

Large Amplitude Oscillatory Shear Studies on the Strain-stiffening Behavior of Gelatin Gels*

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Abstract Linear and nonlinear viscoelasticity of gelatin solutions was investigated by rheology. The dynamic mechanical properties during the sol-gel transition of gelatin followed the time-cure superposition. The fractal dimension d_f of the critical gel was estimated as 1.76, which indicated a loose network. A high sol fraction $w_s = 0.61$ was evaluated from the plateau modulus by semi-empirical models. Strain-stiffening behavior was observed under large amplitude oscillatory shear (LAOS) for the gelatin gel. The strain and frequency dependence of the minimum strain modulus G_M , energy dissipation E_d , and nonlinear viscoelastic parameter N_E was illustrated in Pipkin diagrams and explained by the strain induced helix formation reported previously by others. The BST model described the strain-stiffening behavior of gelatin gel quite well, whereas the Gent and worm-like chain network models overestimated the strain-stiffening at large strains.

Keywords: Gelatin; Large amplitude oscillatory shear; Strain-stiffening.

INTRODUCTION

Gelatin is a water soluble protein obtained by partial hydrolysis of collagen^[1, 2]. When a gelatin solution is cooled below the sol-gel transition temperature, the coil molecules form triple-chain helices, and the solution becomes an elastic hydrogel^[3–5]. Gelatin gels were widely used in food industry as thickening or gelling additives^[6], in drug delivery systems^[7, 8], and in tissue engineering scaffolds^[9, 10]. In all of these applications, the mechanical properties of the gelatin gel played a significant role^[11]. Gelatin gels stiffen at large strains, where the stress increases more than linearly with strain^[12–14]. This nonlinear elastic behavior of gelatin gels was well modeled by the phenomenological Blatz-Sharda-Tschoegl (BST) equation^[15].

The strain-stiffening phenomenon is common among rubber elastic materials^[16]. Phenomenological models describing this property includes the invariant-based models such as the Mooney-Rivlin model^[17, 18] and the Gent model^[19], as well as the models based on principal stretches such as those of Ogden^[20]. Besides these purely phenomenological models, micro-mechanically based network models were also proposed, such as the 3-chain^[21, 22], 4-chain^[23, 24], 8-chain^[25], and full network models^[26], based on non-Gaussian chain assumption. Models considering the topological constraint in the movement of crosslinking junctions and/or the chain segments were also developed^[27–29]. Miehe *et al.*^[30] combined the full network model with the tube model and proposed the micro-sphere model. Comparison and evaluation of these models with experimental data of rubber

* This work was financially supported by the National Natural Science Foundation of China (No. 21204023), the National Basic Research Program of China (973 Program, 2012CB821504) and the Open Fund of the State Key Laboratory of Pulp and Paper Engineering (201346).

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Received March 31, 2014; Revised June 6, 2014; Accepted July 2, 2014

doi: 10.1007/s10118-015-1559-5

elastic materials can be found in the literature of Boyce and Arruda^[31], Gottlieb and Gaylord^[32] and Marckmann and Verron^[33].

In spite of the success of the above mentioned models in describing the nonlinear elastic behavior of synthetic rubber, the gelatin gels possess several differences that require more consideration. First, the gelatin macromolecule seems to be more stiff than the freely joint chain model due to the limited rotation about peptide bonds and the charge repulsion along the chains as a polyelectrolyte^[2]. Therefore, the non-Gaussian distribution of the chain configuration is more important in describing the nonlinear elasticity of gelatin gels. Second, some researchers suggested that the non-affine nature of the network alone was enough to predict the strain stiffening of a cross-linked network sufficiently^[34, 35], as successfully applied to the collagen systems^[36–38]. This implies that the non-affine network may also be a possible contribution to the strain stiffening of gelatin gels. Third, as a hydrogel, the gelatin gel contains a large volume of water. In addition, the gelatin gel contains a part of “sol fraction”, *i.e.*, the dissolved gelatin molecules that have not joined the infinite gel network. These viscous factors contribute to the nonlinear viscoelastic behavior of gelatin gels, which cannot be described with the pure elastic models^[39, 40]. These characteristics are also common in other biopolymer gels such as collagens and F-actin networks^[41, 42]. In the view of the above considerations, the models mentioned above appear to be hardly considered best for the gelatin gel.

On the other hand, despite long history and wide application of gelatin, its nonlinear viscoelasticity has been rarely studied^[12–14, 43]. Recently, the large amplitude oscillatory shear (LAOS) became a new and versatile method for evaluating nonlinear viscoelasticity^[44]. In a LAOS experiment, the extent and rate of deformation are separately controlled. By applying a sinusoidal strain (or stress) with the amplitude higher than the linear viscoelastic limit of the specimen, the resultant non-sinusoidal stress (or strain) signal was collected to extract the nonlinear viscoelastic information further. The analysis methods which were widely used included the Fourier-transform^[45, 46], the stress decomposition^[47–49], and quantitative measures for the Lissajous curve^[48, 50, 51]. We have used the LAOS to study the nonlinear viscoelasticity of hectorite gels^[52], emulsions^[53] and microgel suspensions^[54]. In LAOS experiments, the mechanical response function was suggested to be plotted on the ordinates of frequency ω and strain amplitude γ_0 as contour lines called the Pipkin diagram^[55]. This allowed the nonlinear viscoelasticity of complex fluids to be investigated in much more detail^[48]. It is worth noting that the LAOS analysis distinguishes the nonlinear viscoelasticity into intra- and inter-cycle aspects^[48, 50]. The intra-cycle nonlinear viscoelasticity refers to the non-sinusoidal shape of the Lissajous curve at constant γ_0 and ω , whereas the inter-cycle nonlinear viscoelasticity refers to the mechanical response depending on the γ_0 and ω . These aspects of the gelatin gels have rarely been investigated to the authors' knowledge.

The present paper reports the measurement and modeling of the LAOS behavior of gelatin. Both strain amplitude and frequency dependence was considered quantitatively. Three constitutive models were adopted to describe the strain stiffening, where the LAOS behavior of the gelatin gel was related to the network and molecule properties.

