Nonlinear Viscoelastic Biomaterials:
Meaningful Characterization and Engineering Inspiration

Randy H. Ewoldt, Anette E. Hosoi, Gareth H. McKinley

Hatsopoulos Microfluids Laboratory, Department of Mechanical Engineering,

Massachusetts Institute of Technology, Cambridge, MA 02139

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Synopsis

Nonlinear mechanical properties play an important role in numerous biological functions, for instance the locomotion strategy used by terrestrial gastropods. We discuss the progress made toward bioinspired snail-like locomotion and the pursuit of an engineered fluid that imitates the nonlinear viscoelastic properties of native gastropod pedal mucus. The rheological behavior of native pedal mucus is characterized using an oscillatory deformation protocol known as large amplitude oscillatory shear (LAOS), and we review recently-developed techniques for appropriately describing nonlinear viscoelastic behavior. Whereas materials that exhibit purely elastic and purely viscous nonlinearities are amenable to standard techniques for characterization, pedal mucus samples (and biomaterials in general) are viscoelastic, exhibiting both elastic and viscous nonlinear responses simultaneously and requiring advanced techniques for characterization. We reveal the utility of these new methods by examining trail mucus from the terrestrial slug *Limax maximus* using oscillatory shear rheology. Material responses which previously could only be described mathematically, with little physical insight, can now be interpreted with familiar language such as strain-stiffening/softening and shear-thickening/thinning. The new methodology is applicable to any complex material that can be tested using imposed oscillatory deformations. We have developed data-analysis software to enable wider use of this framework within and beyond the biomaterials community. The functionality of this software is outlined here.

Introduction

Biomaterials are inherently structured, hierarchical, and complex, and often exhibit nonlinear mechanical properties. Such nonlinear viscoelastic properties can be essential for proper biological functioning. For example, the strain-stiffening of arterial walls enables stability to inflation over a range of pressures (Shadwick, 1999), and terrestrial gastropods depend on the dramatic viscous shear-thinning of pedal mucus for their locomotion (Denny, 1980b). The central role of nonlinear viscoelasticity is becoming increasingly apparent with both biological and bioengineered materials. However, in many biophysical processes it is less clear how to most appropriately describe such complex materials. Standard methods for characterizing nonlinear viscoelasticity are mathematically robust (Dealy and Wissbrun, 1990; Wilhelm, 2002), but lack a physical interpretation and have proven to be insufficient descriptors, especially for biological materials (Gardel *et al.*, 2004; Ewoldt *et al.*, 2007a). A new framework, or *ontology*, of descriptive measures has therefore been developed (Ewoldt *et al.*, 2008) in order to meaningfully describe the behavior of materials and enable the sharing of knowledge about nonlinear mechanical properties.

In the present work, the importance of nonlinear mechanical properties is discussed within the context of natural and biomimetic gastropod adhesive locomotion. The mechanical properties of the thin protein-polysaccharide biomaterial responsible for adhesive locomotion, commonly referred to as “pedal mucus,” cannot be adequately quantified using standard linear methodology. They can, however, be clearly and completely described using a nonlinear viscoelastic representation which is based on the deformation protocol known as large amplitude oscillatory shear (LAOS). This recently-developed framework of nonlinear viscoelasticity is reviewed and given context by comparing it to descriptive measures for purely elastic or purely viscous nonlinearities. Software has been developed to make this new ontology for nonlinear viscoelasticity accessible to researchers. This software is introduced and discussed here, with the hope that the framework will find application in, and beyond, the biomaterials research community.

Bioinspired snail-like locomotion requires nonlinear mechanical properties

Terrestrial snails and slugs crawl using a technique called adhesive locomotion, in which a thin layer of pedal mucus connects the foot to the substrate. The typical thickness of the film of pedal mucus is on the order of tens of microns (Denny, 1980b). Pedal mucus is a physically crosslinked gel whose primary constituents are water (96-97 wt%) and high-molecular-weight protein-polysaccharide complexes (3-4 wt%) (Denny, 1983). The protein-polysaccharide complexes found in invertebrate mucus lie somewhere on the spectrum between a glycoprotein and a glycosaminoglycan. While there is a clear distinction between glycoproteins and glycosaminoglycans for vertebrates, that distinction is blurred for invertebrate mucus (Denny, 1983). It has been shown that gastropods can exhibit different formulations of pedal mucus, ranging from a strong glue used for attachment over long timescales to a more slippery *trail mucus* employed during locomotion (Smith *et al.*, 1999; Smith and Morin, 2002).

The current theory of adhesive locomotion is that trail mucus behaves as a yield stress fluid (Denny, 1980b; Denny and Gosline, 1980), a distinct strategy among mechanisms for biological attachment employed by other organisms (Gorb, 2008). A yield stress fluid is a material which reversibly changes from solid-like to liquid-like behavior depending on the applied mechanical stress. The animal therefore applies a high shear stress at various portions of the foot, causing those portions to move forward on a liquid-like layer of mucus, while other portions remain fixed to the substrate by solid-like mucus in the lower stress regions. This transitory attachment mechanism allows terrestrial gastropods to climb inclined surfaces (Figure 1a), an acrobatic ability that is enabled by the nonlinear viscoelastic properties of pedal mucus.

