## **THERMAL MOTION EXPERIMENT**

#### **Abstract**

This experiment aims to test the validity of Albert Einstein's theoretical framework describing Brownian motion. This is done by examining the accuracy with which the Avogadro constant can be verified using said framework in comparison to it's known value of  $6.02214076 \times 10^{23}$ .

#### Introduction

#### **Brownian Motion**

Brownian motion is the motion of an object in a medium due to thermal energy. The mean displacement of a particle in brownian motion should be zero while the mean *squared* displacement increases linearly with time, characterised by the following equation:

$$(1) < x^2(t) > = \frac{\delta^2 t}{\tau} = 2Dt$$

where  $\delta$  is the step distance,  $\tau$  is the time in seconds, t is the step number multiplied by  $\tau$ , and D is the diffusion coefficient. Equation (1) models brownian motion in one spatial dimension. The two-dimensional equivalent equation is:

$$(2) < r^{2} > = < x^{2} > + < y^{2} > = 4Dt$$
(3)

The diffusion coefficient can be calculated using Einstein's relationship:

$$(4) D = \frac{kT}{\gamma}$$

where k is the Boltzmann constant, T is temperature in on the kelvin scale, and  $\gamma$  is the stokes drag coefficient which is calculated using the following equation:

(5) 
$$\gamma = 6\pi\eta r$$

In Equation (4),  $\eta$  is the viscosity of the system's suspending fluid and r is the radius of the object exhibiting the brownian motion.

Another way to calculate the diffusion coefficient is by measuring the distance the brownian motion-exhibiting body travels on a time interval: its step distance. This produces a Gaussian probability distribution modelled by the following equation:

(6) 
$$P(x,t) = \frac{e^{-\frac{x^2}{4Dt}}}{(4\pi Dt)^{1/2}}$$

### Avogadro's Number

Avogadro's number (interchangeably referred to as the *Avogadro constant* throughout this report) describes the number of particles in one mole of a substance. Microscopic molecular motion is connected to macroscopic gas laws by Avogardro's number  $(N_A)$ :

Marta Gonczar (introduction, materials, methodology, results (activity 1), partial code (activity 1))
Nick Garcia (abstract, results (activity 2), analysis and discussion, conclusion, primary code)

$$(7) N_A = \frac{R}{k}$$

Here, once again, k is the Boltzmann constant. Furthermore, the ideal gas law can be written as:

$$(7a) \rho V = nRT$$

where  $\rho$  is pressure, V is volume, n is the number of molecules, R is the gas universal constant, and T is the temperature. Substituting Equation (6) into Equation (7a) yields:

(7b) 
$$\rho V = nkN_A T$$

Finally, from substituting Equation (3) into Equation (6), we are left with the following equation:

$$(8) N_A = \frac{TR}{D\gamma}$$

#### Materials

- Diluted Bead solution
- Microscope (40x magnification)
- X-Cite Series 120 Q Fluorescent Lamp
- Microscope slide
- Glass cover slide\*
- Petroleum Jelly
- Pipette
- Object tracking software

# Methodology

## Sample preparation:

A microscope slide was delimited using several layers of petroleum to create a 3-4cm rectangular space. A pipettes was used to transfer  $50\mu l$  of the diluted bead solution onto the sealed rectangular space. Glass was placed gently over the solution, taking extra care to not disturb the bead solution with the glass cover.

#### Microscope setup:

The fluorescence illumination was switched on in the microscope and the GFP fluorescence cube was rotated into place so that it was in position 2. The fluorescence shutter was set to open using the front lever below the eyepieces. The prepared slide was placed carefully on the microscope stage and the sample was left for a couple minutes to ensure equilibrium of particles after slide movement. The microscope was focussed in phase two with a 40x objective until particles were visible, the microscope was focused on small particles in particular.

## **Data Acquisition:**

To acquire data, the Microscope Camera Controller was run and camera imaging was switched on. Image settings were adjusted to provide the best contrast between beads and the background. The camera was focussed on a few energised small beads in the same focal plane. The microscope camera controller was set to Multiple Image Capture with the number of images set to 120 frames and images per second set to 2 frames. Once data acquisition was completed, the images were saved to a folder. This process was

repeated for 20 energised beads. The data analysis was done using Image Object Tracker. For each folder, the fist image was opened, 5 was clicked in the middle of the bead to be tracked and data was saved as a text file.