MATERIALS AND METHODS

Gelatin was purchased from Shanghai Runjie Chemical Reagent Co. It was an alkaline treated (type B) gelatin from mixed sources of calf and porcine skins. Yoshimura *et al.*^[56] found that alkaline treatment led to degradation of telopeptides in the gelatin molecules, so we assumed that our sample contains little or no telopeptides. NaCl was purchased from Guangzhou Chemical Reagent Factory. Deionized water (Millipore) was used for all experiments.

The isoelectric point (IEP) of the gelatin was 4.6, which was determined as the pH of maximum turbidity for a 0.1 wt% gelatin in ethanol/acetate buffer solution following previous reported method^[57, 58]. The intrinsic viscosity ($[\eta]$) was measured to be 0.47 dL/g using an Ubbelohde viscometer at $(35 \pm 0.05)^\circ\text{C}$ in 0.1 mol/L NaCl.

Samples for rheological measurements were prepared as following: 19 g of vacuum dried (50 °C overnight) gelatin was dissolved in 81 mL of 0.1 mol/L aqueous NaCl solution at 60 °C under stirring to obtain a gelatin solution of weight percent of 19 wt%, which was converted into $c = 202.2$ g/L with the assumption of the solution volume additivity and gelatin density of 1.465 g/mL^[59]. Rheological measurements were conducted on a TA ARES-RFS strain-controlled rheometer. A cone-and-plate geometry with diameter of 50 mm and cone angle of 0.04 rad was used. Samples were melted at 50 °C to fill the gap of the fixture. Silicone oil was applied on the edge of the sample to prevent water evaporation. We observed a significant aging phenomenon after the gelatin solution was cooled to 28 °C, where the storage modulus G' slowly increased with time. We followed this aging process at 28 °C and found that after 30 min the increase in modulus was slow enough to be negligible within the time of a typical experiment. Therefore, all the experiments were performed after cooling the sample to 28 °C and aged for 30 min. Dynamic strain sweep was performed at frequency $\omega = 4.0$ rad/s to check the linear viscoelastic regime. The sol-gel transition of the gelatin solution (aged 30 min at 28 °C) was observed by dynamic frequency sweep in the linear regime ($\gamma_0 = 5.0\%$) at several temperatures from 26 °C to 32 °C. At each temperature, the sample was allowed to equilibrate for 400 s. The LAOS rheology was carried out at $\gamma_0 > 100\%$ and ω of 1.0, 1.6, 2.0, 2.5, and 4.0 rad/s using the arbitrary waveshape procedure of the TA Orchestrator software. In this procedure, a sinusoidal strain input $\gamma(t) = \gamma_0 \sin(\omega t)$ was specified and the response stress $\sigma(t)$ was collected during the measurement. This procedure was found to provide a signal-to-noise ratio (SNR) as high as the data acquisition (DAQ) method proposed by Wilhelm^[45] on the same sample. Arbitrary waveshape test was also used by Ewoldt *et al.*^[48, 60]. Finally, all of the calculations were accomplished with MATLAB.

The response stress of the LAOS is expressed by the Fourier expansion

$$\sigma(t) = \gamma_0 \sum_n \left[G'_n(\omega, \gamma_0) \sin n\omega t + G''_n(\omega, \gamma_0) \cos n\omega t \right] \quad (1)$$

where $n = 1, 3, 5, \dots$ for an isotropic fluid. G'_n and G''_n are the n^{th} -order real and imaginary moduli, respectively. In the linear viscoelastic regime, only the first harmonic is meaningful, and G'_1 and G''_1 are reduced to the storage modulus G' and loss modulus G'' , respectively. Whereas, the higher order harmonic contributions appear in the nonlinear viscoelastic regime.

The nonlinear viscoelastic stress response is also described by Lissajous curve (σ plotted against γ). Distortion appears as the response enters the nonlinear viscoelastic regime. Nonlinear viscoelasticity is thus quantified by the geometric parameters that express the distortion of the Lissajous curve from a perfect ellipse. Ewoldt *et al.*^[48] defined the minimum- and large-strain moduli G_M and G_L , respectively

$$G_M = \left. \frac{d\sigma}{d\gamma} \right|_{\gamma=0} = \sum_{n=1, \text{odd}} n G'_n = e_1 - 3e_3 + \dots \quad (2)$$

$$G_L = \left. \frac{\sigma}{\gamma} \right|_{\gamma=\gamma_0} = \sum_{n=1, \text{odd}} G'_n (-1)^{\frac{n-1}{2}} = e_1 + e_3 + \dots \quad (3)$$

G_M and G_L were proposed together with G'_1 in order to measure deviation of the Lissajous curve from an ellipse. In the linear viscoelasticity regime, G_M , G_L , and the real modulus of the first harmonic G'_1 reduce to the storage modulus G' , *i.e.*, $G_M = G_L = G'_1 = G'$. In the nonlinear viscoelastic regime, higher harmonic components contribute to the stress response, and $G_M \neq G_L \neq G'_1 \neq G'$. G_L is seldom discussed alone because only its deviation compared with G_M and G'_1 reflects the nonlinear viscoelasticity. In the present study, G_L was discussed in comparison with G_M and G'_1 , and was used to calculate N_E . Under the nonlinear viscoelasticity, G'_1 loses its meaning of “storage modulus” and should only denote the real modulus of the first harmonic component.

The overall nonlinear viscoelasticity was measured by a nonlinearity parameter $N_E \equiv (G_L - G_M)/G'_1$ proposed by Sun *et al.*^[52], where detailed derivation was provided. Subtracting Eq. (3) by Eq. (2) results in $G_L - G_M = \sum_{n=3, \text{odd}} [(-1)^{(n-1)/2} - n] G'_n = 4e_3 + \dots$, where the primary harmonic ($n = 1$) was eliminated. In other words,

the quantity $G_L - G_M$ sums up all the higher harmonics except the first one. Therefore, normalized with the first harmonic G_1' , N_E gives a scaled measure for the overall response nonlinearity. Ewoldt *et al.*^[48] proposed the stiffening index $S \equiv (G_L - G_M)/G_L$, which was similar to N_E . A positive value of S indicated an intra-cycle strain stiffening behavior, and *vice versa*. However, S was normalized by G_L instead of G_1' and lost the meaning of overall nonlinear viscoelasticity. Here, we use N_E to characterize the overall viscoelastic nonlinearity.