The locomotory mechanism of terrestrial gastropods has inspired efforts to mimic the adhesive locomotory technique with mechanical crawlers (Chan *et al.*, 2005; Chan *et al.*, 2007) using a suitably designed complex fluid to mimic pedal mucus (Ewoldt *et al.*, 2007a). Adhesive locomotion can be modeled by considering the successive displacement of discrete pads actuated by an internal force (Figure 1b). The internal force F actuates one pad with respect to the rest, and a force balance distributes the load amongst the discrete components. This actuation force is resisted by the shear deformation of the material which connects each pad to the substrate. For a purely viscous fluid connecting the crawler to the substrate, it can be been shown (Ewoldt, 2006) that the velocity of such a crawler on a horizontal surface is

$$V_{cm} = \frac{Fh}{A}\left[\frac{1}{\eta(\sigma_i)} - \frac{1}{\eta(\sigma_n)}\right] \qquad (1)$$

where h is the fluid thickness, A is the total area of all pads, $\eta(\sigma)$ is the stress-dependent viscosity of the fluid, σ_i is the shear stress applied to the fluid under the single pad, and the stress within the fluid under the remaining pads is σ_n. A non-zero velocity of the center of mass results in successful locomotion, which requires that $\eta(\sigma_i) \neq \eta(\sigma_n)$. Thus, two features are required for successful locomotion: (i) differential areas in motion so that different stresses are applied to different portions of the connecting fluid, $\sigma_i \neq \sigma_n$, and (ii) a non-Newtonian fluid with stress-dependent viscosity. From an engineering perspective, it is noteworthy that the viscous nonlinearity can be either shear-thinning ($d\eta/d\sigma < 0$) or shear-thickening ($d\eta/d\sigma > 0$), which broadens the design options for bioinspired locomotion beyond the simple imitation of the shear-thinning behavior observed in natural systems. Nonetheless, two factors argue for the use of shear-thinning materials. First, for biological systems the energy required to produce mucus comprises the largest fraction of the energy required for crawling (Denny, 1980a). It has been shown that shear-thinning properties of trail mucus favor a decrease in the volume of mucus necessary for crawling (Lauga and Hosoi, 2006) and therefore a decrease in the required energy for locomotion. Second, shear-thinning behavior can enable stationary attachment on inclined surfaces. Stable inclined locomotion requires that the low-stress viscosity is exceedingly large, $\eta(\sigma_n) \to \infty$, so that negligible movement occurs at rest. The fluid must still exhibit a stress-dependent viscosity, and therefore the high-stress viscosity must be sufficiently different from the low-stress viscosity to enable locomotion, $\eta(\sigma_i) \ll \eta(\sigma_n)$. This dramatic shear-thinning character approaches the behavior of an ideal viscoplastic yield stress fluid.

It is non-trivial to duplicate biological functionality, given the constraints on conventional engineering components such as motors and actuators (Madden, 2007). For example, it has been exceedingly difficult to mimic flapping wing flight with a self-contained device due to power requirements and actuator efficiency; instead we have found the best engineering success with fixed-wing flight powered by propeller or jet engines. It has recently been shown that adhesive locomotion *can* be used as the locomotion strategy of a self-contained device (Chan, in progress). The crawler (Figure 1c) has been designed to meet the requirements of appropriate motion and geometry for adhesive locomotion. It employs on-board power (two AA batteries), is driven by a single DC motor, and relies on a unique custom-designed cam system that transfers power to discrete foot pads. Furthermore, a suitable non-Newtonian yield stress fluid has been formulated (Ewoldt *et al.*, 2007a) to enable the crawler of Figure 1c to traverse inclined and inverted terrain. The fluid used is an aqueous polymeric microgel at neutral pH, based on Carbopol 940 (4 wt%). This complex fluid has sufficiently large yield stress to support the crawler, a reasonably low flow viscosity after yield, and an adequate self-healing time (the time required to restructure into a solid-like material once a load is removed) (Ewoldt *et al.*, 2007a). It has thus been demonstrated that the adhesive locomotory strategy can be successfully implemented with existing engineering technology. Although a synthetic fluid can be concocted to adequately mimic the nonlinear mechanical properties of pedal mucus, and demonstrate the feasibility of mimicking adhesive locomotion, native slime is still superior to the synthetic self-healing materials explored. Native pedal mucus self-heals more quickly following cessation of deformation and has a sharper yielding transition at the critical stress for flow (or "yield stress"). The superior performance of the natural mucus gel motivates a detailed understanding of the linear to nonlinear viscoelastic properties of pedal mucus, which are discussed in the following sections.

