DETERMINING AVOGADRO'S NUMBER FROM THE RANDOM WALK OF MICROSCOPIC POLYSTYRENE BEADS

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Nathan C. Pierce
Department of Physics (Undergraduate)
University of Michigan - Dearborn
4901 Evergreen Rd, Dearborn, MI 48128
piercen@umich.edu

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ABSTRACT

Avogadro's Number N_A was determined experimentally by observing the Brownian motion of of 6 polystyrene beads and measuring the mean square displacement of each walker over time. A bead and water suspension was placed under a microscope where random walks were observed and recorded with a camera. The mean square displacement was determined directly from the particle trajectories. N_A was extracted from the best fit slope of the plot for the data: $N_A = (1.0 \pm 0.7) \times 10^{23}$. The true value N_A falls within the error of the obtained result.

1 Introduction

The botanist Robert Brown first described the phenomenon of Brownian motion in 1827 by observing pollen submerged in water. The motion is characterized by random fluctuations in a particles position within a fluid that is free of an overall drift, and is a key concept leading to the development of the Equipartition theory in thermodynamics.

Einstein conducted work which also examined Brownian motion. In 1905, he published four papers that transformed contemporary understanding of physics. Among this work was his paper on Brownian motion, which laid the theoretical groundwork for Jean Baptiste Perrin, who's experimental work verified the predictions made by Einstein, namely that observations of the mean square displacement of many particles suspended in a solution would allow calculating atomic dimensions. At the time, the atom was still regarded by many as a necessary mathematical fiction. Einstein's result relied on the acceptance of Maxwell-Boltzmann statistics and its relation to the molecular-kinetic theory of heat. Therefore, Perrin's work also had massive ramifications on thermodynamics - gases, solutions, and suspensions were modelled as if they were composed of many atoms, but prior to this experiment, their existence was not proven.

By measuring the mean square displacement $\langle x^2 \rangle$ of multiple particles undergoing Brownian motion, some physical parameters of the system can be determined, among them Avogadro's number, N_A. The goal of this experiment is to determine N_A using Einstein's theories in a manner similar to Perrin's. Modern technology greatly simplifies the laborious work that Perrin and earlier scientists completed by hand - but the concept is the same.

2 Theory

Einstein assumed suspensions behave as solutions do, i.e. that Brownian motion is a diffusion process [3]. The equation describing the motion of a particle subject to such diffusion through a fluid in the x direction is given by the partial differential equation:

$$D\frac{\partial^2 n}{\partial x^2} = \frac{\partial n}{\partial t} \tag{1}$$

Here, $n = n(x,t)$ and is the number of particles per unit volume around position x at time t. D is the diffusion constant, and is equal to:

$$D = \frac{RT}{N_A}\frac{1}{6\pi\eta a} \tag{2}$$

Here, R is the gas constant, T is the temperature, η is the viscosity, and a is the radius of the particles (spheres) in the suspension.

The solution $n(x,t)$ to Eq 1 appears in the integral form of the expected value for the mean square displacement. The integral form and the approximate solution is:

$$\langle x^2 \rangle = \frac{1}{n}\int x^2 n(x,t)dx \approx 2Dt \tag{3}$$

Substituting Eq 2:

$$\langle x^2 \rangle = \frac{RT}{3\pi N_A \eta a}t \tag{4}$$

In this equation, the following quantities can be measured: η, $\langle x^2 \rangle$, t, T, and a. R is also known. Therefore, Avogadro's number N_A can be solved for:

$$N_A = \frac{1}{\langle x^2 \rangle}\frac{RT}{3\pi\eta a}t \tag{5}$$

By measuring $\langle x^2 \rangle$ for various particle trajectories over time t (i.e., plotting Eq 4), a linear plot can be generated if enough random walks are tracked. The linearity of the displacement squared with time implies that displacement is (on average) proportional to the square root of time. The linearity of random walk displacement with time means we can use the slope of best fit m to simplify Eq 4 to:

$$\langle x^2 \rangle = mt \tag{6}$$

Where m must be equal to the constant factor $m = \frac{RT}{3\pi\eta a N_A}$. Solving for Avogadro's number, N_A:

$$N_A = \frac{RT}{3\pi\eta a m} \tag{7}$$

Finally, the colloidal motion of a particle in a fluid is characterized by a very large number of collisions per second. Water molecules at room temperature undergo around 10^{11} collisions per second[2]. In such a scenario, the motion of a particle can be treated as a sequence of independent events.