The area A enclosed by a Lissajous curve represents the viscous energy dissipation every cycle for the unit volume of sample, which is evaluated by

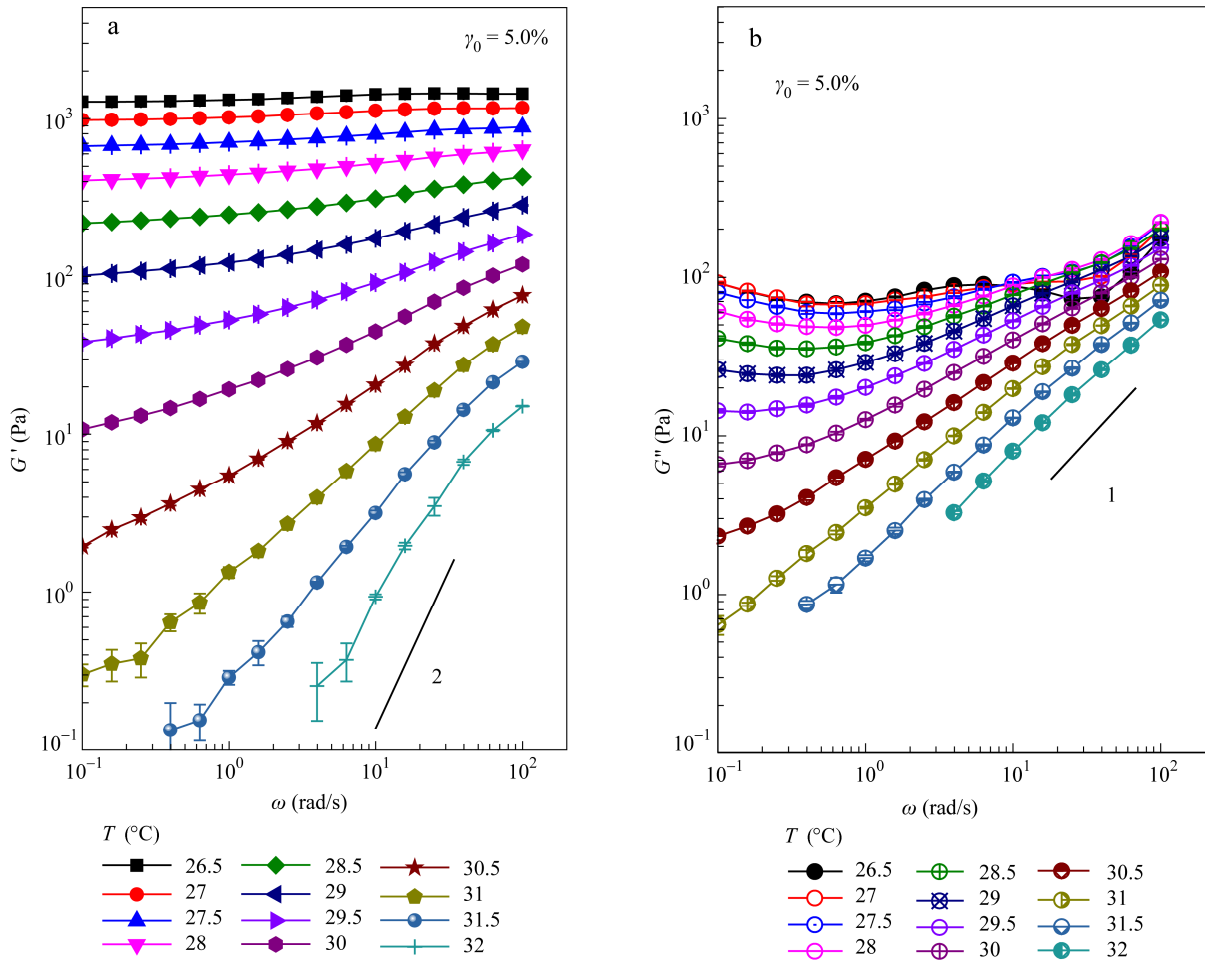
$$A = \oint \sigma d\gamma = \pi \gamma_0^2 G_1'' \quad (4)$$

Ewoldt *et al.*^[60] proposed the related dissipation E_d as the ratio of actual dissipated energy A to the perfect plastic dissipation $A_{pp} = 4\sigma_{\max}$, *i.e.*, $E_d = \pi \gamma_0^2 G_1'' / (4\sigma_{\max})$, where $\sigma_{\max}$ was the maximum stress within one cycle.

RESULTS AND DISCUSSION

Linear Viscoelasticity

The temperature-dependent sol-gel transition of gelatin solution was monitored by dynamic frequency sweep at different temperatures as shown in Fig. 1. At 32 °C, the Maxwell fluid behavior of $G' \sim \omega^2$ and $G'' \sim \omega$ was observed, which is a typical performance of a viscoelastic polymeric fluid without crosslinking^[61]. As temperature decreases, G' became higher than G'' and a plateau of G' appeared in low frequency range, indicating the formation of network. The gel point T_{gel} was determined according to the Winter's criterion^[62, 63]