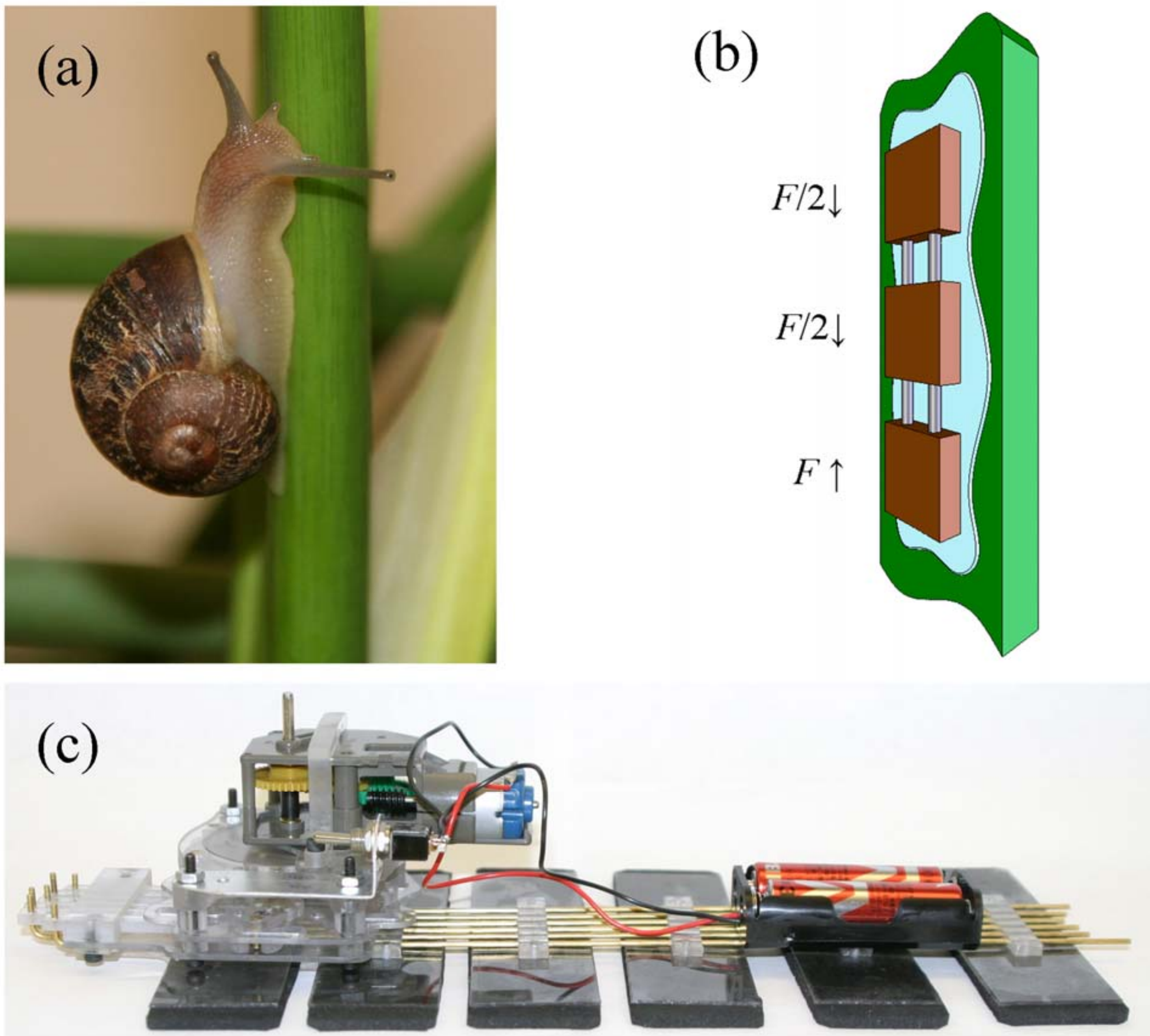


Figure 1. (a) The common garden snail *helix aspera* (and other terrestrial gastropods) use the nonlinear viscoelastic properties of excreted trail mucus for transitory attachment, allowing locomotion on inclined surfaces. (b) A model of adhesive locomotion with discrete components; an internal force *F* actuates one sectional food pad with respect to the rest. Because of the requirements of zero net external force, the force distributed on the remaining *N*-1 pads is *F*/(*N-1*); in this illustration *N*=3. Sections are connected to the underlying surface by a thin layer of nonlinear viscoelastic material. (c) Bioinspired embodiment of a mechanical crawler (*N*=6) that successfully uses adhesive locomotion to traverse inclined and inverted surfaces using appropriately designed complex fluids.

Methods

Trail mucus was collected from the terrestrial slug, *Limax maximus*. A slug was placed on a glass plate in a humid environment. The animal would begin crawling, and after traveling for more than one body length, a razor blade was used to collect trail mucus from behind the slug as it continued to crawl. When a sufficient sample size had been collected, it was immediately placed on the rheometer for testing.

The mechanical properties of native pedal mucus were measured using a displacement-controlled rotational rheometer (ARES, TA Instruments). The temperature was maintained at T=22ºC with a Peltier plate, and a solvent trap was used to inhibit evaporation of the sample. The tests were performed with a parallel pate geometry (diameter D=8mm, gap h=550 μm). Plate surfaces can be modified to increase surface roughness and eliminate slip at the geometry surface (in contrast, this is more difficult with a cone-plate geometry). To eliminate slip, the plate surfaces were covered with adhesive-backed waterproof sandpaper, 600 grit (ARC Abrasives, Inc.). For parallel plates, the shear strain in the samples varies from zero at the center of rotation to a maximum at the edge of the plate. Here we report (and use for analysis) the strain, γ, at the maximum radius $r=R$. The torque response, T, is measured and used to estimate the stress at the location $r=R$, $\sigma = 2T/\pi R^3$. The oscillatory time-series signals of imposed shear strain, $\gamma(t)$, and resulting shear stress, $\sigma(t)$, were acquired by the instrument. The resulting steady state oscillatory response was then used for analysis.

Theory: An Ontology for Nonlinear Viscoelasticity

Pedal mucus was examined with large amplitude oscillatory shear (LAOS) deformation and analyzed with a new theoretical framework for describing nonlinear viscoelasticity. We review this new framework for the specific purpose of describing the nonlinear properties of pedal mucus, and the general purpose of introducing the framework to the biomaterials community, as the framework is universally applicable to any viscoelastic material.

