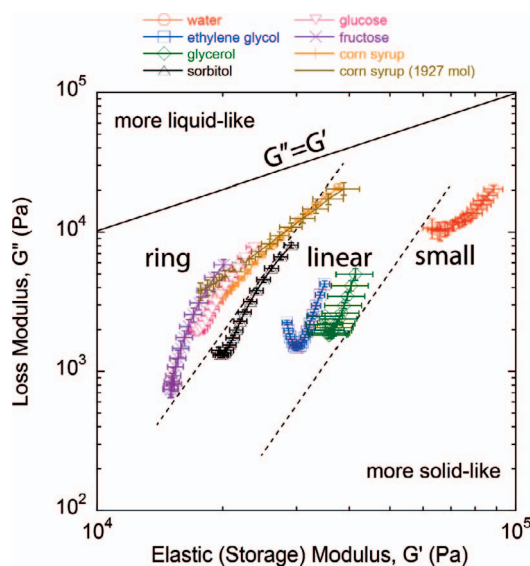


FIG. 12. — Modified Cole–Cole ($\log G''$ – $\log G'$) plot showing plasticized gelatin rubber samples separating into distinct regions for small molecules, linear polyols/sugars, and ringed sugars.

water- and corn syrup-plasticized gelatin rubbers also undergo the largest modulus decrease after the first cycle and have the highest residual strain, x , so these rubbers appear to be more fragile networks that break and dissipate imposed strain energy rather than storing and returning it.

The modified Cole–Cole plot (G'' vs. G' on a log–log scale, Figure 12) is an empirical way to look for rubber structural trends.⁵⁸ There is a trend with water (small molecule), linear polyols/sugars (ethylene glycol, glycerol, sorbitol), and ringed sugars (glucose, fructose, and corn syrup) separating into distinct regions. These regions depend on the molecular weight between crosslinks, M_c , with more crosslinked rubbers lying to the right. However, rubber plastic deformation (residual strain, x) and efficiency (R or $\tan \delta$) depend on other protein molecular features. While modulus, G' and E , can be explained by how each plasticizer creates a network of different M_c , there are additional network features that contribute to the rubber efficiency and residual strain or the ability to store and dissipate energy, respectively. When comparing Figures 3a,c and 4a,c, there is a general trend that less plastically deformable rubber networks are more efficient at storing and returning energy, which makes sense. The results suggest that gelatin rubbers with low crosslink density (or high M_c) far from the glass transition are the most recoverable and efficient. For instance, fructose-plasticized gelatin probed at $\omega = 10$ rad/s is less efficient (has higher $\tan \delta$) than ethylene glycol-plasticized gelatin. While fructose-plasticized gelatin is of lower crosslink density, it is approaching the glass transition faster than ethylene glycol-plasticized gelatin at $\omega = 10$ rad/s. However, when both are probed at $\omega = 0.2$ rad/s, the fructose-plasticized gelatin is outside of the glass transition and in the rubbery region and is more efficient than ethylene glycol-plasticized gelatin, which is slightly below the rubbery region and able to have some viscous loss. For a plasticizer like glucose syrup, there is a completely different gelatin molecule relaxation mechanism at work that makes it less efficient, even though it has a low crosslink density and is far from its glass transition at $\omega = 0.2$ rad/s. While the separation is not as large at high strains, the general trend still holds. The ability to damage the network at high strain does not appear to be related to the plasticizer molecular weight or hydrogen bonding potential. It could be related to how the plasticizer exists in the rubber, that is, how much it interacts with other plasticizer molecules vs. gelatin.

CONCLUSIONS

Protein rubbers are valued for their high efficiencies *in vivo*. Small amounts of protein rubbers like elastin and resilin have been harvested or fermented for use *in vitro*. Typically, the protein rubbers require immersion in aqueous solution to function properly. Here, it is shown that a commodity protein rubber, gelatin, can be plasticized and not immersed in various liquids to realize a highly efficient usable rubber. The choice of plasticizer affects the overall rubber modulus, with larger molecular weight plasticizers containing more hydrogen bond donors and acceptors forming softer networks with larger molecular weight between crosslinks, M_c . Plasticizers like ethylene glycol, glycerol, and fructose syrup (Group A) create gelatin networks that can store and return a large portion of an imposed strain energy. Other plasticizers like water and corn syrup (Group B) create gelatin networks that dissipate more imposed strain energy. The efficiency is dependent on the gelatin molecule relaxation mechanism elicited by the plasticizer. Being below the glass transition and firmly in the rubbery region is one important aspect to high efficiency, although there seem to be others not yet understood. All gelatin rubbers exhibit plastic deformation at high strain but to very different extents, with less plastic deformation contributing to higher efficiency. A simple and usable biological rubber of controllable modulus and efficiency is created that might find use in engineered bioinspired applications such as emerging soft machines. These devices could work on elastic recoil with high efficiency without having to be fully immersed in aqueous solution.