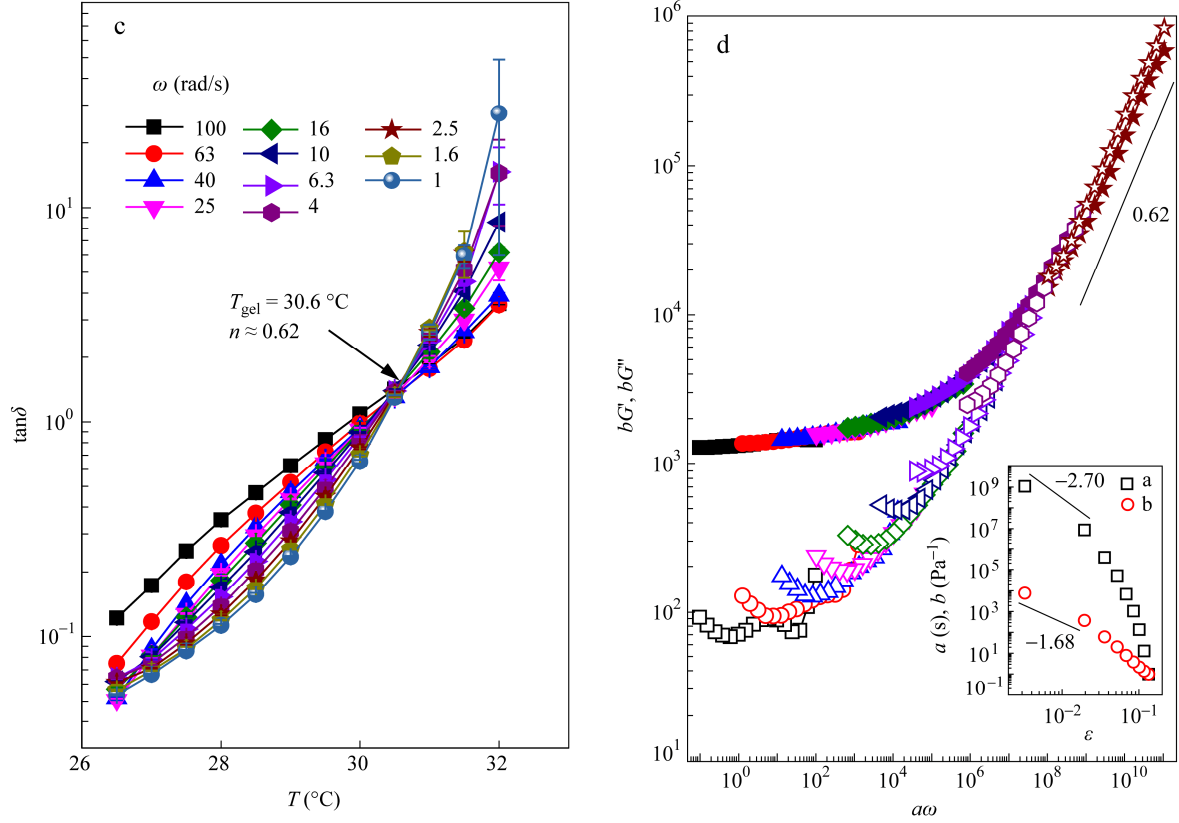


Fig. 1 (a) Storage modulus $G'(\omega)$ and (b) loss modulus $G''(\omega)$ at indicated temperatures as functions of frequency ω ; (c) loss angle $\tan \delta$ plotted against T for indicated ω ; (d) master curve of $G'(\omega)$ and $G''(\omega)$ at $T < T_{\text{gel}}$, inset: horizontal and vertical shift factor a and b plotted against the relative distance ϵ to the gel point T_{gel} ($\epsilon = |(T - T_{\text{gel}})/T_{\text{gel}}|$). The error bars are the standard deviation among data of three repeated experiments. Some error bars are too short to be visible.

of frequency-independent $\tan \delta$, where $\tan \delta = \tan(\pi/2)$. Figure 1(c) depicts the $\tan \delta$ against T at several frequencies. The gel point $T_{\text{gel}} = 30.6^\circ\text{C}$ and the critical exponent $n = 0.62$ were determined from the intersection point of all curves. The G' and G'' curves below T_{gel} can be superposed into a master curve in Fig. 1(d), which was called time-cure superposition^[64]. The time-cure superposition was previously reported for the sol-gel transition of both chemical^[65–67] and physical gels^[67–72]. The horizontal and vertical shift factors a and b were plotted against the relative distance to the gel point $\epsilon = |(T - T_{\text{gel}})/T_{\text{gel}}|$ in the inset of Fig. 1(d). a is proportional to the longest relaxation time τ_{max} of the system, and b is inversely proportional to the equilibrium modulus G_∞ ^[64]. The scaling theory of sol-gel transition predicts that $\tau_{\text{max}} \sim \epsilon^{-y}$ and $G_\infty \sim \epsilon^{-z}$ at the limit of $\epsilon \rightarrow 0$, and the critical exponent $n = z/y$ ^[64]. As shown in the inset of Fig. 1(d), the curves of a and b approach respectively to slopes near $y = 2.70$ and $z = 1.68$ at the lower ϵ limit, which lead to $n = 0.62$, consistent with the result from Fig. 1(c). The shape of the master curve is similar to the typical dynamic response of entangled or cross-linked polymer systems with a plateau region at low frequencies and a transition region at high frequencies^[61]. According to the Winter criteria, at the gel point the dependence of G' and G'' on ω becomes $G' \sim G'' \sim \omega^n$, as is the case in Fig. 1(d) at the high frequency regime; meanwhile the loss angle becomes independent of ω , i.e. $\tan \delta = \pi/2$ as is the case of Fig. 1(c). The slope of G' and G'' in the transition zone reflects the fractal nature of the molecular strands between the cross-linking points^[73]. We estimated the slope of 0.62 in Fig. 1(d), consistent with the n value obtained in Fig. 1(c). Groot *et al.*^[13] related the slope n to the fractal dimension d_f of the polymer as $n = (3 - d_f)/2$. This relation was found successful for gelatin in predicting the wave vector q dependence of the neutron scattering intensity $I(q) \sim q^{d_f}$ and the concentration c dependence of the

Young modulus $E \sim c^{3/(3-d_f)}$ [74]. Therefore, this relationship was adopted in the present study to derive the d_f value of 1.76.

Modulus of gelatin gels of such high concentration (19 wt%) was seldom reported in the literatures^[59, 75–77]. Of the limited number of reports, values of the gel moduli differed largely with varying animal sources or processing methods^[76, 77]. Even for the same sample, the gel modulus still depended on the molecular weight of gelatin and such conditions as temperature, concentration, pH, ionic strength and the time of aging before measurements^[75, 78–83]. On the other hand, it was reported that the gel strength at the critical gel point, S_c , as defined by $S_c = G' \omega^{-n} \Gamma(1-n)^{-1} \cos(n\pi/2)$ ^[62], was independent of pH and ionic strength^[84], which makes S_c a better quantity for comparison of the present study with other's reports. Hsu and Jamieson^[84] measured S_c for gelatin gels of concentration from 4 wt% to 14 wt% and found the relationship $S_c = 0.085c^{1.33}$ Pa sⁿ where c was used in wt%. Extending this relationship to 19 wt% gives $S_c = 4.27$ Pa sⁿ. The S_c value obtained in the present study was about 1.00 Pa sⁿ, smaller than the prediction of Hsu and Jamieson^[84]. This may be due to different source of gelatin samples used in the studies.

