Application of Viscoelastic Transformations to Rheological Analysis of Human Biological Fluids

*Steven R. Aubuchon, Ph.D.
TA Instruments, 109 Lukens Drive, New Castle, DE 19720, USA
&
Bruce K. Rubin, MD
Wake Forest University School of Medicine
Medical Center Boulevard, Winston-Salem, NC 27157 USA

* corresponding author

ABSTRACT

Controlled-stress rheology is used to investigate the viscoelastic parameters of human biological fluids and simulants. These fluids include human airway secretions (mucus), and a simulant of human meconium, specifically a vegetable-based food product. In light of volume restrictions of viable specimens, analyses are performed on <30 μL of material. This small volume of sample makes it difficult to collect data in oscillation mode as inertial effects often predominate. Hence, Polymer Transformation Software is used to generate oscillation data from collected creep data. The generated spectra are shown to be in good agreement with data collected directly.

INTRODUCTION

Rheology is often used to examine the viscoelastic nature of liquids such as inks, paints and personal care products. These materials are precisely engineered to exhibit a desired mechanical behavior under a specific set of circumstances. For example, a high-quality paint will flow readily under the high stress (and shear rate) of brushing or rolling, then stick to the wall like a high-viscosity solid at rest.

Many biological fluids and semi-solids exhibit a similar viscoelastic nature. Mucus is a good example of rheological engineering. The viscoelastic nature of mucus had been documented and described in detail by Rubin and coworkers (*1*). "Mucus is a viscoelastic gel consisting of water and high relative molecular mass (M_r), cross-linked glycoproteins mixed with serum and cellular proteins (albumin, enzymes, immunoglobulins), and lipids…. The liquid like behavior of mucus is useful in understanding the role in cough in the clearance of secretions. The near-explosive expulsion of air from the lung imparts very-high shearing forces to the mucus that is lining the upper airways. Exposed to high-shear stress, the mucus flows easily forward, since its effective viscosity (flow resistance) in this condition is low. After the cough, exposed only to the modest shear stress of gravity, it does not flow back into the lung because it's effective viscosity is high again, although because of shear thinning, it is perhaps not as high as before the cough".

In the development of new pharmaceuticals and treatments for pulmonary disorders, it is imperative to measure the viscosity and viscoelasticity of mucus samples. However, it is often difficult to collect *in situ* volumes large enough for traditional analysis (especially from pediatric patients). For this reason, it is necessary to perform rheological analyses on sample volumes typically less than 30 μL. The AR1000 rheometer was chosen for this work due to its superior low-end sensitivity facilitating analyses of these ultra-low volumes. Even with the superior sensitivity, inertial effects can periodically and adversely affect the oscillation measurement. For this reason, the TA Instruments' Polymer Transformations Software is employed to generate oscillation data from creep data (which is not influenced by instrument inertia). To check the accuracy of the transformation routine, a meconium simulant sample was analyzed under both oscillation and creep transformation. The meconium simulant has sufficient viscosity and shear modulus to be immune from inertial effects. The resultant data were compared and found to be in excellent agreement.

EXPERIMENTAL

All data were collected on a TA Instruments model AR1000 Controlled Stress Rheometer running the Rheology Advantage 3.0 software. A low inertia, 20 mm, 0.5° aluminum cone was employed as the measurement geometry that requires a small (23 μL) volume. Samples were provided by Dr. Bruce Rubin of Wake Forest University Baptist Memorial Hospital (Winston-Salem, NC). Data were collected at 37 °C.

