

Using Optical Tweezers as a Non-Invasive Microrheometer

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In this lab, we learn about the theory and the principles of use of optical tweezers. We then employ the optical tweezers as a microrheometer, to determine the storage modulus (G') and loss modulus (G'') of a 4 weight percent solution of bovine gelatin gel, via the optical trapping and oscillation of a $1.5\mu\text{m}$ silica beads at various frequencies. In order to do this, the system was first calibrated with a solution of the same silica beads in water. Both the calibration data and the subsequent gelatin gel data are presented.

I. INTRODUCTION AND MOTIVATION

Rheology, the study of the deformation matter given an applied stress, has historically been a macroscopic science, in that typical rheometers, devices used to measure the deformation properties of matter, typically required macroscopic sample quantities. A typical macroscopic rheometer consists of two coaxial cylinders, in between which is placed a macroscopic quantity of sample (usually a few liters). The inner cylinder is then oscillated by a motor at a given frequency, and the relative phase and amplitude of the oscillation of the outer cylinder to the inner cylinder's motion can be used to define the storage modulus and loss modulus of the sample. The storage modulus of the sample is measure of the elastic energy stored per cycle, and the loss modulus is a measure of the amount of energy lost as heat per cycle, and each is measured in Pa.

Not only does this process require a macroscopic sample quantity, it is also very invasive, in that all of the sample is significantly disturbed in the process. Both of these potential problems are overcome by using optical tweezers as a rheometer. Using a microscope and laser, a dielectric bead in a very small volume of sample can be trapped in the path of the beam. Oscillation of the beam causes oscillation of the bead, and the phase and amplitude of the two are completely analogous to the macroscopic rheometer. Thus, optical tweezers may serve as a microrheometer, in that sample sizes may be on the order of microliters, and the bead sizes are typically on the micron scale, and the disturbance due to the bead is extremely localized (on the order of nanometers) thus noninvasive. The rheology of many regimes of study, such as live biological samples and delicate polymers, are now permitted by optical tweezers.

II. OPTICAL TRAPPING AND THE OPTICAL TWEEZERS SETUP

When a dielectric bead (of radius on the order of the waist diameter of the beam) is placed near the waist of a highly focused laser beam, a force on the bead toward the center of the waist is observed, independent of the relative positions of the bead and beam waist. Thus, as this center-seeking force is noted for a bead displaced any direction away from the center, for small displacements, the bead is essentially trapped in the waist of the beam, and can be manipulated by manipulating the beam. This force can be thought of in two different ways. For a Gaussian beam and dielectric particle, the dielectric electric energy has a minimum at the center of the waist, and thus the negative of the energy gradient defines a force toward the center of the beam. Similarly, the force may also be thought of as derived by the change in momentum of photons refracted by the dielectric particle. Ray diagrams show that for particles near the waist center, momentum imparted to the bead by the photons is always toward the waist center. This is the principle of optical trapping.

The setup of the optical tweezers inherently affects the theory of it's operation. The optical tweezers setup used in this lab was as follows:

Laser light emitted at 1064 nm and 44 mW is sent through a beam expander, expanding the millimeter sized beam to a diameter of nearly a centimeter. The beam is then incident upon a two-axis piezo steering mirror. The piezo mirror is attached to the function generator of a lock-in amplifier. The 3 V signal applied to the mirror at a given frequency causes the mirror to oscillate in one (or, possibly two) dimension with an amplitude on the order of nanometers at the frequency supplied. This beam is then combined with a second beam of different wavelength, at a fraction of the intensity of the first beam. The combined beam is then incident upon a confocal microscope, which intensely focuses the beam inside of a sample on a microscope slide. After bead capture, the 1064 nm beam provides the sinusoidal force on the bead, and the scattered beams are then passed through a polarizer, which filters out

the 1064 nm beam, leaving only the stationary second beam. This beam is then incident upon a position sensitive detector, and the scattering of this beam is converted by the PSD to a voltage, which is then sent to the lock-in, where the output amplitude and phase may be read.

