

# Measuring Avogadro's Number Using Brownian Motion

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## ABSTRACT

We determine Avogadro's number by measuring the mean-squared displacement of colloidal particles 100 nm and 1  $\mu\text{m}$  in size suspended in water. This is essentially a reproduction of the experiment for which Jean Perrin won the Nobel Prize in Physics in 1926. However, we also attempt to explore the effect of solvent particle size on Brownian motion by suspending the particles in glycerol and polyvinylpyrrolidone (PVP) at viscosities of  $\eta = 0.0025, 0.0083,$  and  $0.250 \text{ kg m}^{-1} \text{ s}^{-1}$ .

## 1. Introduction

At the turn of the 20<sup>th</sup> century, the concept of the atom was not universally accepted as a physical reality. It was not until Albert Einstein wrote a paper in 1905 showing that the microscopic motion of particles suspended in solution could be explained in the context of the molecular-kinetic theory of heat and Jean Perrin experimentally confirmed Einstein's predictions that the existence of the atom was firmly established.

Before Einstein, it was thought by some that the microscopic random motion of *colloidal particles* are a result of the collisions between the particles and the thermally agitated solvent molecules. The opponents of this explanation contested that the minuscule size of the solvent molecules in comparison to the particles could not yield a change in position large enough to be perceptible and even if they could the extremely high collision rate ( $\sim 10^{12}$  for water molecules) would not allow an observer to resolve the events.

Einstein realized that although each individual displacement is too small and occurs too quickly to be detected, an observer is able to see the larger displacements that occur when a series of collisions pushes the particle in one given direction. These larger displacements are rare because the solvent molecules collide with the colloidal particle randomly from every direction. In other words, they occur less frequently and at a rate which can be resolved by an observer. Using these key arguments, Einstein reasoned that the colloidal particles are executing a random walk and he predicted that their mean-squared displacement to be given by the Einstein relation

$$\langle x^2 \rangle = \frac{RT}{3\pi\eta N_A r} t \quad (1)$$

where,  $R$  is the ideal gas constant,  $T$  is the temperature,  $\eta$  is the viscosity of the solution,  $N_A$  is Avogadro's number,  $r$  is the radius of the particle, and  $t$  is the sample time.

The Einstein relation is crucial because it provided a method for measuring  $N_A$  not just  $k_B N_A$ , where  $k_B$  is the Boltzmann constant. Avogadro's number is the quantity that dictates the size of atoms and molecules. Thus, the existence of atoms was established when Perrin used Eq. (1) to find that  $N_A$  is in fact a very large number. In this experiment, we follow Perrin and examine the validity of Eq. (1) using 100 nm gold nanoparticles and 1  $\mu\text{m}$  polystyrene spheres in a total of seven solutions of varying viscosity for each particle. We will record the Brownian motion of the particles and compute the mean-squared displacement for several sample rates. Then we plot  $\langle x^2 \rangle$  as a function of  $t$  with error bars and perform a weighted least-squares linear regression. From the slope of the regression line, we are able to compute the value of  $N_A$ .

## 2. Theory

### 2.1. Random Walk

The ideas of a random walk are fundamental to the discussion of Brownian motion so we discuss them in some detail here. We begin with a one-dimensional random walk. Suppose we have a particle located at an origin of the real line at time  $t = 0$ . Next, suppose that after some discrete time interval  $\Delta t$ , the particle can either move to left or right in a discrete step of physical length  $\Delta s$ . If the particle is allowed to take a total of  $N$  steps, then the particle displacement is given by  $X = (R - L)\Delta s = (2R - N)\Delta s$ , where  $R$  is the number of steps taken to the right and  $L$  is the number of steps taken to the left. Where is the particle after time  $t$  has elapsed?

The number of steps to the right  $R$  is discrete random variable whose distribution is binomial( $N, p$ ), where  $N$  is the number of trials and  $p$  is the probability of success. The expectation value of any random variable that follows a binomial( $N, p$ ) distribution is  $\mu = Np$  and the variance is  $\sigma^2 = Np(1 - p)$ . Since  $R$  follows binomial( $N, 1/2$ ) distributions,  $\langle X \rangle = 0$ . This is equivalent to saying that we expect to find the particle near the origin. However we will show that  $\langle X^2 \rangle \neq 0$ , which indicates that the particle is not stationary at the origin but rather moving randomly about.