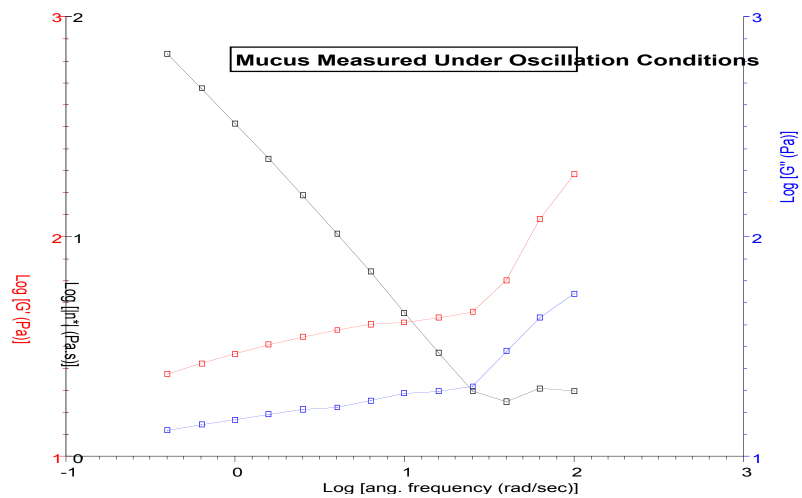

Figure 1 – Typical Oscillation Data of Mucus Sample

RESULTS AND DISCUSSION

The intention of this work is to directly measure viscoelastic properties of mucus and other biological fluids. In the case of mucus, the parameters of interest are primarily shear modulus (G'), loss modulus (G"), and complex viscosity (η*). Measurement of these parameters at a variety of frequencies indicates the viscoelastic tendency of the mucus under simulated respiratory conditions. For example, the shear rate experienced during a cough is *ca*. 100 rad/s, whereas at rest the mucus is subjected to only about 1 rad/s of shear. To simulate these conditions, the mucus is measured with a frequency sweep between 100 and 1 rad/s, at a controlled strain of 1% (previously determined to be within the linear viscoelastic region). Figure 1 contains representative data for mucus analyzed under these conditions. Because of the small volume of sample, the measuring system often suffered from a lack of consistent drag. This is manifested as negative phase-lags, and inconsistent values of G', especially at higher frequencies. To negotiate this problem, TA Instruments Polymer Transformations Software is used.

TA Instruments Polymer Transformations Software utilizes the theory of interconversion of linear viscoelastic functions. Materials have only one set of viscoelastic properties but there are many ways of investigating them, such as oscillation, creep or stress relaxation. In principle, each of these investigative tools delivers the same information, although each technique provides a different section of total information and presents information in different form. The theory of interconversion of linear viscoelastic functions holds that data collected from one technique can be transformed into data as collected from an alternative technique. For example, creep recovery data can be collected and transformed into oscillation data, or *vice-versa*. These conversions cannot be made directly. The first step is to calculate the core function or discreet relaxation spectrum for the material. The relaxation spectrum is a property of the material that is not directly measurable but must be calculated. It is a plot of the relative magnitude of all of the discreet molecular relaxation processes as a function of their individual timescales. Once the relaxation spectrum is calculated, any viscoelastic function can be generated. Figure 2 contains a schematic that illustrates the capabilities of the Polymer Transformations Software package.

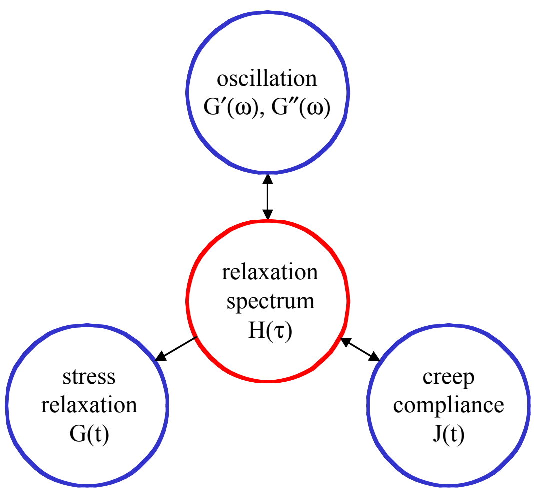

Figure 2 - Available Viscoelastic Interconversion Paths

This software automatically calculates the relaxation spectrum from either creep or oscillation data and can thus interconvert the two. As the creep experiment is not susceptible to inertial effects, it is much easier to collect creep data on the small-volume mucus sample. Representative data are shown in Figure 3. This creep data are collected at a stress of 0.5 Pa, (within the linear viscoelastic region for the sample). The data were collected in "fast-sampling" mode, that allows for excellent resolution at very short times. (Note the ringing behavior in the first second of data, which arises from the elasticity of the sample). The first step in the interconversion process is to calculate the discreet retardation spectrum from the creep data. This model is overlaid on the data in Figure 3. The discreet retardation spectrum is calculated down to about 0.05 s in time, as this will convert to the desired maximum of 100 rad/s in frequency.

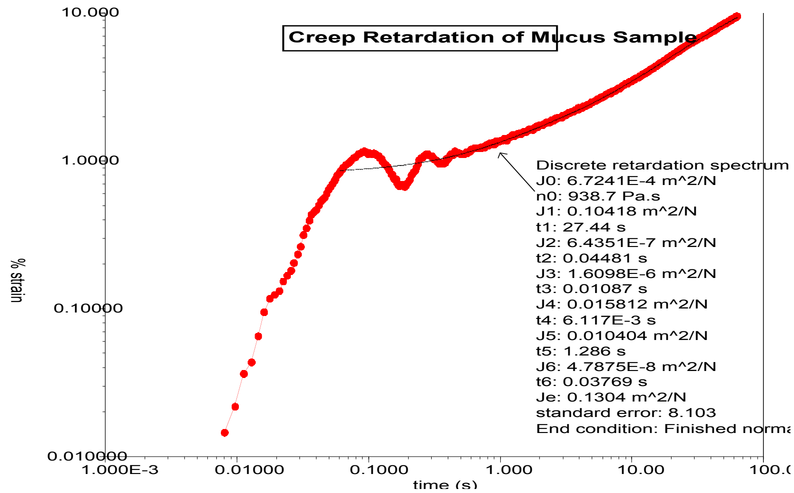

Figure 3 - Creep Data of Mucus Sample with Discreet Retardation Spectrum

Figure 4 contains the oscillation data as generated from the Polymer Transformations function within the Rheology Advantage software. The relative magnitudes of G' and G" are useful parameters in quantifying the expected behavior of the mucus *in situ*. The magnitude of η^* undergoes a two-decade decrease over this frequency range, illustrating the shear thinning nature of the mucus. The data from all three parameters is much more stable at high frequencies.