3 Methods

The experimental setup consisted of two primary components: a Digital Monocular Microscope KEN-A-VISION T19542CP, and a Point Grey Firefly MV 0.3 MP FireWire camera. The camera was attached to a tripod, which was clamped and fastened to a heavy stand. By this arrangement, the camera was held in place over the ocular lens. The camera view was displayed on a PC, where video could be recorded at 60 frames per second (fps). The camera's resolution is 640 x 480 pixels. Using a K-Type thermocouple and Keithley 2000 DMM, the room ambient temperature (the suspension was soaked in this temperature) was recorded at the time of measurement to be $T = 294.05\,\mathrm{K}$. See Fig 1 for a line drawing of the apparatus.

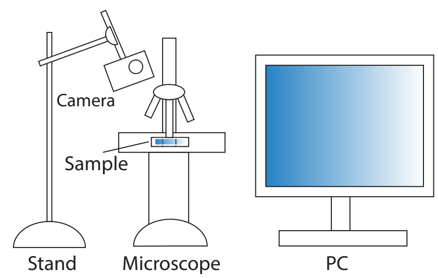

Figure 1. Line diagram of the experimental setup.

The particles used are $1\,\mu\mathrm{m}$ (diameter) polystyrene beads suspended in a water suspension. The exact concentration is unknown, although it is fair to approximate the number of beads being much less than the number of water molecules. Two drops of the suspension contained enough beads to easily fit many beads within the field of view of the microscope. The suspension was stored at room temperature for an unknown time prior to use in this experiment. This may have been a contributing factor to the clustering of beads together. However, this was not a significant problem in the experiment, as only a few clusters were observed.

ImageJ/Fiji was used to track the particles using a plugin called Mosaic. Before any particle tracking could be done, the camera frames needed to be spatially calibrated. This could be done by capturing an image of a calibration slide under the scope (Fig 3). It is important that the image be captured using the same objective lens used in tracking the particles. This lens was the Plan Achromatic Objective 100X (100/0.65). A line could be drawn between consecutive divisions in ImageJ/Fiji to obtain the pixel count. A conversion factor of $0.65 \frac{\mu m}{pixel}$ was found.

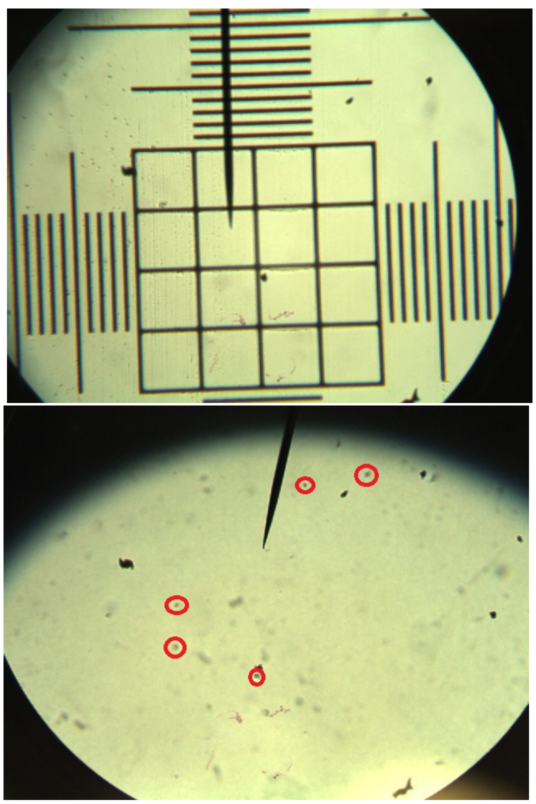

Figure 2. $\underline{Top}$: View of the calibration slide under 100X magnification. Each division is $0.01\,\mathrm{mm}$. $\underline{Bottom}$: Raw image of the polystyrene beads under the microscope (circled in red). Note that not all of the beads tracked in the experiment are outlined