The small value of d_f indicates that the gelatin gel near the gel point consists of a very loose network. Considering the high concentration (19 wt%) used in the present work, a large portion of gelatin molecules may not join to the network but exist in the partial associated state. The plateau value of G' in Fig. 1(a) continues to increase with decreasing temperature below T_{gel} , which implies that more sol fraction joins into the network. The amount of sol fraction w_s was calculated from the plateau modulus G_p according to the network theory proposed by te Nijenhuis^[85]:

$$G_p = \frac{2cRT}{M} \left(\frac{M}{M_c} \frac{f-2}{f} - 1 \right) (1-w_s) \quad (5)$$

where c (g/m³) is the concentration of gelatin, $R = 8.314$ J·K⁻¹·mol⁻¹ is the gas constant, T is the temperature, M is the molecular weight of gelatin. The functionality of crosslinking f is the average number of branches per crosslinking point. For gelatin gels, the crosslinking junction is formed by the triple helix of three chains^[3]. Therefore, the value of f is chosen to be 6 for gelatin gels. M_c is the average molecular weight between the crosslinking points related to w_s by te Nijenhuis^[85]

$$\frac{M}{M_c} = - \frac{\ln w_s}{1-w_s} \frac{1-w_s^{0.5f}}{1-w_s^{0.5f-1}}$$

The molecular weight of gelatin M is assumed to follow the empirical relationship proposed by Ferry and Eldridge^[81]:

$$\frac{\sqrt{G_p}}{c} = 1.22 \times 10^{-4} \left[M - 3.1 \times 10^5 \exp \left(- \frac{7900}{RT} \right) \right] \quad (6)$$

where the units of G_p and c was 0.1 Pa and g/L, respectively. Taking the case of $T = 28$ °C for example, calculation from Eqs. (5) and (6) gives $w_s = 0.61$, *i.e.*, a large amount of sol fraction presents in the gelatin gel at $T = 28$ °C.

Large Amplitude Oscillatory Shear Rheology

Figure 2(a) depicts the Lissajous curves of gelatin gel at $T = 28$ °C measured at $\omega = 1.0$ rad/s and $100\% \leq \gamma_0 \leq 281\%$. At $\gamma_0 = 100\%$, the curve is an ellipse with a small hysteresis, indicating that the sample is mostly elastic in the linear regime. As γ_0 increases, the shape of the curve distorts increasingly, showing an intra-cycle strain-stiffening behavior. Similar results were observed at $\omega = 1.6, 2.0$, and 2.5 rad/s (data are not shown here). The intra-cycle strain stiffening was also observed from gels of other biological molecules or tissues, including gluten gels^[86], fibrin gels^[87], actin filament network^[41], pedal mucus of snails^[48], bovine brain tissues^[88] and myocardium isolated from rat heart septum^[89]. Storm *et al.*^[90] reported the strain stiffening of various biological gels including actin, collagen, fibrin and vimentin. The strain above which the strain stiffening behavior occurs

for the gelatin studied here (*ca.* 100%) is much higher than those reported by Storm *et al.*^[90]. This may due to less stiffness of the gelatin molecules than those of actin, collagen, fibrin and vimentin.