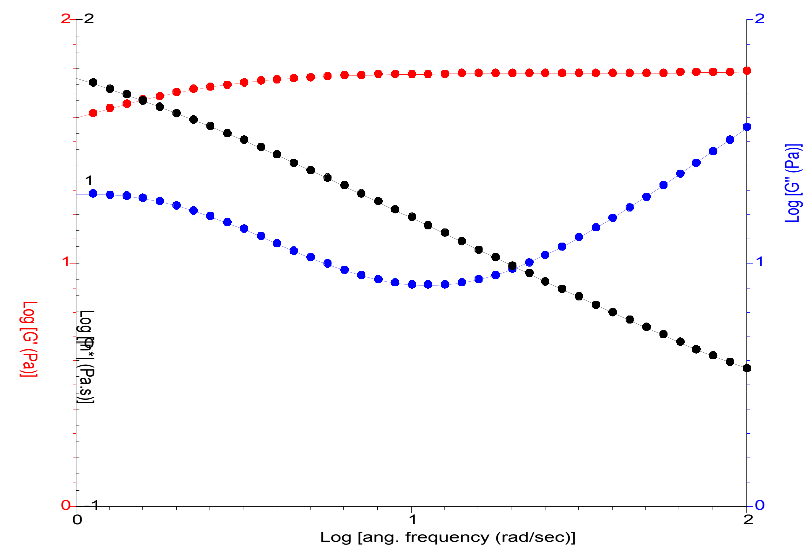

Figure 4 - Oscillation Data Generated from Creep

In order to check the accuracy and validity of the Polymer Transformations functions, a known sample was analyzed by both direct oscillation and creep-transformation techniques. The sample was a common vegetable-based spread that is a suitable simulant for meconium (a fetal waste product which is expelled shortly after birth). Meconium possesses a stronger structure and higher viscosity that allows for oscillation study without inertial effects. Figure 5 contains the data comparing G', G" and η^* for the meconium simulant. Note that the agreement in the values is very good, given the relative historical difficulty in generating repeatable data. This confirms the utility of the polymer transformation routine for this study.

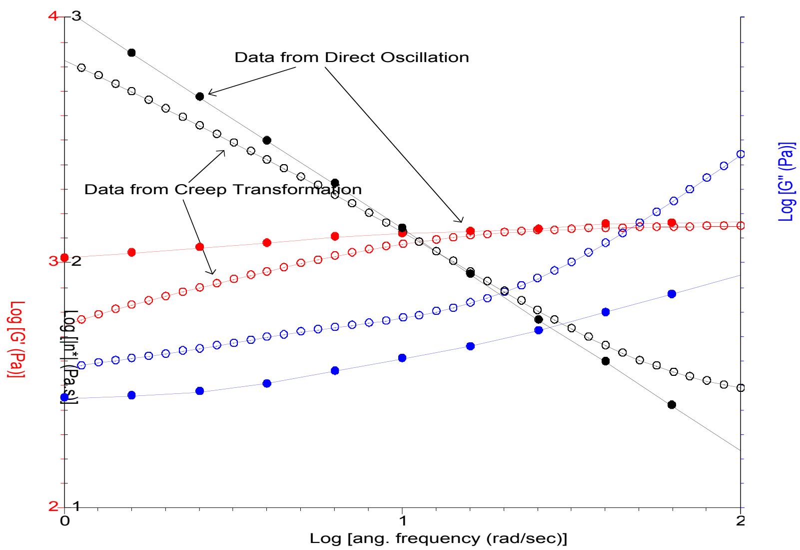

Figure 5 - Comparison of Data Collected from Oscillation and Generated from Creep

SUMMARY

The AR1000 Controlled Stress Rheometer is shown to measure the viscoelastic parameters of human mucus. Volumes as low as 23 µL are analyzed. Polymer Transformation Software has been proven to be a valuable tool for generating oscillation functions when inertial effects are present in the measurement. The data generated is shown to be in excellent agreement with data collected directly.

REFERENCES

1. M. King and B. Rubin, "Rheology of Airway Mucus", *Airway Secretion: Physiological Bases for the Control of Mucus Hypersecretion,.* T. Takishima and S. Shimura (Eds.), **1994**, pp. 283-314.

KEYWORDS

biologicals, creep and stress relaxation, flow properties, rheology, viscosity