As the output of the system, V is a voltage and not a distance, the system must first be calibrated with a water-bead sample to determine the maximum voltage, A_v , corresponding to maximum displacement, so that $\frac{V}{A_v}$ might serve as a dimensionless displacement. This is assuming that the PSD output is proportional to the bead movement, which is valid for the displacement ranges used in this experiment. Further necessity for system calibration is found when analyzing the equation of motion. For small displacements from the center of the beam waist, the trapping force may be considered linear in displacement, and thus an inherent force constant of the tweezers, k_{ot} , must be determined. Thus, the equation of motion of the bead under the sinusoidal trapping force is:

$$m \frac{d^2x}{dt^2} = -6\pi a(\eta_0 + \eta(\omega)_{material}) \frac{dx}{dt} - k_{material}x + k_{ot}(Ae^{i\omega t} - x) \quad (1)$$

where x is the displacement of the bead, A is the maximum displacement of the bead, a is the radius of the bead, and the first two terms on the right hand side are the drag and elastic forces of the material, respectively. Assuming the solution of the form $x(\omega, t) = D(\omega) \cos(\omega t - \delta(\omega))$, we find that $D(\omega) = A/\sqrt{\tau^2\omega^2 + 1}$, and $\delta(\omega) = \tan^{-1}(\tau\omega)$, where $\tau = 6\pi\eta_0 a/k_{ot}$. Thus we may determine k_{ot} by fitting the both the phase and amplitude data of a bead in water to these formulae. Then, for an arbitrary material:

$$G'(\omega) = \frac{k_{ot}}{6\pi a} \left(\frac{\cos(\delta(\omega))}{D(\omega)/A} - 1 \right) \quad (2)$$

$$G''(\omega) = \frac{k_{ot}}{6\pi a} \left(\frac{\sin(\delta(\omega))}{D(\omega)/A} \right) \quad (3)$$

However, the raw data obtained from the lock-in cannot be used immediately for modulus calculations, as system corrections need to be taken into account. The phase and amplitude displayed by the lock-in are systematically off by a given amount due to mechanical effects (such as phase lag of the piezo mirror), and prescribed correction factors must first be applied before data analysis.

III. PROCEDURE

A. Water Calibration: Sample Preparation

To prepare a sample for water calibration, a glass slide was obtained, upon which was placed two thin pieces of double sided tape (thickness: 100 microns). A slip cover was then placed on top of the double sided tape, and using a micropipette, solution containing 1.5 micron-radius beads was applied to the exposed edge of the slip cover, until liquid filled the area underneath the slip cover. To prevent evaporation, vacuum grease was then placed on the two exposed edges of the slip cover.

B. Water Calibration: Bead Capture and Measurement

The glass slide was placed in the stage of the microscope and fastened in place, making sure enough index-matching oil was placed between the microscope objective and the slide. Adjusting the focus knob counterclockwise moved the microscope lens (and thus the laser beam waist) up such that one division on the knob was approximately one micron. Using the focus, the top and bottom of the sample were located by identifying concentric beam reflections on the glass surfaces of the boundaries. Due to gravity, in water, most of the beads tend to be near the bottom of the sample. Thus, using the x-y stage movement controls, a plane about 5 microns above the bottom was scanned (using the microscope video display) for beads, avoiding fouling the objective with vacuum grease. The beads, in proper focus, are spherical; all other objects were sample fouling (bacteria, dust, etc). Once a bead was located, it was trapped by overlaying the beam "crosshair" on the video monitor on the bead. After trapping, the bead and beam wait were raised 20 microns to remove any frictional effects from the glass bottom of the sample. At this point, the Labview lock-in control program (used previously) was run to take three data points at each of 30 frequencies. These data were imported into Excel, the average at each frequency point was taken, and subsequent system corrections

were applied. The corrected data were then imported into Origin, in which both the dimensionless displacement and phase were plotted as a function of $\log_1 0(\omega)$, to which one-parameter fits for k_{ot} were applied using the expressions above. Thus, k_{ot} was determined independently from both displacement and phase data.

C. Gelatin Gel: Sample Preparation

A sample of 4 weight percent of bovine gelatin gel was prepared as follows: 9.6 grams of water-silica bead solution were placed in a vile, to which was added 0.4 grams of powdered gelatin. Magnetic stirrers were added to the vile, and the solution was stirred and heated gently for 20 minutes. After this, one end of a capillary tube was placed in the gel until the tube was filled. The inside cavity of the capillary tube was precisely 100 microns in thickness. One third of the capillary was broken off and discarded, and the remainder of the tube was placed in the center of a glass slide. Vacuum grease was then applied to the ends both to seal them and to secure the capillary on the slide.

D. Gelatin Gel: Bead Capture and Measurement

The same procedure was followed for bead capture and data acquisition as in the water calibration bead capture and measurement. However, after data were taken, imported into excel, averaged, and corrections applied, the previously obtained values of k_{ot} and the voltage, A_v , corresponding to the maximum displacement of the bead in water were imported also, and from these data, values of the storage modulus and loss modulus as a function of frequency were obtained and plotted.