Glass slides with a single concave depression were used. This aids in preventing convection within the sample. The suspension was stirred, then a stirring rod was used to transfer two drops to the divot. A cover slip was then placed over the divot and pressed into the divot to create a seal. Care was taken to prevent the formation of any air bubbles within the divot. The stage position was adjusted until the beads came into focus (3). The motion of the beads was monitored to ensure that there was no overall drift to the particles, as it was necessary to ensure Brownian motion was being observed. Some samples were discarded because drift was observed. Additionally, a large number of detected particles within the field of view is preferred. For this experiment, 6 particles were caught within the view of the microscope. Once a suitable sample was observed, footage was recorded at 60 fps for $40\,\mathrm{s}$.

The video was saved in an .avi format. The video file was decompressed into an ordered sequence of frames in the form of .jpgs. This was achieved using VLC. The image sequence was then imported into ImageJ/Fiji. Additionally, every 12th frame was saved - the others were discarded. In all, 200 frames were collected, separated by $0.2\,\mathrm{s}$ intervals. However, only the first 116 of those frames are used in the analysis, as it was found that one of the particle trajectories was lost by the particle linking algorithm at frame 117.

The plugin Mosaic was used for the particle tracking. Mosaic had difficulty detecting the particles for the unprocessed, raw frames from the video. To correct this, the image sequence was converted to grayscale and then inverted, since Mosaic looked for white particles on a black background by default. Some time was taken to adjust the particle detection and particle linking settings within mosaic to ensure that all particles recorded in the sample image sequence were being detected (see the caption of Fig 3 for these parameters), and that particles would be properly tracked for most of the image sequence. After finding the suitable detection/linking parameters, ImageJ/Fiji generated data for the trajectory of each detected particle. Only the data for those detected particles which appeared to randomly walk were saved. Over the course of the experiment, some hydrodynamic effects were observed, such as some particles adhering to the cover slip. Some beads also were clumped together, affecting the motion. The radius parameter of the particle detection algorithm allowed the filtering out of such interacting beads, since it set a maximum limit on the size for each spot on the image it would consider to be a particle. All trajectories that Mosaic generated were sifted through by hand to ensure the data was representative of single beads undergoing random walks.

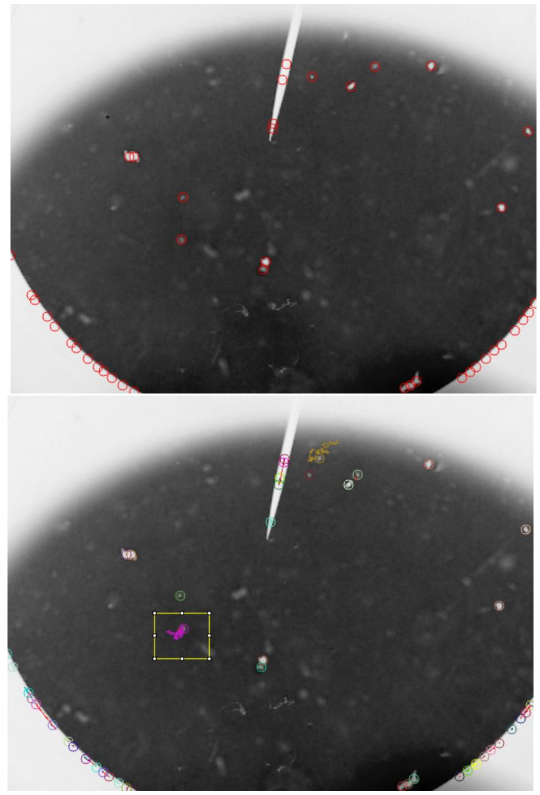

Figure 3. *Top* : Preview image of the detected particles. The red circles indicate the particles detected using the user provided particle detection parameters (Radius = 5, Cutoff = 0.001, Per/Abs = 4 was used for this experiment). *Bottom* : Image displaying some of the particle trajectories. The colored lines indicate trajectories of particles undergoing a random walk through the fluid. Note these previews do not show all of the trajectories/particles analyzed in the experiment. Color spots without lines indicate a particle was detected by the algorithm but no trajectory could be traced out (likely a false-positive on a detected particle).