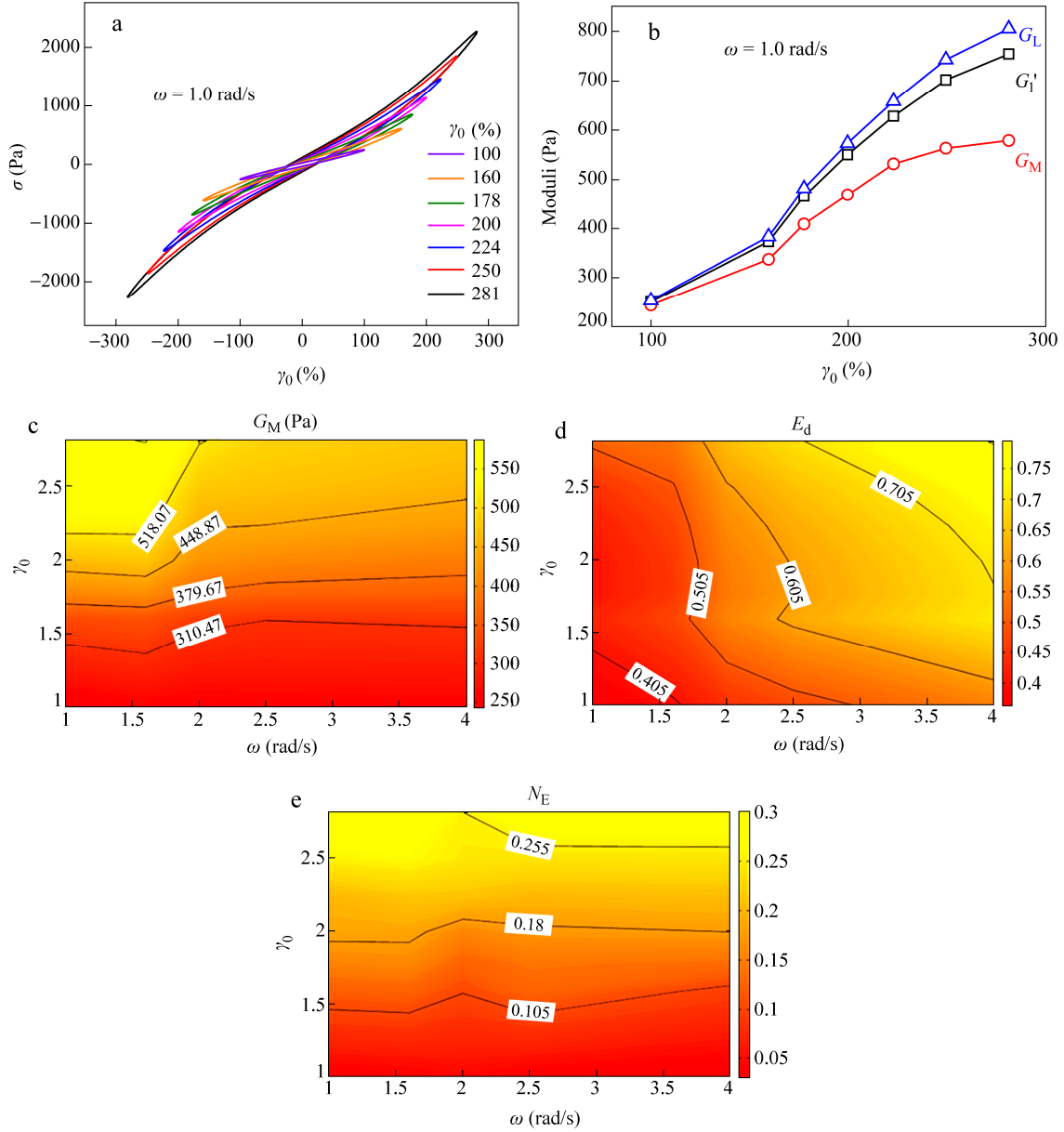


Fig. 2 (a) Lissajous curves of gelatin at angular frequency $\omega = 1.0$ rad/s and the indicated strain amplitude γ_0 ; (b) minimum strain modulus G_M , large strain modulus G_L and the first order real modulus G_1' as functions of γ_0 ; (c), (d) and (e) contour plots of G_M , relative energy dissipation E_d and nonlinear parameter N_E , respectively, against γ_0 and ω axes. The contour lines are labeled with the corresponding values of G_M , E_d and N_E , respectively.

The extent of distortion is quantitatively described by G_1' , G_M , and G_L defined by Eqs. (1) through (3). Figure 2(b) displays the γ_0 dependence of these parameters evaluated from the Lissajous curves. All the three moduli increase with increasing γ_0 . At $\gamma_0 = 100\%$, the values of the three moduli are close to each other, reminiscent of the linear viscoelasticity. As γ_0 increases, G_L and G_M deviate from G_1' in the order of $G_L > G_1' > G_M$. This order of the moduli is consistent with that obtained from the Lissajous curves of some strain stiffening

materials^[48, 86]. The minimum strain modulus G_M increases with increasing γ_0 for gelatin gel, indicating an inter-cycle stiffening behavior. Both intra- and inter-cycle stiffening were reported for fibrin gels^[87]. However, actin filament network^[41], bovine brain tissues^[88] and gluten gels^[86] were reported to be intra-cycle stiffening while inter-cycle softening. According to the theory of rubber elasticity, the minimum strain modulus G_M is proportional to the cross-linked chain density ν of the network by $G_M = \nu RT$. The cross-linking in the gelatin gel was provided by the inter-chain helices^[4, 5]. Both theoretical^[91] and experimental^[92, 93] studies indicated that extension would help the helix formation above but close to the coil-helix transition temperature due to the reduction of the chain entropy. Under LAOS deformation, the value of G_M reflects the stationary value of ν under the repeated helix forming-unwinding cycle at a specific amplitude and frequency. The increase of G_M by γ_0 may be attributed to the strain-induced helix formation, leading to more helices at larger strains.

To demonstrate the γ_0 and ω dependence, G_M is expressed as contours in Fig. 2(c), where the brighter area indicates higher G_M values. High G_M appears at the high-strain and low-frequency region (top left). In other words, the increase of G_M with γ_0 is increasingly impeded at higher ω , which implies that the strain induced cross-linking is less favorable at high deformation rate. Both the formation and unwinding processes were assumed to be time dependent. If the strain induced helix formation were faster than the unwinding process, a large intra-cycle hysteresis would be expected, and a decrease in G_M with increasing ω would be observed. In the present study, however, the opposite was observed, which means, under the same assumption, a slower helix formation than the unwinding.

The related dissipation ratio E_d is illustrated in the contour plots of Fig. 2(d). The highest E_d value locates at the high-strain and high-frequency region, different from the ω dependence of G_M . This indicates that the energy dissipation increases with both increasing γ_0 and ω , or with the strain rate $\dot{\gamma}_0 = \gamma_0 \omega$, characteristics of the viscous behavior. Increase in dissipation with γ_0 was also reported in literature for filled elastomers^[94, 95], whereas the dependence on ω is rarely reported. In the present study, the intra-cycle dissipation was generally small. As estimated above, the gelatin gel contains a high sol fraction, which contributes mainly to the cyclic dissipation.

Finally, the degree of the overall viscoelastic nonlinearity is characterized by N_E as shown in Fig. 2(e). N_E reflects the distortion of the Lissajous curve and quantifies the degree of strain-stiffening. Figure 2(e) shows that N_E increases with increasing γ_0 , but its frequency dependence is not so obvious, as the bright spot for high N_E spans horizontally over the top of this contour plot.

Model Interpretation of LAOS Results

To model the LAOS behavior of the gelatin gel, the shear stress σ is divided into an elastic (in-phase) part and a viscous (out-of-phase) part: $\sigma = \sigma_e + \sigma_v$, similar to a Kevin-Vogt model. $\sigma_v(\gamma_0, \omega)$ is directly substituted by the experimental dissipation energy A defined with Eq. (4), $\sigma_v(\gamma_0, \omega) = A(\gamma_0, \omega) / \pi \gamma_0$, while σ_e is modeled with three hyperelastic constitutive equations: the Blatz, Sharda and Tschoegl (BST) model^[15], the Gent model^[19] and the worm-like chain (WLC) network model by Palmer and Boyce^[96].

The BST model^[13, 15] in simple shear deformation is

$$\sigma_e = \frac{2G_M}{n_{BST}} \frac{\left(\frac{\sqrt{\gamma^2 + 4}}{2} \right)^{n_{BST}} - \left(\frac{\sqrt{\gamma^2 + 4}}{2} \right)^{-n_{BST}}}{\left(\frac{\sqrt{\gamma^2 + 4}}{2} \right) + \left(\frac{\sqrt{\gamma^2 + 4}}{2} \right)^{-1}} \quad (7)$$

where n_{BST} is the elasticity parameter of the BST model. The value of n_{BST} characterizes the non-linear elastic behavior at large deformation. When $n_{BST} = 2$, the equation reduced to the neo-Hookean model. $n_{BST} > 2$ is an indicator of strain stiffening^[15]. Previous study showed that this model perfectly described the stretching behavior of gelatin gels^[12–14]. Groot *et al.*^[13] compared the BST model with the result from a scaling theory of polymer network and obtained the relation of $n_{BST} \approx d_f / (d_f - 1)$. In other words, the BST model approximately

predicted the large deformation based on the knowledge of the fractal dimension d_f of the network^[13].