Viscous and elastic mechanical properties can be simultaneously probed by subjecting a material sample to oscillatory deformation. For shear deformation, one requires an input shear strain of the form $\gamma(t) = \gamma_0 \sin \omega t$, where γ_0 is the strain amplitude and ω is the frequency of deformation. This oscillatory shear strain consequently imposes an oscillatory strain-rate $\dot{\gamma}(t) = \dot{\gamma}_0 \cos \omega t$, where $\dot{\gamma}_0 = \gamma_0 \omega$ is the amplitude of the strain-rate. The stress resulting from this deformation will also be oscillatory, and will have a component in phase with the strain (i.e. elastic stress, $\sigma' = f(\gamma)$) and a component in phase with the strain-rate (i.e. viscous stress, $\sigma'' = f(\dot{\gamma})$). Note that the inputs of strain and strain-rate are exactly out of phase, which allows for the decomposition of the elastic and viscous stresses since elastic stress should only be a function of strain (e.g. $\sigma \sim G\gamma$ where G is the shear modulus) and viscous stress should only be a function of strain-rate (e.g. $\sigma \sim \eta\dot{\gamma}$ where η is viscosity).

In the linear viscoelastic regime, the stress response is simply represented by

$$\sigma(t) = \gamma_0 \left(G' \sin \omega t + G'' \cos \omega t \right), \qquad (2)$$

where G' is the elastic modulus and G'' is the viscous modulus (Ferry, 1980). For a nonlinear viscoelastic response, the stress response is more complicated and the viscoelastic moduli, G' and G'', are no longer well defined. An adequate mathematical representation for such a nonlinear viscoelastic stress response is a Fourier series, and the term Fourier-transform rheology (or FT-rheology) (Dealy and Wissbrun, 1990; Wilhelm, 2002) refers the practice of representing the oscillatory stress response as

$$\sigma(t) = \gamma_0 \sum_n \left(G_n' \sin(n\omega t) + G_n'' \cos(n\omega t) \right). \qquad (3)$$

The first-harmonic coefficients, n=1, of this Fourier series representation are often used as measures of viscoelastic moduli for a nonlinear response. For example, most commercial rheometers output the first-harmonic moduli, G_1' and G_1'', and interpret these measures as the viscoelastic moduli. This is a common, but arbitrary, choice since G' and G'' are not precisely defined in the nonlinear viscoelastic regime. We have shown that reporting the first-harmonic moduli is insufficient for describing the properties of native pedal mucus (Ewoldt *et al.*, 2007a). Thus, a new theoretical framework was developed such that a meaningful representation of nonlinear viscoelastic properties could be achieved (Ewoldt *et al.*, 2008). Table 1 shows this current framework (ontology) for describing linear to nonlinear viscoelasticity.

The various measures of reporting nonlinear viscoelasticity are put into context by first considering nonlinear material responses that are either purely viscous or purely elastic. For example, the nonlinear response of a purely elastic material is described by multiple measures of elastic modulus, including the secant shear modulus $G(\gamma) \equiv \sigma / \gamma$ and the tangent shear modulus denoted $G_K(\gamma) \equiv d\sigma/d\gamma$. G_K is also known as the differential modulus, often denoted as K' (e.g. Gardel et al., 2004). In the linear elastic regime these two measures are equivalent, $G = G_K$, but in the nonlinear regime they are different and have distinct interpretations. Analogous measures exist for a purely viscous material. A nonlinear purely viscous material can be described by the (secant) viscosity $\eta(\dot{\gamma}) \equiv \sigma / \dot{\gamma}$ and tangent viscosity $\eta_K(\dot{\gamma}) \equiv d\sigma/d\dot{\gamma}$. A viscoelastic material simultaneously exhibits elastic and viscous properties, and therefore may contain both elastic and viscous nonlinearities. Multiple measures of elastic modulus and multiple measures of dynamic viscosity can be used for describing such nonlinear viscoelastic materials, just as multiple measures are useful for the purely elastic or purely viscous scenarios. The measures available for describing nonlinear viscoelasticity are outlined in Table 1.

An elastic nonlinearity is said to be *strain-stiffening* if the elastic modulus increases as strain increases, and *strain-softening* if the modulus decreases as strain increases. In the case of a *purely elastic* material, one may compare the modulus at zero strain with the modulus at a larger strain, e.g. comparing the tangent moduli $G_K(\gamma = 0)$ and $G_K(\gamma \neq 0)$ (note that we use G_K, without the prime mark, for a purely solid material measure but add the prime mark for a viscoelastic measure, e.g. G'_K). Similar comparisons can be made for interpreting the elastic nonlinearity of a viscoelastic response, e.g. comparing $G'_M(\gamma_0, \omega)$ and $G'_K(\gamma_0, \omega)$ at a specified strain amplitude γ_0 and frequency ω. As noted in Table 1, $G'_M(\gamma_0, \omega)$ represents the tangent elastic modulus at instantaneous strain equal to zero, $\gamma = 0$ (the *minimum* imposed strain), whereas $G'_K(\gamma_0, \omega)$ captures the tangent elasticity at the extreme of imposed shear-strain, $\gamma = \gamma_0$. Thus strain-stiffening is indicated for $G'_K > G'_M$, and strain-softening for $G'_K < G'_M$. Such elastic nonlinearities are independent of the viscous nonlinearities, which are described by separate terminology and material parameters. Nonlinear viscous properties can be either *shear-thickening* or *shear-thinning*, if the dynamic viscosity increases or decreases, respectively, as a function of strain-rate. For the viscous contribution to a viscoelastic response, a comparison can be made between $\eta'_M(\gamma_0, \omega)$ and $\eta'_K(\gamma_0, \omega)$. Shear-thickening is indicated by $\eta'_K > \eta'_M$, and shear-thinning for $\eta'_K < \eta'_M$.

When comparison of these various measures is made as described above, at fixed values of the input parameters strain-amplitude and frequency $\{\gamma_0, \omega\}$, the resulting nonlinearities are termed *intra-cycle* nonlinearities. This terminology is used because only a single oscillatory cycle is required to observe such nonlinear behavior and the nonlinearity appears within the cyclic loading history. In contrast, parameters that change as a function of the amplitude or frequency of the imposed loading, $\{\gamma_0, \omega\}$, are termed *inter-cycle* nonlinearities, since multiple cycles, or multiple values of the input parameters, are required to observe the dependency.