#### Results

### **Activity 1**

Of the 20 recorded data sets, the first data set will be the one discussed in detail as it yielded the least-inaccurate results. This experiment and it's results were respectively concocted and derived with the assumption that the system's temperature was 296.5  $\pm$  0.5 K and that the diameter of the observed beads was 1.9  $\pm$  0.1  $\mu$ m. For all data points, the mean squared displacement,  $r^2$ , was calculated using the following equation:

$$r^2 = x^2 + y^2$$

where  $x^2$  and  $y^2$  are the mean squared horizontal displacement x and vertical displacement y respectively. The calculation of these values using the known conversion factor between the number of pixels in the captured images and the actual length,  $0.1208 \pm 0.003 \,\mu m$  per pixel, is as follows:

$$x^{2} = ((x_{i} - x_{0})(0.1208 \pm 0.003 \frac{\mu m}{pixel}))^{2}$$

$$y^2 = ((y_i - y_0)(0.1208 \pm 0.003 \frac{\mu m}{pixel}))^2$$

Above,  $x_0$  and  $y_0$  are the respective initial horizontal and vertical positions of the bead and  $x_i$  and  $y_i$  are the respective horizontal and vertical positions of the bead in the i<sup>th</sup> image. A sample calculation of the mean squared position of bead 1 in the second image is as follows:

$$r^2 = x^2 + y^2 = (((588.01) - (591.46))(0.1208 \frac{\mu m}{pixel}))^2 + (((538.85) - (541.53))(0.1208 \frac{\mu m}{pixel}))^2$$
  
 $r^2 = 0.277 \ \mu m^2$ 

The uncertainty of mean squared displacement, using the known uncertainty in the measurement software of 0.005 pixels, was propagated as such:

$$u(r^{2}) = \sqrt{\left(2\left(\left(x_{i} - x_{0}\right)(0.12048)\right)\sqrt{\left(\frac{\sqrt{\left(\frac{0.005}{x_{i}}\right)^{2} + \left(\frac{0.003}{x_{0}}\right)^{2}}{\left(x_{i} - x_{0}\right)}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}} + \left(2\left(\left(y_{i} - y_{0}\right)(0.12048)\right)\sqrt{\left(\frac{\sqrt{\left(\frac{0.005}{y_{i}}\right)^{2} + \left(\frac{0.003}{y_{i}}\right)^{2}}{\left(y_{i} - y_{0}\right)}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}}{u(r^{2})} + \left(\frac{0.003}{\left(\frac{0.005}{y_{i}}\right)^{2} + \left(\frac{0.003}{y_{i}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}}{\left(\frac{0.005}{y_{i}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}}\right)^{2}} + \left(\frac{0.003}{0.12048}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}$$

$$u(r^{2}) = \sqrt{\left(\frac{\sqrt{\left(\frac{0.005}{y_{i}}\right)^{2} + \left(\frac{0.003}{y_{i}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}}}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2} + \left(\frac{0.003}{0.12048}\right)^{2}}$$

$$u(r^{2}) \approx 0.03$$

$$\Rightarrow u(r^2) = 0.277 \pm 0.03 \,\mu\text{m}^2$$

The mean squared displacement was graphed against time for all beads. Figure 1 is the best graph acquired:

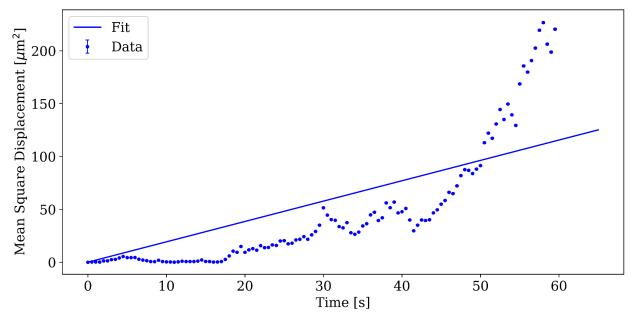
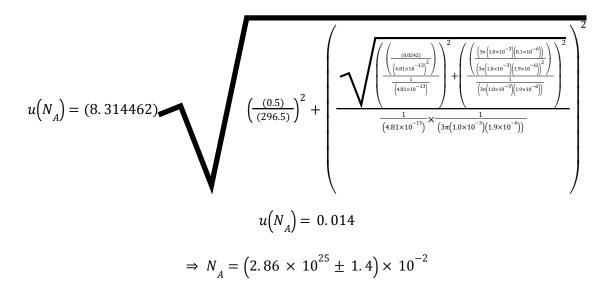


Figure 1: Mean displacement squared of a fluid-suspended bead exhibiting brownian motion as a function of time. Vertical error bars represent combined propagated measurement and calculated uncertainty, but are negligible given their relative minisculeness (< 1).  $\chi^2_{reduced} = 1.71 \times 10^5$ .

The relationship between time and mean displacement squared is linear. Theoretically the linear relationship should model Equation (4), the slope should equal 4D. Using the regression fit, D was calculated to be one quarter of the slope times  $10^{-12}$ :  $4.81 \times 10^{-13} \pm 0.02$ , with the uncertainty coming from the linear fit's covariance. Thus, by substitution into Equation (8), and taking the suspending fluid viscosity to be  $1.0 \times 10^{-3}$  kg m<sup>-1</sup> s<sup>-1</sup>, the Avogadro constant was calculated to be  $2.86 \times 10^{23} \pm 0.0014$ , with the uncertainty propagating as follows:

$$u(N_A) = u\left(\frac{TR}{D\gamma}\right) = R \qquad \left(\frac{u(T)}{T}\right)^2 + \left(\frac{\left(\frac{(u(D)}{D^2}\right)^2 + \left(\frac{\left(\frac{(3\pi\eta u(a_{bead}))}{(3\pi\eta a_{bead})^2}\right)^2}{\frac{1}{(3\pi\eta a_{bead})}}\right)^2}{\frac{1}{D} \times \frac{1}{(3\pi\eta a_{bead})}}\right)$$



# **Activity 2**

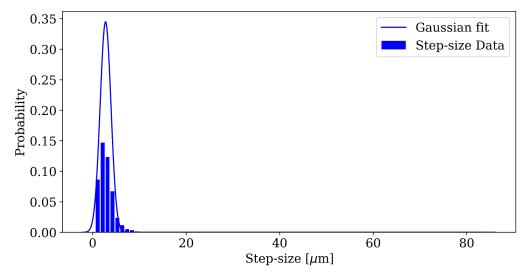


Figure 2: Probability distribution with Gaussian fit of step sizes per 0.5 s time interval of brownian motion-exhibiting microscopic bead across all data sets.

The diffusion coefficient D was calculated to be 1.34  $\times$  10<sup>-12</sup> as follows, where  $\bar{S}$  represents the mean of the 21 step data sets:

$$D = \left(\sqrt{\bar{S} - \frac{1}{2}} - \frac{1}{2}\right)^2 \times 10^{-12}$$

$$D = \left(\sqrt{(5.24) - \frac{1}{2}} - \frac{1}{2}\right)^2 \times 10^{-12}$$

$$D = 1.34 \times 10^{-12}$$

By substituting this value into Equation (8), and taking the suspending fluid viscosity to be  $1.0 \times 10^{-3}$  kg m<sup>-1</sup> s<sup>-1</sup>, the Avogadro constant was calculated to be  $(1.03 \times 10^{25} \pm 1.4) \times 10^{-2}$ , with the uncertainty for *D* propagating as follows:

$$u(D) = 10^{-12} \times \left( \sqrt{\left(\bar{S} - \frac{1}{2}\right)} - \frac{1}{2} \right)^{2} \times 2 \times \frac{\left( \sqrt{\left(\bar{S} - \frac{1}{2}\right)} \times \frac{\left(\frac{\left(\bar{S} - \frac{1}{2}\right)}{\sqrt{21}}\right)}{\left(\sqrt{\left(\bar{S} - \frac{1}{2}\right)} - \frac{1}{2}\right)}} \right)}{\left(\sqrt{\left(\bar{S} - \frac{1}{2}\right)} - \frac{1}{2}\right)}$$