The trajectory information for the 6 different particles were saved in the form of 6 .csv files containing pixel data. The row index represented the time index, $t = n\Delta t$ where n

is the nth row in the file, and $\Delta t = 0.2\,\mathrm{s}$. This time index constitutes the t value in the mean square displacement function. Both Δx and Δy displacements are considered. The pixel data was converted to μm. The first measurement at $t = 0$ was used to set the origin of each particles displacement. The mean square displacement was then calculated numerically:

$$\langle x_j^2 \rangle = \frac{1}{N} \sum_{i=1}^{N} |x_{i,j}(t) - x_i(0)|^2 \tag{8}$$

Where the number of particles $N = 6$, and the index j is the time index (from 0 to 116). Note that $x_i(0)$ is the reference position of the ith particle. Using the square of the mean displacement ensures that the average does not approach 0, since roughly half of displacement will be negative with respect to the reference position, and half positive. Both $\langle x(t)^2 \rangle$ and $\langle y(t)^2 \rangle$ was then plotted. Finding the best fit slope for the line was accomplished using SciPy's optimize.curve_fit function. A linear trend is expected by Eq 4. Finally, the average slope of best fit for $\langle x^2 \rangle$ and $\langle y^2 \rangle$ was used to determine N_A.

4 Results and Discussion

The objective and condenser lenses on the microscope should have been cleaned prior to video recording. A quick look at Fig 3 shows plenty of dust and other debris in the field of view. This complicated detecting and tracking particles in ImageJ/Fiji. While all of the beads could be detected, some of the trajectories were lost over the frame sequence, as described in Sec 3. The particles that passed into the splotches caused by dust and debris (even if only for a brief time) would sometimes be lost by the tracking algorithm. This resulted in the loss of good data points, and is particularly crucial since only one sample (with 6 particles total) was recorded and analyzed.

The slope of best fit m of the plot $\langle x(t)^2 \rangle$ vs. t is the key to determining Avogadro's number N_A by the methods of this experiment. See Fig 4 for this plot. The slope of the best fit line is forced to go through the origin. With m, we can solve for N_A directly with Eq 7. The calculation requires knowledge of several parameters. Here, $\eta = 1.00 \times 10^{-3}\,\mathrm{Pa\,s}$, $R = 8.314\,\mathrm{J\,mol^{-1}\,K^{-1}}$, $a = 5 \times 10^{-7}\,\mathrm{m}$, and $T = 294.05\,\mathrm{K}$ (as measured by a K-Type thermocouple connected to a Keithley 2000 DMM).

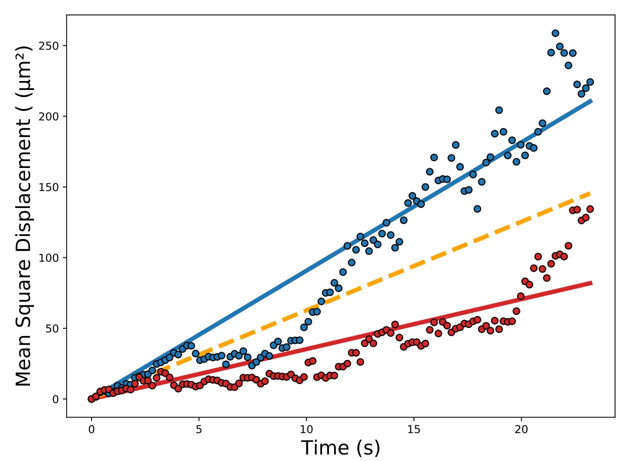

Figure 4. Plot of the mean square displacement for 6 different $1\,\mu\mathrm{m}$ beads as a function of time. The red points/line represent the y direction, and the blue points/line represent the x direction. The dashed orange line is the averaged slope for x and y. A linear trend is predicted.