For many materials the nature of intra-cycle nonlinearities can be captured by two parameters, the elastic Chebyshev coefficient e_3 and the viscous Chebyshev coefficient v_3 (Ewoldt *et al.*, 2008). These Chebyshev parameters are defined by considering the single valued functions of elastic stress $\sigma'(\gamma)$ and viscous stress $\sigma''(\dot{\gamma})$, which can be represented by polynomials. A series of orthogonal Chebyshev polynomials of the first kind has been found to work particularly well. For elastic stress, $\sigma'(\gamma) = e_1 x + e_3(4x^3 - 3x) + \ldots$, where $x = \gamma / \gamma_0$, and the first- and third-order Chebyshev polynomials are $T_1(x) = x$, and $T_3(x) = 4x^3 - 3x$ (higher harmonics are also included but omitted here for clarity). The viscous stress is similarly represented as $\sigma''(\dot{\gamma}) = v_1 y + v_3(4y^3 - 3y) + \ldots$, where $y = \dot{\gamma} / \dot{\gamma}_0$.

It is the sign of curvature (positive or negative) which indicates the nature of the nonlinearity of the elastic and viscous stress. To leading order, the sign of the curvature (or concavity, d^2/dx^2) of the elastic stress $\sigma'(\gamma)$ is captured by the sign of e_3, and the curvature of the viscous stress $\sigma''(\dot{\gamma})$ is described by the viscous Chebyshev coefficient v_3. Although the magnitude of curvature may change along the curve (as a function of x or y), to leading order the sign of the curvature everywhere correlates with the sign of e_3 or v_3. We summarize the interpretation of the Chebyshev coefficients in Table 1, and note that such interpretations would identically result from comparing the various measures of elastic moduli or dynamic viscosities.

Table 1. Ontology for characterizing linear to nonlinear viscoelasticity with oscillatory shear deformation

Purely elastic	**Purely viscous**
(load ~ deformation)	(load ~ rate of deformation)
Secant shear modulus: $G(\gamma) \equiv \sigma/\gamma$	Viscosity: $\eta(\dot{\gamma}) \equiv \sigma/\dot{\gamma}$
Tangent shear modulus: $G_K(\gamma) \equiv d\sigma/d\gamma$	Tangent viscosity: $\eta_K(\dot{\gamma}) \equiv d\sigma/d\dot{\gamma}$
(equivalent in linear elastic regime, $G = G_K$)	(equivalent in linear viscous regime, $\eta = \eta_K$)

Viscoelastic

load = f(deformation, rate of deformation)

Impose oscillatory deformation: $\gamma(t) = \gamma_0 \sin \omega t$, and therefore $\dot{\gamma}(t) = \dot{\gamma}_0 \cos \omega t$

Measure $\sigma(t) = \gamma_0 \sum_n \left(G_n' \sin(n\omega t) + G_n'' \cos(n\omega t) \right)$

Elastic character $\sim \gamma$	Viscous character $\sim \dot{\gamma}$
First-harmonic modulus: G_1'	First-harmonic dynamic viscosity: $\eta_1' = G_1''/\omega$
Minimum-strain modulus: $G_M' = d\sigma/d\gamma\big\|_{\gamma=0}$	Minimum-rate viscosity: $\eta_M' = d\sigma/d\dot{\gamma}\big\|_{\dot{\gamma}=0}$
Large-strain modulus: $G_L' = \sigma/\gamma\big\|_{\gamma=\gamma_0}$	Large-rate viscosity: $\eta_L' = \sigma/\dot{\gamma}\big\|_{\dot{\gamma}=\dot{\gamma}_0}$
Differential modulus: $G_K' = d\sigma/d\gamma\big\|_{\gamma=\gamma_0}$	Differential viscosity: $\eta_K' = d\sigma/d\dot{\gamma}\big\|_{\dot{\gamma}=\dot{\gamma}_0}$
(equivalent in linear viscoelastic regime, $G' = G_1' = G_M' = G_L' = G_K'$)	(equivalent in linear viscoelastic regime $\eta' = G''/\omega = \eta_1' = \eta_M' = \eta_L' = \eta_K'$)
Intra-cycle elastic nonlinearity	Intra-cycle viscous nonlinearity
$e_3 \begin{cases} > 0 & \text{stiffening} \\ < 0 & \text{softening} \end{cases}$	$v_3 \begin{cases} > 0 & \text{thickening} \\ < 0 & \text{thinning} \end{cases}$

