Measurement of the Viscosity of Biological Mediums Using Brownian Motion

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INTRODUCTION

The viscosity of artificial biological mediums can determined by examining the Brownian motion of suspended polystyrene micro beads. These measurements will allow for comparison of the viscosity of the artificial medium, used in in-vitro protein expression, with extracted cytoplasm used in cellular protein expression. An optical microscope is used to measure the horizontal displacement of the microspheres, undergoing confined Brownian motion, from which the viscosity of the medium is determined using the Stokes-Einstein Law. This measurement wll allow for a comparison of the viscosity of the mediums used for *in virtro* protein expression to *in vivo* protein expression.

THEORY

Brownian motion is charachterized by solutions to the Langevin Equation, for which [1] derived a statistical solution, Equ. 1, expresing the probability of finding a particle as a function of the square of the displacement and time. The diffusion coefficient, D_0, arises from Stokes' Law where η_0 is the viscosity of the fluid and a is the radius of the sphere. The common form of the Stokes-Einstein Law, Equ. 2, describes the average of the displacement in any one dimension as a linear function of time. For a two-dimenstional space (or the horizontal projection of three dimensional motion, the Stokes-Einstein Law is represented by Equ. 3. Deviations from the Stokes-Einstein Law occur due to finite boundaries. [2] gives a simple model, Equ. 4, expressing the deviation in the viscosity parallel to a plate boundary below the sphere as a series in the ratio of the radius to the average height of the bead. The average height is calculated from the Boltzmann density profile, Equ. 5.

$$P(\mathbf{r},t;\mathbf{r}_0) \cong \frac{1}{(4\pi D_0 t)^{\frac{3}{2}}} \exp\left(-\frac{(\mathbf{r}-\mathbf{r}_0)^2}{4D_0 t}\right) \quad D_0 = \frac{k_B T}{6\pi\eta_0 a} \quad (1)$$

$$\langle x^2 \rangle = \frac{1}{3}\langle (\mathbf{r}-\mathbf{r}_0)^2 \rangle = 2D_0 t \ (2) \qquad \langle r^2 \rangle = 2D_0 t \ (3)$$

$$\eta_{\|^b} = \eta_0[1 - \frac{9}{16}(a/\langle z \rangle) + \frac{1}{8}(a/\langle z \rangle)^3 - \frac{45}{256}(a/\langle z \rangle)^4 - \frac{1}{16}(a/\langle z \rangle)^5]^{-1} \ (4)$$

$$P_B(z) = \frac{1}{L}\frac{e^{-z/L}}{e^{-a/L} - e^{(a-h)/L}} \quad L = \frac{k_B T}{4/3\pi a^3(\rho - \rho_0)g} \ (5)$$

METHODS

The horizontal projection of the Brownian motion of polystyrene microspheres suspended in the medium in question will be observed using an optical microscope equipped with a 100X magnification oil immersed objective. The microspheres are distributed highly concentrated in a solution that is dilluted by a factor of 10-3. The solution is then centrifucated, the surfactant is removed, and replaced by the medium in question.

Thirty microliters of the prepared suspention of microspheres is confined between two glass slides, separated by a spacer approximately 250 micrometers thick. The spacer has adhesive on both sides that sticks to the top and bottom plate, sealing the system and preventing evaporation. Fig. 1 shows a schematic of a prepared slide viewed by the microscope.

From the Boltzmann density profile, most of the beads are found near the mean height. Upon locating and focusing on a microbead, time lapse images recording the location of the microbead are recorded with a computer controlled camera that takes an image at a known time interval.

Images are then analyzed using ImageJ to determine successive horizontal displacements of the microspheres. Calibration has found that 15.2 pixels corresponds to a one micrometer displacement. The mean square of the displacement is then used to calculate the viscosity of the medium in question.

METHODS (CONTINUED)

Calibration to verify the validity of the procedure is achieved by measuring the viscosity of known substances of similar viscosity to the mediums in question. Calibration has been done using water, ethanol, and isopropanol.

The mediums in question include feeding buffer, feeding buffer with polyethelyne glycol, and cell-free extract. Feeding buffer is used to provide the molecules necessary to express proteins to an expression system. At a point in the expression process, polyethelyne glycol is added to increase the viscosity and change the rate of diffustion of the necessary molecules. The cell-free extract is material extracted from cells that contains the DNA plasmids the system is to express. See [3] and [4] for further reading.