$$u(D) = 10^{-12} \times \left( \sqrt{\left((5.243) - \frac{1}{2}\right) - \frac{1}{2}\right)^{2}} \times 2 \times \frac{\left(\sqrt{\left((5.243) - \frac{1}{2}\right)} \times \frac{\left(\frac{\left((5.243) - \frac{1}{2}\right)}{\sqrt{21}}\right)}{\sqrt{21}}\right)}}{\left(\sqrt{\left((5.243) - \frac{1}{2}\right)} - \frac{1}{2}\right)}} \right)$$

$$u(D) = 0.014$$

Upon calculating D and u(D), the Avogadro constant's uncertainty was propagated using the same method as in Activity 1:

$$\Rightarrow N_A = (1.03 \times 10^{25} \pm 1.4) \times 10^{-2}$$

## **Discussion & Analysis**

The more precise of the two respective estimations of the Avogadro constant from each activity,  $1.03 \times 10^{23}$ , is 82.9% accurate with respect to the known value of the Avogadro constant,  $6.02214076 \times 10^{23}$ . This suggests two possibilities: the first is that Einstein's model of Brownian motion does not apply to this experimental system, as 12% is a significant margin off of the true value. The second is that significant uncertainty in measurement and analysis, alongside lack of experimental control, contributed to this discrepancy. Either way, Einstein's theoretical framework describing Brownian motion was not verified by this experiment due to this significant discrepancy of the Avogadro constant of unknown precise origin.

Multiple sources of error arose from the physical set-up of the experiment. First, the microscope stage was slightly tilted. This had the effect of ruining data sets by reducing the negligence of the force of gravity on the microscopic beads, causing them to slide along straight trajectories as they simultaneously exhibited their brownian motion. This error could have been alleviated by ensuring beforehand that the microscope stage was level using a digital level measurement device.

Second, the camera used to capture the motion of the particles produced two-dimensional images. Because of this, Equation (2) was used to model the motion as opposed to its three-dimensional counterpart. This would not have been an issue if the motion of the observed particles was controlled to only occur in two measurable spatial dimensions. Instead, the motion in the third spatial dimension (along the axis pointing directly towards and away from the camera) was approximated to be negligible, despite

the thickness of the fluid being many times greater than the width of the beads. All of this means that there was almost certainty unobserved brownian motion along an unobservable axis that would have contributed to the  $r^2$  value yet could not be accounted for. This could have been fixed by forcing the motion of the beads to be fixed in two dimensions. This could be achieved by, say, thinning the fluid sample (as it was not controlled) or pressing the sample down using some transparent medium until the particles could not move towards or away from the camera lense.

The Image Object Tracker also contributed to error in this experiment. Given the inherently blurry nature of the images, the software had a hard time maintaining its tracking point in the centre of the particle of focus it was measuring, and would often go as far as change focus to a non-existent particle mid-analysis.

Finally, the decision to use a *linear* regression fit on the data in Activity 1 certainly significantly contributed to the experiment's error as it was the slope of the fit line that was used to calculate D for that activity. With a  $\chi^2_{reduced}$  value of  $\sim 1.71 \times 10^5$ , the model was a bad fit for the data set.

Overall, the error in this experiment was not statistical in nature but rather dominated by software bugs, physical hardware limitations, a poor fit, and lack of variable control.

#### Conclusion

This experiment could not verify Einstein's theoretical model describing brownian motion. The primary reason for this was a lack of experimental control regarding the particle's axes of motion, including unmeasured motion along a third axis of translation and drift caused by gravity acting down-slope on the fluid sample. In Activity 1, using the linear fit through mean squared displacement of the data recorded, the Avogadro constant was calculated to be  $(2.86 \times 10^{25} \pm 1.4) \times 10^{-2}$ , and likewise in Activity 2, using a Gaussian fit on a histogram of the recorded step data, it was calculated to be  $(1.03 \times 10^{25} \pm 1.4) \times 10^{-2}$ .