Nonlinear Viscoelastic Properties of Gastropod Pedal Mucus

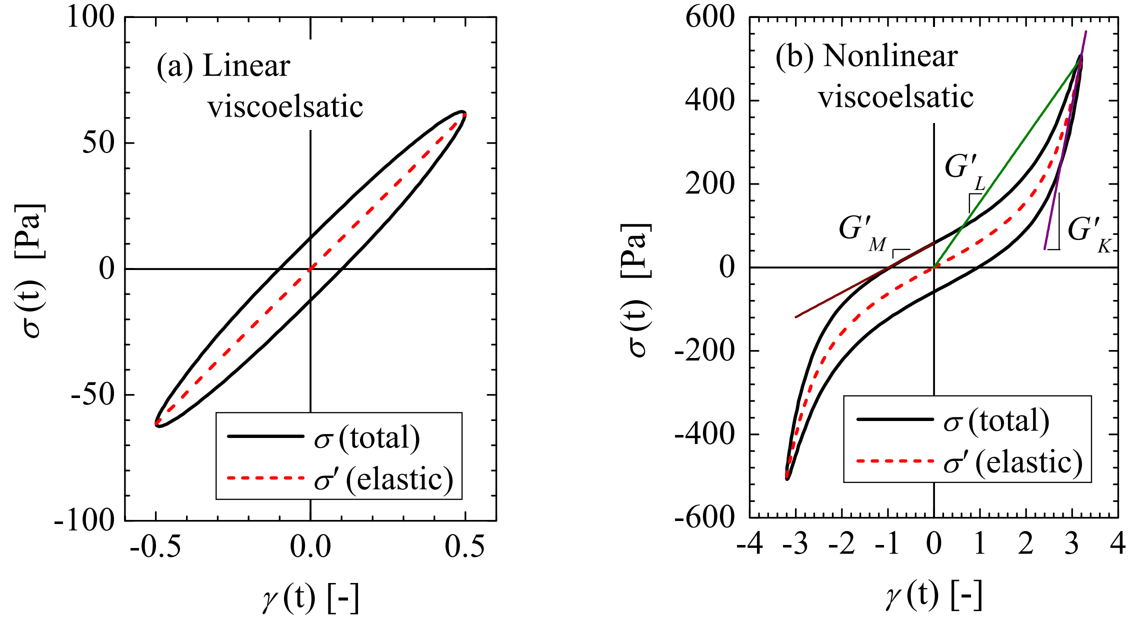
The new framework for describing nonlinear viscoelasticity (Table 1) is generally applicable to any material that can be tested with oscillatory deformation, e.g. simple shear, torsion, bending, or axial loading. Here we describe some of the nonlinear viscoelastic properties of native pedal mucus from the terrestrial slug *Limax maximus*.

A useful way to display the results from an oscillatory test is in the form of parametric plots of the output stress $\sigma(t)$ against input strain $\gamma(t)$ (or stress $\sigma(t)$ against strain-rate $\dot{\gamma}(t)$), properly known as Lissajous-Bowditch curves (Crowell, 1981; Whitaker, 2001). Results from two tests of native pedal mucus are shown in Figure 2, in which the total stress $\sigma(t)$ and the elastic stress $\sigma'(t)$ are plotted against the input strain $\gamma(t)$. The latter contribution is determined by decomposing the total stress into elastic stress and viscous stress (Cho *et al.*, 2005), which are single-valued functions of strain and strain-rate respectively, $\sigma(t;\gamma_0,\omega)=\sigma'(\gamma)+\sigma''(\dot{\gamma})$. The tests in Figure 2 are performed at a frequency $\omega = 1\ \text{rad.s}^{-1}$ but at different strain amplitudes, $\gamma_0 = 0.5$ and $\gamma_0 = 3.2$ ($\gamma_0 = 320\%$). The test at $\gamma_0 = 0.5$ (Figure 2a) is in the linear viscoelastic regime, as evidenced by the elliptical shape of the curve of total stress vs. strain, and by the linear shape of the elastic stress vs. strain curve. The test at $\gamma_0 = 3.2$ (Figure 2b) is clearly a nonlinear response as indicated by the non-elliptical shape of the total stress curve and the nonlinear shape of the elastic stress curve.

Results for the various measures of elastic modulus are shown for each test in Figure 2. Each measure of modulus is effectively equivalent for the linear viscoelastic response at $\gamma_0 = 0.5$, i.e. $G_1' = G_M' = G_L' = G_K'$. However, the measures of elastic modulus take distinct values for the nonlinear viscoelastic response ($\gamma_0 = 3.2$). Note that the prior standard for reporting elastic modulus, the first-harmonic elastic modulus G_1', is approximately the same in both the linear and nonlinear regime for this material, which would incorrectly imply a linear viscoelastic response at $\gamma_0 = 3.2$ if no other measures were considered. The additional measures of elastic modulus (G_M', G_L', G_K') address the true nonlinearity of the response, and correctly indicate intra-cycle strain-stiffening in the nonlinear viscoelastic regime.

The elastic nonlinearity of pedal mucus is a complex response that is not fully described by any single measure. Intra-cycle stiffening is indicated by the positive numerical value of the elastic Chebyshev coefficient $e_3 > 0$, or equivalently by visually comparing the elastic moduli indicated in Figure 2b, which show that $G'_L > G'_M$ and $G'_K > G'_M$. This intra-cycle stiffening likely results from the large extensions of constituent polymeric components within the pedal mucus gel which exhibit stretch-stiffening. The elasticity at the maximal imposed strains within each cycle (i.e. G'_L and G'_K which capture the elasticity at $\gamma = \gamma_0$) increase as the strain amplitude γ_0 is increased from $\gamma_0 = 0.5$ to $\gamma_0 = 3.2$, thus indicating *inter*-cycle stiffening of G'_L and G'_K. That is, the stiffness at the largest deformations increases as the strain amplitude increases. This inter-cycle stiffening is consistent with the hypothesis of constituent polymeric components which themselves stiffen. However, the structure of the polymeric mucus gel must be more complicated than affine stretching of polymeric components to account for the distinct behavior revealed by the minimum-strain modulus G'_M, which strain-*softens* as the strain amplitude γ_0 is increased. Because G'_M represents the elasticity at an instantaneous strain equal to zero ($\gamma = 0$), the softening may result from temporary disruption of inter-molecular bonds that decreases the small-strain elasticity. The emerging picture is that the interconnected structural components of mucus are strain-stiffening, and destruction of inter-molecular interactions is initiated gradually with increasing strain. The combined stiffening and softening counteract each other, and the average elasticity G'_1 is approximately constant. The mucus eventually yields and flows at a sufficiently large deformation amplitude, and the progression towards that ultimate failure, as shown in Figure 2, provides insight into the nature of the yield transition. Such insight is useful for understanding the structure and properties of this biomaterial, and also provides guidance in selecting material systems for emulating the mechanical behavior.