The Gent model^[19] for simple shear deformation is

$$\sigma_e = \frac{G_M J_m \gamma}{J_m - \gamma^2} \quad (8)$$

where J_m sets a maximum value for the strain γ at which the strain energy approaches infinity. The Gent model is a commonly used limiting chain extension model for strain stiffening of elastomers based on the concept of limiting chain extensibility^[31, 97, 98]. Horgan and Saccomandi^[99] showed that the Gent model was a very accurate approximation of the full network model of Beatty^[100], which assumed a non-Gaussian distribution of the end-to-end distance of the chains. Under this approximation, J_m is related to the average number of Kuhn segments between cross-linkers N_k by $J_m = 3(N_k - 1)$ ^[99].

The WLC network model^[96] is

$$\sigma_e = \frac{G_M}{3l_p} \frac{r_0 \gamma}{\lambda_c} \left(\frac{1}{4 \left(1 - \frac{\lambda_c r_0}{L_c} \right)^2} \right) \left(\frac{\frac{L_c}{l_p} - 6 \left(1 - \frac{\lambda_c r_0}{L_c} \right)}{\frac{L_c}{l_p} - 2 \left(1 - \frac{\lambda_c r_0}{L_c} \right)} \right) \quad (9)$$

where r_0 is the end-to-end distance of the chain under zero stress, λ_c is the stretch ratio of the cross-linked chain with $\lambda_c = [(3 + \gamma^2)/3]^{0.5}$, and L_c is the contour length of chain between two cross-linkers. According to the WLC model,

$$r_0^2 = 2l_p L_c \left[1 - \frac{l_p}{L_c} \left(1 - e^{-\frac{L_c}{l_p}} \right) \right] \quad (10)$$

where l_p is the persistence length. The average molecular weight M_c between crosslinking points was calculated by Eqs. (5) and (6) as *ca.* 8700. Light and small angle neutron scattering measurements on gelatin solutions by Pezron *et al.*^[101] provided the average molecular weight per unit length of chain $M_1 = (28 \pm 8) \text{ g mol}^{-1} \text{ \AA}^{-1}$. So, $L_c = M_c/M_1 = 31.1 \text{ nm}$. The WLC network model was based on the single filament behavior of semi-flexible chain^[90, 102] comprising the Arruda-Boyce 8-chain network^[96]. This model was used in expressing strain stiffening of various biological gels and soft tissues including F-actin network^[96], and ligaments and tendons^[103].

The nonlinear viscoelastic parameter N_E was calculated using these three models with parameters obtained from experiments and only one parameter in each model was determined by curve fitting: n_{BST} in the BST model, N_k in the Gent model, and l_p in the WLC network model. In fact, the number of the Kuhn segments between cross-linkers N_k was related to the contour length between cross-linkers L_c by $N_k = L_c/b$, where $b = 2l_p$ was the Kuhn segment length. Thus, the parameter l_p was fitted by the Gent model and WLC network model for comparison.

The evolution of the nonlinear viscoelasticity parameter N_E with strain amplitude γ_0 at each frequency ω was fitted with these models. Figure 3 illustrates the fitting results at $\omega = 1.0 \text{ rad/s}$ for an example. The BST model predicts the change of N_E most closely, while the Gent and WLC network models overestimate the increasing tendency of N_E with γ_0 . The frequency dependence of the fitting parameter is not obvious. The elastic parameter n_{BST} determined from this work is around 2.4, indicating a strain stiffening behavior. The d_f value evaluated from our n_{BST} is 1.71, which agrees very well with the value of 1.76 from the critical exponent n . The values of l_p obtained from the best fit to the data are also listed in Table 1 for Gent and WLC network models. Because the fitting of Gent and WLC network models is not satisfactory, the fitting value of l_p is only regarded as an adjusting parameter. In the literature, values of l_p for the gelatin molecule were reported to be 1.13 nm in 1 mol/L in KCNS solution^[104] and 2.0 nm in 0.1 mol/L NaCl solution^[101].

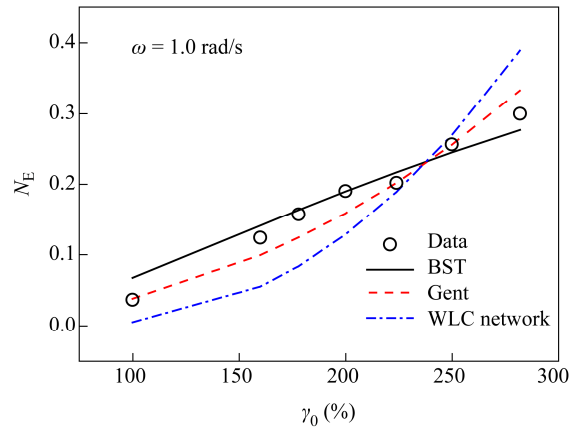


Fig. 3 Nonlinear viscoelastic parameter N_E as a function of strain amplitude γ_0 at frequency $\omega = 1.0$ rad/s (Circles: experimental data; lines: fitting results by the indicated models with parameters listed in Table 1)

The Lissajous curve was modeled using the same fitting parameters. Figure 4 shows the results at $\gamma_0 = 282\%$ and $\omega = 1.0$ rad/s for an example. Both elastic Lissajous curves (stress-strain curves) and the viscous Lissajous curves (stress-strain rate curves) are shown. The BST model describes the experimental data quite well, while the Gent model and WLC network model overestimate the intra-cycle strain stiffening effect. Pipkin diagrams in Figs. 5 through 7 present the comparison of the observed Lissajous curves at several γ_0 and ω with calculated results of the BST model, Gent model and WLC model, respectively. Again, the BST model with the fitting parameter n_{BST} describes all the observed curves well. In contrast, the Gent model and WLC network model predict the observed curves at low strain quite well, but overestimate the strain stiffening at high strains.