By the definition of variance, it can be shown that the variance of any random variable  $A$  is given by

$$\sigma^2 = \langle A^2 \rangle - \langle A \rangle^2 \quad (2)$$

For a binomial( $N, 1/2$ ) random variable we have

$$\langle A^2 \rangle = \sigma^2 + \langle A \rangle^2 = Np(1 - p) + N^2 p^2 = \frac{N}{4}(1 + N)$$

The expectation value of  $X^2$  is

$$\begin{aligned} \langle X^2 \rangle &= \langle (2R - N)^2 (\Delta s)^2 \rangle \\ &= (\Delta s)^2 \langle 4R^2 - 4NR + N^2 \rangle \\ &= (\Delta s)^2 [4\langle R^2 \rangle - 4N\langle R \rangle + N^2] \\ &= (\Delta s)^2 [N(N + 1) - 2N^2 + N^2] = N(\Delta s)^2 \end{aligned}$$

The number of steps taken by the particle is not a measurable physical parameter. We write  $N$  in terms of the sample time  $t$ , which *is* physically measurable. The number of steps taken by a particle in time  $t$  is  $N = t/\Delta t$ . There is no reason for us to know the physical step size of the particle  $\Delta s$  or the time between steps  $\Delta t$  a priori. We choose to define the *diffusion coefficient* of the random walk to be

$$D \equiv \frac{(\Delta s)^2}{2\Delta t} \quad (3)$$

The diffusion coefficient hold all the physical information of the random walk. Making the substitution for the diffusion coefficient, the equation for the mean square displacement of a particle undergoing a one-dimensional random walk is

$$\langle X^2 \rangle = 2Dt \quad (4)$$

Our random walk is two-dimensional so we extend this result to two-dimensions. Let  $Y$  be the random variable that represents the vertical displacement of a particle in the Euclidean plane. The particle's position is given by  $(X, Y)$  or  $\mathcal{R}^2 = X^2 + Y^2$ . We assume that the random variables  $X$  and  $Y$  are independent i.e. the two dimensions are independent. If  $X$  and  $Y$  are independent random variables, then the expectation of the same is just the sum of the expectation. The mean square displacement for a particle undergoing a two-dimensional random walk is given by

$$\langle \mathcal{R}^2 \rangle = \langle X^2 + Y^2 \rangle = \langle X^2 \rangle + \langle Y^2 \rangle = 4Dt \quad (5)$$

## 2.2. Einstein Relation

We have derived a general equation that applies to any two-dimensional random walk process. To make things more physical, we would like to know how the diffusion coefficient varies as a function of particle size, solution viscosity, and temperature. Thus far, it is a function of the intrinsic physical step size  $\Delta s$  and the time between collisions  $\Delta t$ , which are not macroscopically measurable quantities. We eliminate  $\Delta s$  and  $\Delta t$  and find an expression for  $D$  in terms of the particle size, solution viscosity, and temperature.

Return to the one-dimensional random walk. Suppose that one collision occurs per  $\Delta t$ . The motion of the particle after a single collision is

$$\Delta x = v_0 \Delta t + \frac{F}{2M} (\Delta t)^2$$

where  $v_0$  is its random initial velocity,  $F$  is the magnitude of average force during the collision, and  $M$  is the mass of the particle. The average value of  $\Delta x$  is  $\langle \Delta x \rangle = F/2M(\Delta t)^2$  because we assume that  $\langle v_0 \rangle = 0$  by assuming each collision is independent and the initial velocity vector is equally likely to be pointing in either direction. Thus, the particles gain a net *drift velocity*

$$v_d = \frac{\langle x \rangle}{\Delta t} = \frac{F\Delta t}{2M} = \frac{F}{\zeta} \quad (6)$$

where

$$\zeta = 2M/\Delta t \quad (7)$$

is the *viscous friction coefficient*. The Stokes Formula gives us the viscous friction coefficient in terms of the viscosity of the solution ( $\eta$ ) and the radius of the particle ( $r$ ).

$$\zeta = 6\pi\eta r \quad (8)$$

Note that  $\zeta$  is a macroscopically measurable quantity because it is a function of viscosity and particle size.

Combining Eq. (3) and Eq. (7) yields

$$D\zeta = \frac{(\Delta s)^2 M}{(\Delta t)^2} \quad (9)$$

Einstein's stroke of genius was to notice that from the molecular-kinetic theory of heat

$$\langle v_0^2 \rangle = \left\langle \frac{(\Delta s)^2}{(\Delta t)^2} \right\rangle = \frac{(\Delta s)^2}{(\Delta t)^2} = \frac{k_B T}{M} \quad (10)$$

Combining Eqs. (7), (9), and (10) we get

$$D\zeta = k_B T = \frac{RT}{N_A} \quad (11)$$

Thus, we have an expression for  $D$  that shows it depends on particle size and solution viscosity. Substituting Eq. (11) into Eq. (5) gives the Einstein Relation in Eq. (1). We now have all the ingredients for a Nobel Prize winning experiment.

### 3. Equipment

The main piece of equipment used in this experiment is the Zeiss Aviovert 200 microscope. Attached to the microscope is a CCD camera that captures the motion of the particles under the microscope. The output of the CCD camera is sent to a data processing unit. The data processing unit consists of a Dell computer and a movie making program called Octopus111. The Octopus111 front-end allows the user to specify the number of frames to be captured and the frame rate. The movies are specifically designed to be compatible with Matlab data analysis software. The experimental setup is shown in a block diagram in the Appendix.