The suggested structure-property relations can be tested by developing a microstructural constitutive model and characterizing its nonlinear viscoelastic behavior using the same descriptive measures of nonlinear elastic moduli as described above. For such a full analysis, a material can be subjected to various strain amplitudes, γ_0, and frequencies, ω, to map the full range of possible oscillatory shear deformation. This new framework thus guides formulation of hypotheses regarding structure-property relationships and simultaneously will facilitate the comparison of models and experiments.



$\gamma_0 = 0.5$		$\gamma_0 = 3.2$	
123	G'_1 [Pa]	121	(prior standard)
121	G'_M [Pa]	59.1	tangent, minimum strain
123	G'_L [Pa]	157	secant, large strain
122	G'_K [Pa]	580	tangent, large strain
+0.4%	$e_3 / e_1 *100$ [%]	+23.8%	intra-cycle elastic nonlinearity

Figure 2. Experimental results from the large amplitude oscillatory shear (LAOS) test of the pedal mucus from *Limax maximus* (ω=1 rad.s^{-1}) using the new framework for nonlinear viscoelasticity. (a) Elastic Lissajous-Bowditch plot in the linear viscoelastic regime, γ_0=0.5. (b) Elastic Lissajous-Bowditch plot in the nonlinear viscoelastic regime, γ_0=3.2. (table) Local measures of elastic modulus (G'_M , G'_L , and G'_K) reveal the dramatic nonlinear response of this complex biological material, whereas the prior standard for reporting nonlinear elastic modulus, the first-order modulus G'_1 , is approximately the same for each curve. The nature of the intra-cycle nonlinearity is represented by the curvature of the elastic stress response, and to first order is captured by the sign of the third-order Chebyshev coefficient e_3. In this case intra-cycle strain-stiffening is indicated by $e_3 > 0$.

Data analysis software

The descriptive framework for nonlinear viscoelastic characterization is sufficiently general to be applied to any viscoelastic material, ranging from purely elastic to purely viscous, and any complex viscoelastic response in-between. The framework has been packaged into a distributable data analysis program to widen its use in both academic and industrial settings (Ewoldt *et al.*, 2007b). This MATLAB-based software, "MITlaos," is freely available for use by anyone.[1] A screenshot of the software user interface is shown in Figure 3a.

The MITlaos software requires time-series signals of strain and stress, along with user-specification of some analysis parameters. A flowchart showing the program sequence is shown in Figure 3b. The input data must be in the form of a plain text file, but the software is sufficiently flexible to accept various formats and layouts, including the standard text file export of some commercial instrumentation. Output from the software includes data files and image files. The data file outputs can be modified at the user's discretion, and include calculated viscoelastic parameters and time-series signals of elastic and viscous stresses, $\sigma'(\gamma(t))$ and $\sigma''(\dot{\gamma}(t))$ respectively. Image file output is also useful for documenting the analysis. Figure 4 shows one of the optional image outputs from the software, which includes an overview of the analysis (here for the pedal mucus data at $\gamma_0 = 3.2$, $\omega = 1$ rad.s^{-1}, c.f. Figure 2b). The top left subplot in Figure 4 includes the input time-series data of strain $\gamma(t)$ and shear stress $\sigma(t)$ used for the analysis. The lower left subplot of Figure 4 is a Fourier spectrum of the stress response represented in terms of a power spectrum density $P(\omega)$. This Fourier spectrum provides a mathematically robust description of the response in terms of the individual harmonic contributions to the system response. However, interpreting the physical meaning directly from the information on phase and amplitude encoded in the higher harmonics has proven elusive. The physically meaningful characterization is represented in the middle and far-right columns of Figure 4. The middle column of Figure 4 captures the elastic behavior of the sample response, showing the elastic Lissajous-Bowditch curve at the top, and at the bottom containing the spectrum of elastic Chebyshev coefficients and the value of the different measures of elastic

[1] Contact MITlaos@mit.edu to request the "MITlaos" software.

moduli. The solid red line in the elastic Lissajous-Bowditch curve, the elastic stress $\sigma'(\gamma)$, clearly shows the strain-stiffening nature of the material response. The dashed-green line labeled "Chebyshev 1+3" is the representation of the elastic stress using only first-harmonic and third-harmonic Chebyshev coefficients e_1 and e_3; higher harmonics also exist (e_5, e_7, etc.) but these are negligible as shown by the near overlap of the approximate (dashed line) and actual (solid line) elastic stress $\sigma(\gamma)$. The far-right column of Figure 4 contains the corresponding viscous characterization of the sample response, showing the viscous Lissajous-Bowditch curve at the top and at the bottom displaying the viscous Chebyshev spectrum and the different local measures of dynamic viscosities. The software can save the overview plot of Figure 4 for each test at a given amplitude and frequency, $\{\gamma_0, \omega\}$. All of the data displayed in the overview plot of Figure 4 can be output to data files.