IV. EXPERIMENTAL RESULTS

A. Water Calibration

Two different runs were performed for water calibration. The first was with an already prepared slide, and the second was with a slide of my own preparation. Plots of the displacement and phase are found in Figures 1-4. Each value of k_{ot} is reported in units of dyne/centimeter. We note that not only do the values of k_{ot} vary largely between runs, the values also vary between displacement and phase determinations. This is indicative of slight misalignment of the optical apparatus. Precise alignment produces very similar results of k_{ot} for both displacement and phase determinations.

B. Gelatin Gel

At the time of measurement of G' and G'' for the gelatin gel, only Run 2 calibration data was available. The value of $k_{ot} = 0.01462$ dyne/cm (from the displacement data) was chosen. A plot of the calculated G' and G'' for the gelatin gel is found in FIG 5.

V. CONCLUSIONS

In one aspect, this experiment was successful as I was able to use the principles of optical trapping to calibrate an optical tweezers system functioning as a microrheometer, and then to subsequently measure the storage modulus and loss modulus of a bovine gelatin gel. However, over two runs of calibration with nearly identical samples, a range of k_{ot} values were calculated, even between both displacement and phase determinations, indicating misalignment of the optical elements in the apparatus. Thus, the values of the storage modulus and loss modulus were indeed measured successfully, however, the validity of the measurement is doubted given imprecise calibration.

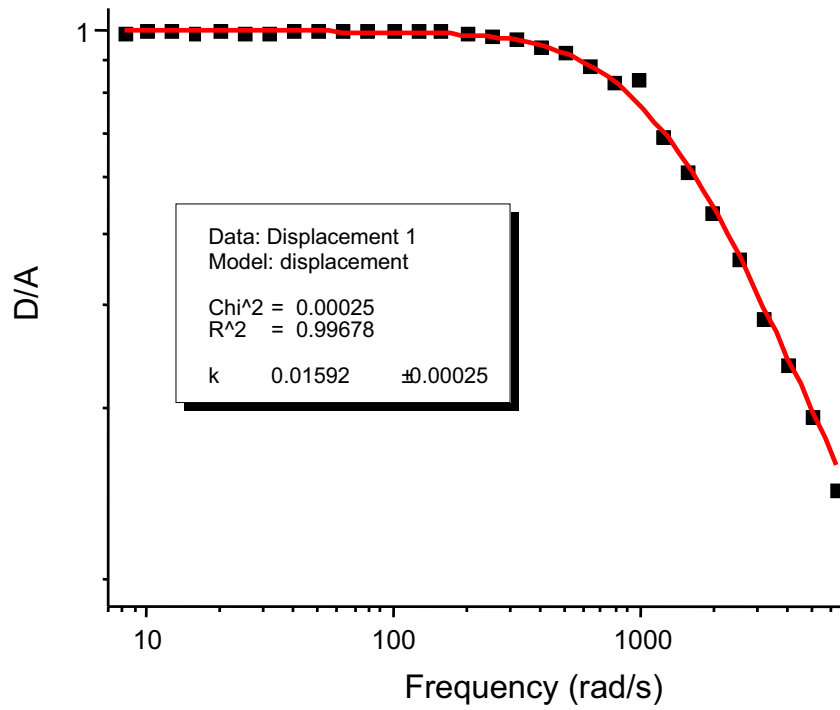


FIG. 1: Run 1 displacement data including the one parameter fit for k_{ot} (solid line).