REFERENCES

- ¹J. Vincent, *Structural Biomaterials*, Princeton University Press, Princeton, NJ, 1990.
- ²T. Weis-Fogh, *RUBBER CHEM. TECHNOL.* **36**, 90 (1963).
- ³R. Schrieber and H. Gareis, *Gelatine Handbook*, Wiley-VCH Verlag GMBH & Co., Weinheim, Germany, 2007.
- ⁴R. M. Alexander, "Functions of Elastomeric Proteins in Animals," in *Elastomeric Proteins*, P. R. Shewry, A. S. Tatham, A. J. Bailey, Eds., Cambridge University Press, Cambridge, 2003, p 1.
- ⁵R. M. Alexander, *J. Exp. Biol.* **44**, 119 (1966).
- ⁶J. M. Gosline, M. A. Lillie, E. Carrington, P. Guerette, C. Ortlepp, and K. Savage, *Phil. Trans. R. Soc. London B: Biol. Sci.* **357**, 121 (2002).
- ⁷T. Weis-Fogh, *J. Exp. Biol.* **37**, 889 (1960).
- ⁸T. Weis-Fogh, *J. Mol. Biol.* **3**, 520 (1961).
- ⁹G. A. Kahler, F. M. Fisher, and R. L. Sass, *Biol. Bull.* **151**, 161 (1976).
- ¹⁰M. Denny and L. Miller, *J. Exp. Biol.* **209**, 4503 (2006).
- ¹¹E. Wöhlisch, H. Weitnauer, W. Grüning, and R. Rohrbach, *Kolloid-Zeitschrift* **104**, 14 (1943).
- ¹²C. A. J. Hoeve and P. J. Flory, *J. Am. Chem. Soc.* **80**, 6523 (1958).
- ¹³P. R. Shewry, N. G. Halford, P. S. Belton, and A. S. Tatham, "Gluten, the elastomeric protein of wheat seeds," in *Elastomeric Proteins*, P. R. Shewry, A. S. Tatham, A. J. Bailey, Eds., Cambridge University Press, Cambridge, U.K., 2002, p 279.
- ¹⁴J. M. Gosline, *RUBBER CHEM. TECHNOL.* **60**, 417 (1987).
- ¹⁵Q. Cao, Y. Wang, and H. Bayley, *Curr. Biol.* **7**, R677 (1997).
- ¹⁶S. M. Partridge, "Elastin," in *Advances in Protein Chemistry*, Vol. 17, C. B. Anfinsen, K. Bailey, M. L. Anson, J. T. Edsall, Eds., Academic Press, New York, 1963, p 227.
- ¹⁷D. W. Urry, T. Hugel, M. Seitz, H. E. Gaub, L. Sheiba, J. Dea, J. Xu, T. Parker, A. J. Bailey, J. Macmillan, P. R. Shewry, and A. S. Tatham, *Phil. Trans. R. Soc. London B: Biol. Sci.* **357**, 169 (2002).
- ¹⁸C. M. Elvin, A. G. Carr, M. G. Huson, J. M. Maxwell, R. D. Pearson, T. Vuocolo, N. E. Liyou, D. C. C. Wong, D. J. Merritt, and N. E. Dixon, *Nature* **437**, 999 (2005).
- ¹⁹S. Ricard-Blum, *Cold Spring Harbor Persp. Biol.* **3**, a0044978 (2011).
- ²⁰A. S. Tatham and P. R. Shewry, *Trends Biochem. Sci.* **25**, 567 (2000).