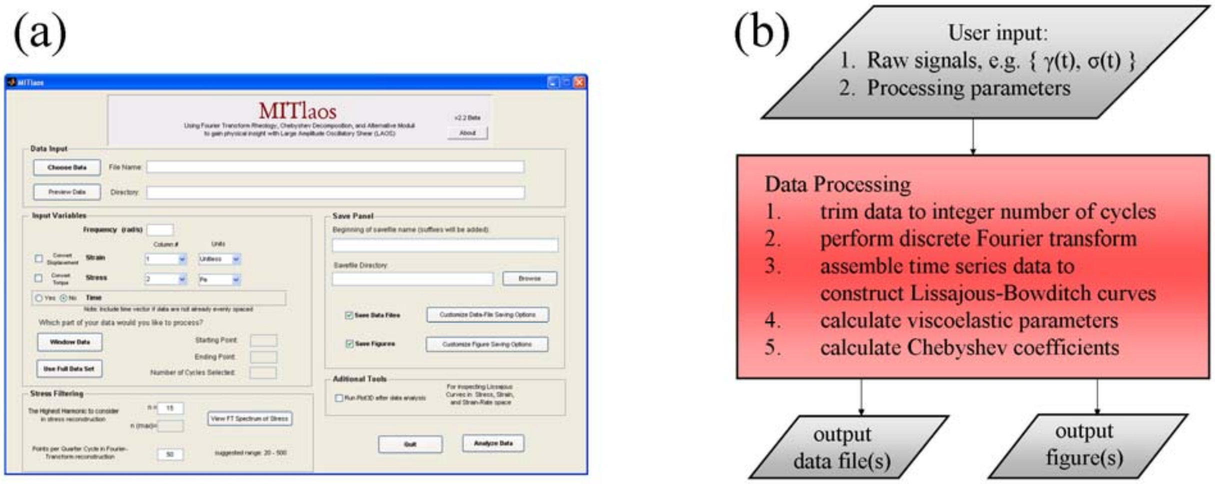


Figure 3. “MITlaos” data analysis software is freely available to assist in implementing the framework for nonlinear viscoelasticity (contact MITlaos@mit.edu to request). (a) Screenshot of the main user interface, (b) flow chart of the processing sequence.

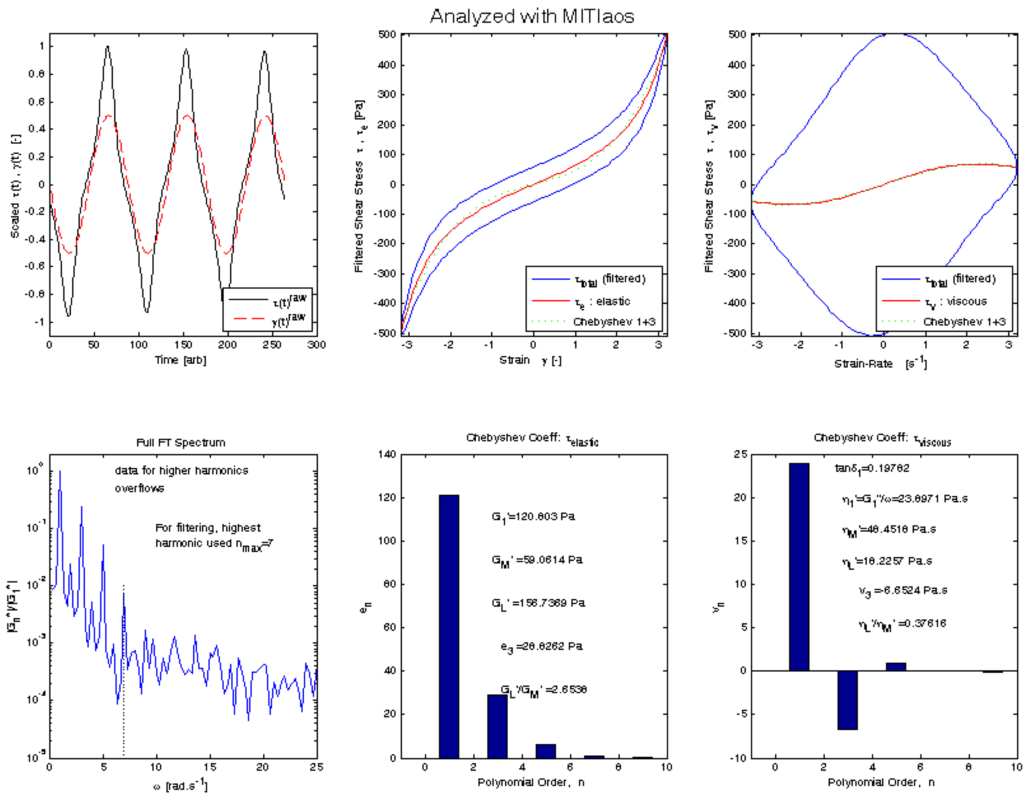


Figure 4. Sample output from the MITlaos software for the *Limax maximus* pedal mucus (γ_0=3.2, ω=1 rad.s^{-1}, c.f. Figure 2b).

Conclusions

Biomaterials are typically complex viscoelastic systems and are often exposed to sufficiently large deformations to elicit a nonlinear viscoelastic response. This nonlinear viscoelasticity is important for physiological functions and motivates engineering designs that imitate unique biological functionality. Systematic understanding of such materials has been hindered in the past by the lack of an appropriate means of describing complex mechanical behavior. The new viscoelastic measures discussed here are generally applicable to any material that can be tested with imposed oscillatory deformation (in shear, torsion, bending, or extension). The new ontology of nonlinear viscoelasticity will enable a better understanding of biological materials by improving the ability to distinguish between different materials and providing insight into the physical mechanisms that cause the observed material response. We hope that this framework will support further refinements in our understanding of structure-property relationships and help the biomaterials community at large to share knowledge about complex nonlinear viscoelastic materials in a clear, concise, and complete way.

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