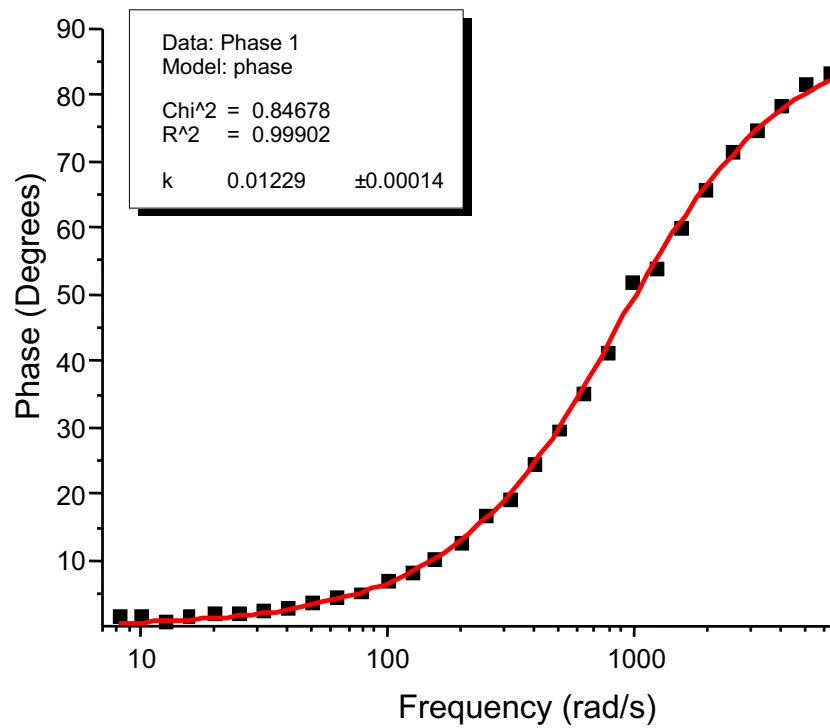


FIG. 2: Run 1 phase data including the one parameter fit for k_{ot} (solid line).

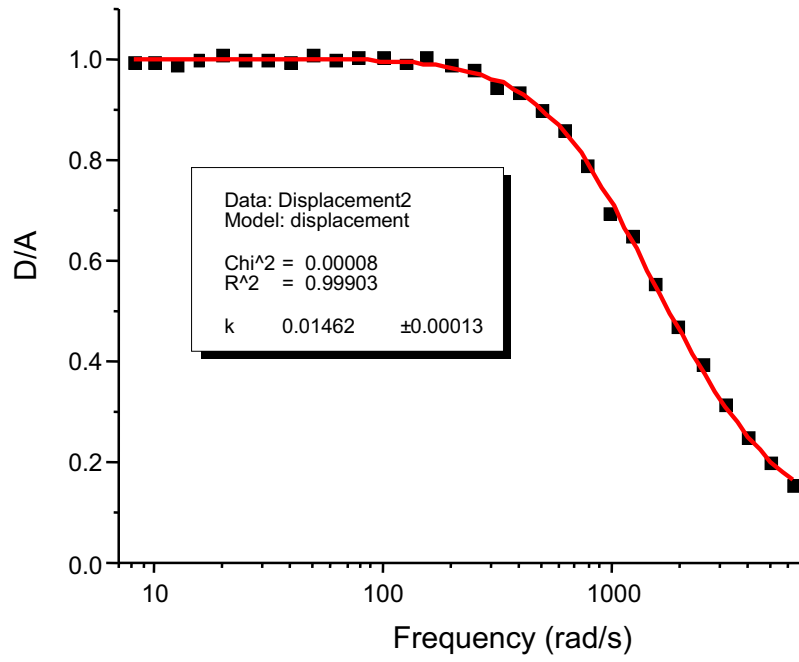


FIG. 3: Run 2 displacement data including the one parameter fit for k_{ot} (solid line).

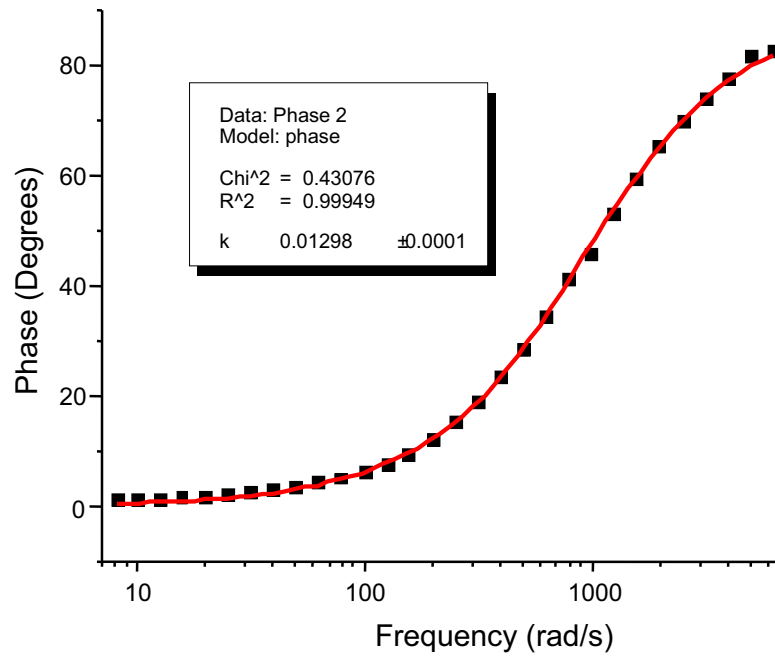


FIG. 4: Run 2 phase data including the one parameter fit for k_{ot} (solid line).