- ²¹A. S. Tatham and P. R. Shewry, *Phil. Trans. R. Soc. London B: Biol. Sci.* **357**, 229 (2002).
- ²²S. Rauscher, S. Baud, M. Miao, F. W. Keeley, and R. Pomès, *Structure* **14**, 1667 (2006).
- ²³S. Rauscher and R. Pomès, “Structural Disorder and Protein Elasticity,” in *Fuzziness: Structural Disorder in Protein Complexes*, M. Fuxreiter, and P. Tompa, Eds., Springer US, New York, 2012, p 159.
- ²⁴C. A. J. Hoeve and P. J. Flory, *Biopolymers* **13**, 677 (1974).
- ²⁵F. Mistrali, D. Volpin, G. B. Garibaldo, and A. Ciferri, *J. Phys. Chem.* **75**, 142 (1971).
- ²⁶G. Qin, X. Hu, P. Cebe, and D. L. Kaplan, *Nature Commun.* **3**, 1003 (2012).
- ²⁷C. L. Martin, M. R. Bergman, L. F. Deravi, and J. A. Paten, *Bioelectricity* **2**, 186 (2020).
- ²⁸T. Weis-Fogh, *J. Mol. Biol.* **3**, 648 (1961).
- ²⁹C. A. J. Hoeve and M. B. J. A. Hoeve, *Polymer Eng. Sci.* **20**, 290 (1980).
- ³⁰A. L. Andrady and J. E. Mark, *Polym. Bull.* **27**, 227 (1991).
- ³¹M. A. Lillie and J. M. Gosline, *Biorheology* **30**, 229 (1993).
- ³²W. F. Harrington and P. H. Von Hippel, “The Structure of Collagen and Gelatin,” in *Advances in Protein Chemistry*, Vol. 16, C. B. Anfinsen, M. L. Anson, K. Bailey, and J. T. Edsall, Eds., Academic Press, New York, 1962, p 1.
- ³³I. V. Yannas, *J. Macromol. Sci. C* **7**, 49 (1972).
- ³⁴D. W. Jopling, *Rheol. Acta* **1**, 133 (1958).
- ³⁵I. V. Yannas and A. V. Tobolsky, *J. Appl. Polym. Sci.* **12**, 1 (1968).
- ³⁶G. V. S. Henderson, D. O. Campbell, V. Kuzmicz, and L. H. Sperling, *J. Chem. Ed.* **62**, 269 (1985).
- ³⁷A. Bot, I. A. van Amerongen, R. D. Groot, N. L. Hoekstra, and W. G. M. Agterof, *Polym. Gels Networks* **4**, 189 (1996).
- ³⁸J. A. Deiber, M. L. Ottone, M. V. Piaggio, and M. B. Peirrotti, *Polymer* **50**, 6065 (2009).
- ³⁹J. H. Mangalala and D. S. Simmons, *ACS Macro Lett.* **4**, 1134 (2015).
- ⁴⁰L. E. Hanzly, K. A. Kristofferson, N. Chauhan, and J. R. Barone, *Green Mater.* **9**, 157 (2021).
- ⁴¹L. R. G. Treloar, *The Physics of Rubber Elasticity*, Oxford University Press, Oxford, 1975.
- ⁴²C. J. Geankoplis, *Transport Processes and Separation Process Principles*, Prentice-Hall, Upper Saddle River, NJ, 2003.
- ⁴³G. Sworn and S. Kasapis, *Food Hydrocolloids* **12**, 283 (1998).
- ⁴⁴I. S. Arvanitoyannis, “Formation and Properties of Collagen and Gelatin Films and Coatings,” in *Protein-Based Films and Coatings*, A. Gennadios, Ed. CRC Press, Boca Raton, FL, 2002, p 27.
- ⁴⁵D. Kim and S. C. Min, *J. Food Sci.* **77**, E240 (2012).
- ⁴⁶N. Suderman, M. I. N. Isa, and N. M. Sarbon, *Food Biosci.* **24**, 111 (2018).
- ⁴⁷F. Liu, B.-S. Chiou, R. J. Avena-Bustillos, Y. Zhang, Y. Li, T. H. McHugh, and F. Zhong, *Food Hydrocolloids* **65**, 1 (2017).
- ⁴⁸H. J. Zhang, L. Wang, X. Wang, Q. Han, and X. You, *Soft Matter* **16**, 4723 (2020).
- ⁴⁹B.-S. Chiou, R. J. Avena-Bustillos, P. J. Bechtel, H. Jafri, R. Narayan, S. H. Imam, G. M. Glenn, and W. J. Orts, *Eur. Polym. J.* **44**, 3748 (2008).
- ⁵⁰M. H. M. Nor, N. N. M. Nazmi, and N. M. Sarbon, *Int. Food Res. J.* **24**, 1910 (2017).
- ⁵¹M. Jensen, T. Weis-Fogh, and J. W. S. Pringle, *Phil. Trans. R. Soc. London B: Biol. Sci.* **245**, 137 (1962).
- ⁵²H. C. Jones and E. G. Snyder, *RUBBER CHEM. TECHNOL.* **16**, 881 (1943).
- ⁵³L. E. Hanzly, N. Chauhan, and J. R. Barone, *Smart Mater. Struct.* **31**, 085005 (2022).
- ⁵⁴NIH (National Institutes of Health). PubChem. <https://pubchem.ncbi.nlm.nih.gov/compound/962>, Water; <https://pubchem.ncbi.nlm.nih.gov/compound/174>, Ethylene Glycol; <https://pubchem.ncbi.nlm.nih.gov/compound/753>, Glycerol; <https://pubchem.ncbi.nlm.nih.gov/compound/5780>, Sorbitol; <https://pubchem.ncbi.nlm.nih.gov/compound/5793>, D-Glucose; <https://pubchem.ncbi.nlm.nih.gov/compound/2723872>, D-Fructose; <https://pubchem.ncbi.nlm.nih.gov/substance/472387359>, Corn Syrup. Accessed June 24, 2022.
- ⁵⁵S. Kasapis and S. S. Sablani, *Int. J. Biol. Macromol.* **36**, 71 (2005).
- ⁵⁶S. Kasapis, *Food Hydrocolloids* **15**, 631 (2001).
- ⁵⁷I. M. Al-Marhoobi and S. Kasapis, *Carb. Res.* **340**, 771 (2005).
- ⁵⁸N. Nakajima, J. J. Scobbo, Jr., and E. R. Harrell, *RUBBER CHEM. TECHNOL.* **60**, 742 (1987).