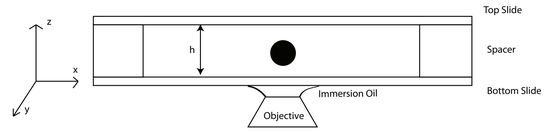

Figure 1: Schematic of prepared microscope slide. Suspention containing polystyrene microspheres is confined between two glass slides sparated by a spacer of thickness, h. An oil immersed 100x objective allows for tracking the horizontal projection of the Brownian motion.

DATA OBTAINED

Figure 2: Shown are two successive time lapse images with a Δt of 1s. The arrow points to a microsphere observed that is in focus. Using ImageJ, and calibration indicating 15.2px/µm, the measured displacement is 1.0±0.6 µm, due to uncertainty in visually locating the center of the bead. Note the out of focus microsphere that appears as a ring in the bottom right quadrant. The other dark spots in the image are artifacts on the camera plate.

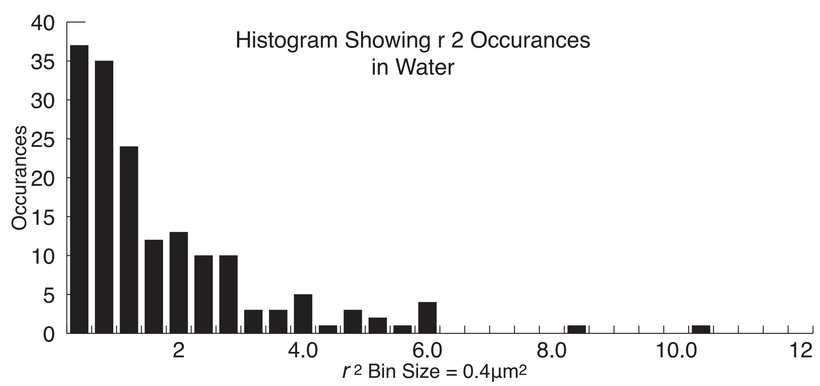

Figure 3: Occurances of the square of the displacement after 1s in µm² for a bin size of 0.4µm². Data obtained for a 1µm radius polystyrene microsphere in water, at a temperature of 276K. Data indicates a viscosity of (1.18±0.10) □ 10^{-3} Pa s.

PRELIMINARY RESULTS

Table 1: Prelimary Calculations of Viscosities for qater, ethanol, and isopropanol. Data analysis for ethanol is at time of publication unfinished. Even though the value for water is 2.88 σ from the accepted value, it is still within 30% and demonstrates that the technique can distingush viscosities that only vary by a factor of two. Uncertainty on the viscosity is has a linear relationship with viscosity. Data takes into account a correction factor due to the breakdown of the Stokes-Einstein Law due to a boundary.

Substance	Visosity (Pa s)	Accepted Value (Pa s)	σ's from Accepted Value
Water	$(1.18 \pm 0.10) \times 10^{-3}$	8.90×10^{-4}	2.88
Ethanol	Unfinished	1.20×10^{-3}	N/A
Isopropanol	$(2.10 \pm 0.24) \times 10^{-3}$	1.96×10^{-3}	0.61

DISCUSSION

The prelimary results indicate a systematic that results in a measurement of viscosity that is higher than the accepted values. However, two data points is not enough to determine a trend. The propagation of the error used is derived from statistics that assume a symertric distribution - this is not the case for the exponential distribution. However, despite this, and the lack of an accurate way to determine the center of the bead, the results are still on the correct order of magnitue and show the correct multiplicative factor between the two datapoints.

REMAINING WORK

Data for the biological mediums still remains to be collected. However, it is expected that this will be a quick process as this procedure has achieved a high percentage of successful data collection runs.

The image processing technique will be refined to attempt to decrease the uncertainty on the measurement of the displacement. MATLAB image analysis has allowed for a successful subtraction of the background. An algorith to accurately determine the center of the bead is being developed. Analysis of the data can then be automated, greatly reducing the time required to obtain measurements from a data collection run.

Refiguring the uncertainty taking into account the asymetric nature of the distribution will allow for a a more accurate quantification of the uncertainty.

REFERENCES

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