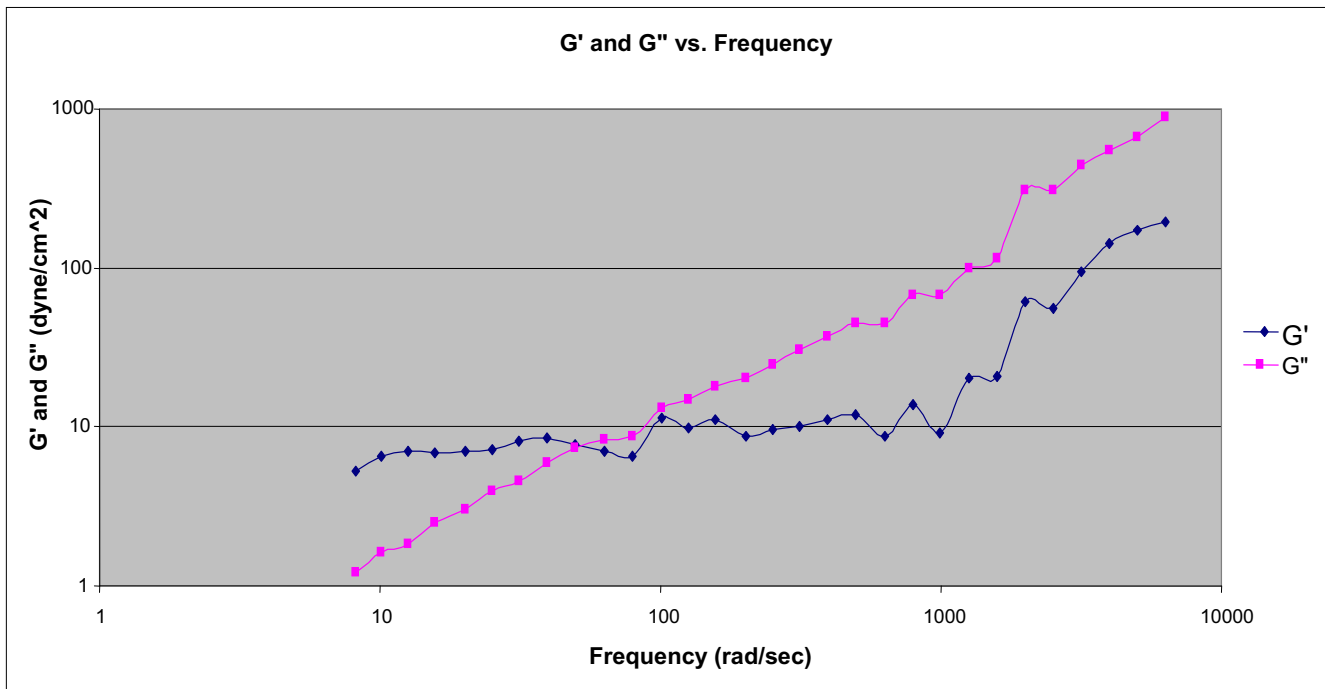


FIG. 5: The calculated values of G' and G'' as a function of frequency, using $k_{ot} = 0.01462$ dyne/cm, and maximum displacement data from Run 2.