The results of the tabulation are given in Tab I. The mean square displacement for displacement of the beads in the x and y directions are given in Fig 4. The trend in the mean square displacement vs. t plot is strongly linear over the five seconds of measurement for both of the plots. The displacement from the reference position for these lower values of t must be small. As time passes, the motion of each particle can take any random path - some particles will walk back towards their reference point while others continue to move away. Over short time scales, the inertia of the bead should drive its displacement linearly in t. It is not clear over which timescales a momentum shift should occur - while many collisions occur per second, the bead is much more massive than the water molecules it is colliding against.

Perhaps the most noticeable feature of the plot is the difference in the slopes for $\langle x^2 \rangle$ and $\langle y^2 \rangle$. It might be expected that the slope which accurately predicts Avogadro's number N_A is somewhere between the two lines. However, a look at Tab I shows this is not the case, since the slope should be $less$ than the slope for $\langle y^2 \rangle$, because the obtained N_A is about one order of magnitude less than the accepted value, and the slope term m appears in the denominator in Eq. 7. The results obtained by Newburgh et. al. in their work on Brownian motion also suggests this is the case [1].

While it can be inferred that the mean square displacement in the y direction is the preferred data because it produces a more accurate result, the other data (x data) cannot be ignored. Because $\langle y^2 \rangle$ and $\langle x^2 \rangle$ are independent, their average can be used as the experimental conclusion for Avogadro's number: $N_{A,Experimental} = (1.0 \pm 0.7) \times 10^{23}$. While the uncertainty is large due to the small sample size of particles $N = 2$, the accepted value does fall within the error of the experimentally determined value. The accepted value is $N_{A,Accepted} = 6.022\,13 \times 10^{23}$.

Dimension	m ($\frac{m^2}{s}$)	$N(10^{23})$
$\langle x^2 \rangle$	$(9.006 \pm 0.016) \times 10^{-12}$	$(5.760 \pm 0.010) \times 10^{22}$
$\langle y^2 \rangle$	$(3.530 \pm 0.011) \times 10^{-12}$	$(1.47 \pm 0.05) \times 10^{23}$
$\langle r^2 \rangle$	$(6.27 \pm 0.04) \times 10^{-12}$	$(1.0 \pm 0.7) \times 10^{23}$

TABLE I. The mean square displacement in a given dimension, the slope of the best fit line from mean square displacement vs. t, and the estimate of Avogadro's number N using Eq 7. $\langle r^2 \rangle$ is the average of the $\langle x^2 \rangle$ and $\langle y^2 \rangle$ values.

Perrin observed 200 particles in his experiment, where he obtained $N_A = 7.15 \times 10^{23}$ particles. This experiment tracks only 6 particles. The reliance on statistical methods suggests strongly that increasing the number of particles tracked will produce a slope which more accurately predicts the accepted value for Avogadro's number. Time constraints prevented the tracking of more particles. To track more particles, more samples would need to be prepared, and each sample would require the repetition of the experimental method all over again - quite a time consuming process, despite the immense time-saving that offloading particle tracking to an algorithm affords. Newburgh et. al. also used 200 particles, and arrived at a value much closer than that determined here.

5 Conclusion

Avogadro's number was obtained by measuring the mean square displacement of microscopic random walkers, just as Einstein predicted should be possible. Furthermore, the experimental result agrees with Einstein's physical explanation for Brownian motion. Admittedly, the experimental result could be improved by tracking more particles, but the experimental result is off by a single digit factor. Perrin's experiment was successfully emulated using modern technology.

References

[1] Newburgh, Ronald, et al. Einstein, Perrin, and the Reality of Atoms: 1905 Revisited. AAPT, 13 July 2015.

[2] Nakroshis, Paul, et al. Measuring Boltzmann's Constant Using Video Microscopy of Brownian Motion. AAPT, 6 Dec. 2002.

[3] A. Pais, Subtle Is the Lord (Oxford U. P., New York, 1982), pp. 88–92.