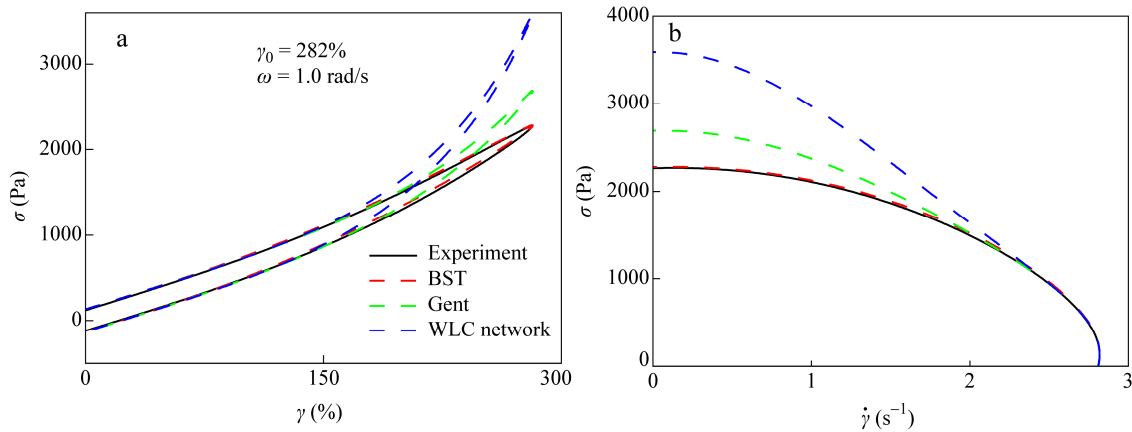


Fig. 4 Half cycles of the Lissajous curves at strain amplitude $\gamma_0 = 282\%$ and frequency $\omega = 1.0$ rad/s: (a) elastic Lissajous curve and (b) viscous Lissajous curve

The BST model is based on generalization of the strain invariants. The success of BST model in describing the mechanical properties of gelatin gels has been demonstrated already on uniaxial tensile test and simple shear test^[12, 14]. However, the fitting parameter n_{BST} does not have any intrinsic structural origin, except the approximate relation with the network fractal dimension d_f proposed by Groot *et al*^[13]. The present results manifest that this relation is valid for gelatin gels under LAOS. The Gent and WLC network models also describe the experiment fairly well, except at the highest strains. Both models are based on the limited extensibility of chains at large strains. Therefore, the different features of these models are mostly exposed at large strains. The Gent model was considered to be approximately equivalent to a full network (affine) model,

whereas the WLC network model was an 8-chain (non-affine) model. This difference in the assumption of these two models may lead to the different predictions at large strains. The less success of these two models in describing the large-strain behavior of gelatin implies that the non-Gaussian or non-affine property may not be the dominant factor, but further research is needed to confirm this.

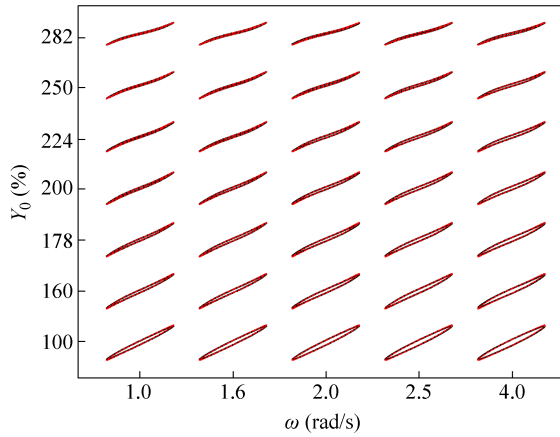


Fig. 5 Pipkin diagram of the experimental (solid) and BST model predicted (red dash) Lissajous curves

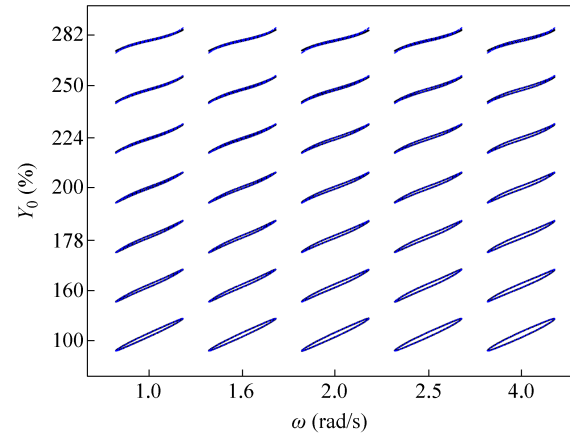


Fig. 6 Pipkin diagram of the experimental (solid) and Gent model predicted (blue dash) Lissajous curves

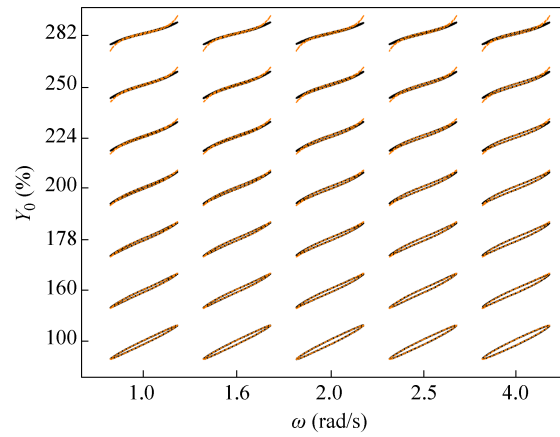


Fig. 7 Pipkin diagram of the experimental (solid) and WLC network model predicted (orange dash) Lissajous curves

CONCLUSIONS

For 19 wt% gelatin solution with 0.1 mol/L NaCl, there are the critical gelation temperature $T_{\text{gel}} = 30.6$ °C and the critical gel exponent $n = 0.62$ with the fractal dimension $d_f = 1.76$. High sol fraction of $w_s = 0.61$ was estimated for the gel at 28 °C. The LAOS measurements indicated that the gel at 28 °C behaved strain-stiffening at large strains. The minimum strain modulus G_M increased with increasing strain amplitude γ_0 , but almost independent of frequency ω . The related cyclic hysteresis E_d increased with both increasing γ_0 and ω . The γ_0 and ω dependence of G_M and E_d was explained with the strain induced helix formation of the gelatin chains. The overall extent of the strain-stiffening was characterized by the nonlinear viscoelastic parameter N_E , which increased with increasing γ_0 . The BST model, Gent model and WLC network model were adopted to simulate the γ_0 dependence of N_E . A constant elastic parameter n_{BST} was evaluated from the BST model fitting, and a reasonable value of the persistence length l_p was obtained from the Gent and WLC network model fitting. The Lissajous curve calculated by the BST model described the data well at several γ_0 and ω , while the Gent and

WLC network models overestimated the strain-stiffening at large strains. Also, the LAOS appears to be powerful in characterizing the strain-stiffening for hydrogels under large deformation.

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