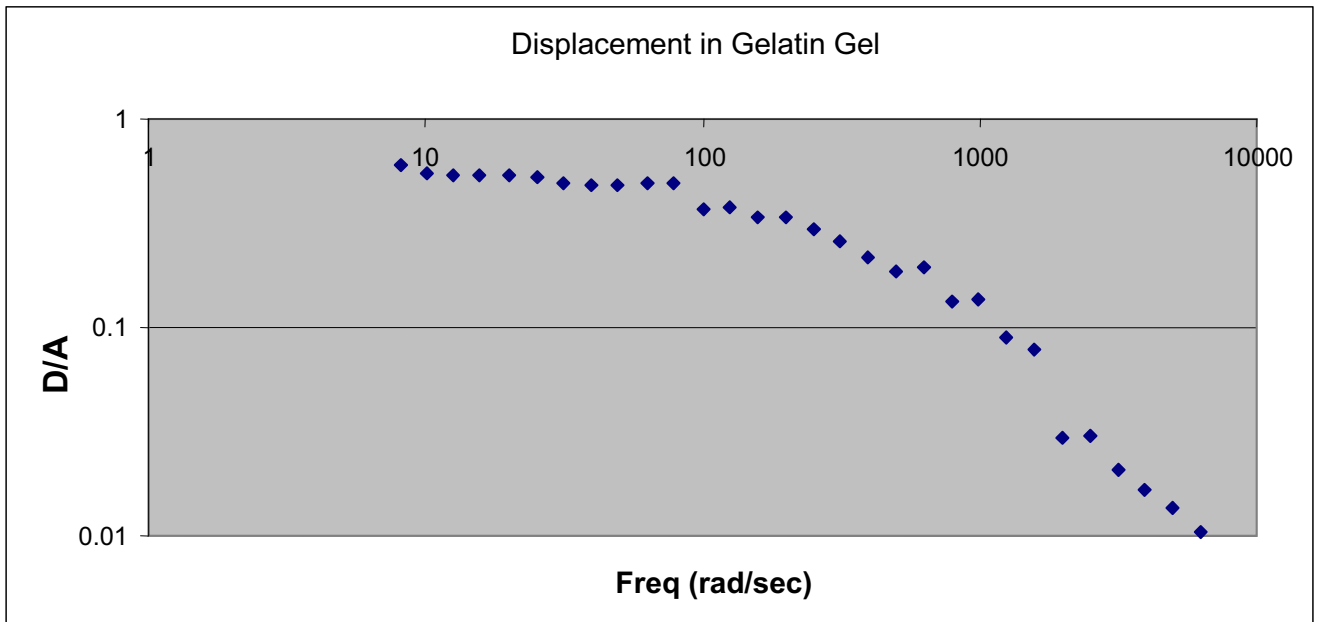


FIG. 6: Displacement of the bead in gelatin gel versus frequency.

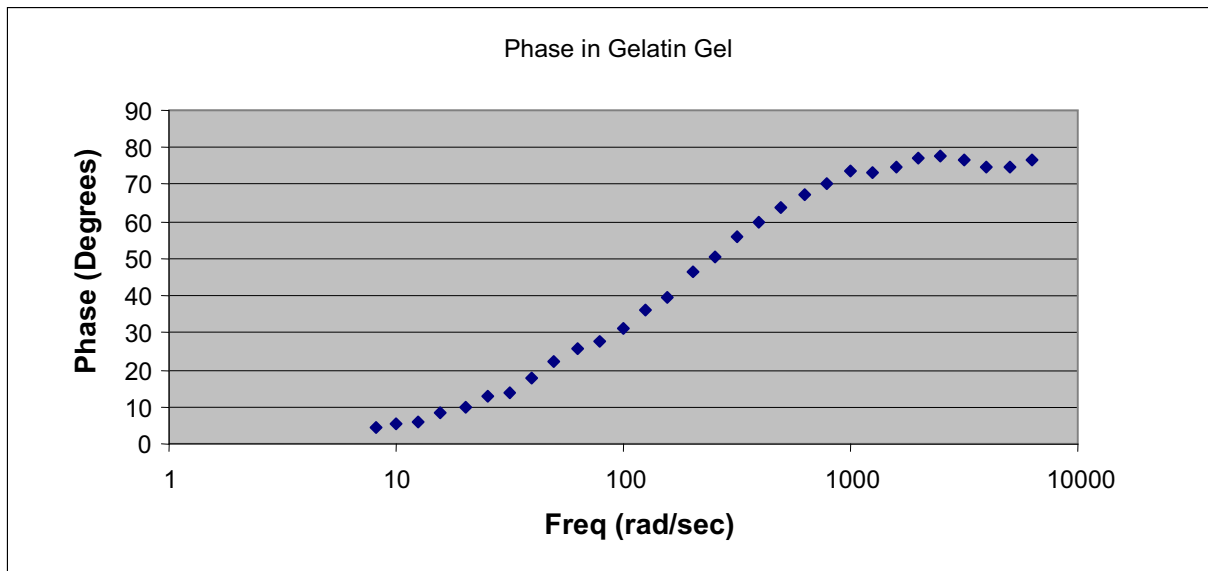


FIG. 7: Phase of the bead in gelatin gel versus frequency.