## 4. Observations

### 4.1. CCD Plate Scale

Octopus111 and the Matlab tracking software returns pixel positions of the particles. We would like to translate this to physical displacement. Thus, we need to measure the pixel to nanometer

conversion for the CCD. We use the 20x objective on the microscope for all observations. We prepare a slide with  $9.86 \pm 0.65 \mu\text{m}$  polystyrene spheres. We measure the pixel diameter of five different polystyrene spheres: 30, 27, 29, 27, 29 pixels. The mean and standard deviation of these values give us a CCD plate scale of  $347.18 \pm 1.3416 \text{ nm pixel}^{-1}$ .

## 4.2. Making Slides

For the experiment, two particle sizes are used: 100 nm gold particles and 1  $\mu\text{m}$  polystyrene spheres. For each particle size we select seven different solvents of different viscosity. The solutions are water, three different viscosity glycerol solutions, and three different viscosity PVP solutions. Table 1 lists the viscosity of the different solutions.

	H <sub>2</sub> O	PVP 1	PVP 2	PVP 3	Gly 1	Gly 2	Gly 3
$\eta$ (Pa s)	$10^{-3}$	0.0025	0.25	0.0083	0.0025	0.25	0.0083

Table 1: The viscosities of the seven solutions used in the experiment.

Slides of each particle in each solution are prepared. The preparation of the slides differs slightly for the two particle sizes. The 100 nm gold particles are so tiny that a buffer of 10 $\mu\text{m}$  polystyrene spheres must be used in order for the gold particles to but freely suspended in solution. After the coverslip is applied, the edges of the slide are cover with nail polish to prevent the solution from evaporating and causing a net flow.

## 4.3. Making Movies

The slides are placed under the microscope and we setup Kohler illumination with the 20x objective. The 1  $\mu\text{m}$  polystyrene spheres are viewed under light-field illumination (dark particles, bright background). The 100 nm gold particles cannot be view under these condition because their physical size is much smaller than the wavelength of visible light. We must use a technique called dark-field illumination(bright particle, dark background) to resolve the tiny gold particles.

The motion of the particle varies depending on the solution. We vary the total movie time and frame rate to what we believe is optimal for each condition. Table 2 is a log of the number of movies, total time, and frame rate for each condition.

Size (nm)	Solution	Frame rate (ms)	# Frames	# Movies
1000	H <sub>2</sub> O	200	50	10
1000	PVP1	500	20	10
1000	PVP2	1000	20	10
1000	PVP3	100	50	5
1000	Gly1	200	50	10
1000	Gly2	500	20	10
1000	Gly3	100	50	10
100	H <sub>2</sub> O	100	10	10
100	PVP1	100	40	10
100	PVP2	500	20	7
100	PVP3	100	50	11
100	Gly1	100	40	12
100	Gly2	100	40	11
100	Gly3	100	50	12

Table 2: A log of the data we took for each particle in each solution.

## 5. Analysis

### 5.1. Do It All

Once we have all the videos for each particle, we use a Matlab program provided to us called DOITALL to extract the pixel position of each particle in each video. The program first filters the raw movie based on two parameters: noise diameter and object diameter. Next the program takes the filtered movie and creates a bi-level movie where there are only two pixel values. This part of the program depends on the parameters standard deviation and minimum deviation. Next, the program does a clean-up on the edges based on the parameter minimum size. This portion is here because we may want to disregard certain particles if they get too close to the edges of the movie. Next, the program finds the pixel position of the particles by centroiding. Lastly, the program produces a track for each particle by logically connecting the positions from one frame to the next based on the parameter maximum displacement. The output of the program is a cellular matrix where each column represents the track of a single particle through the entire movie. Each cell contains the  $(x_i, y_i)$  pixel position of the particle in the  $i^{th}$  frame. We can extract the track of a single particle from this cellular matrix using the Matlab function CELL2MAT.

## 5.2. The Diffusion Coefficient

From the track of the particles, we compute the mean-squared displacement for a given time interval for each particle size in each solution. This is done for multiple time intervals and we plot the mean-squared displacement as a function of the time interval. We see from Eq. (5) that the slope of this plot will give us the diffusion coefficient for a particular particle size in a particular solution. We write our own Matlab program called DIFFUSIONPLOT to do this.

The first parameter DIFFUSIONPLOT accepts is the root name of a series of movies for a given particle size in a given solution. Each movie is named by the root and some integer. Thus, we allow DIFFUSIONPLOT to take a maximum and minimum integer value. This is here so that it can iterate through an entire series of videos. We pick a set of appropriate values for DOITALL and DIFFUSIONPLOT performs DOITALL on each video in that series.

At the end of each iteration, DIFFUSIONPLOT concatenates the output cellular matrix with the particle tracks to the previous iteration’s output cellular matrix. The result is a master cellular matrix with the particle tracks for each particle in each movie for a given particle size in a given solution.

Some of the particles do not have a continuous track through the entire movie because they may go out of focus or move off the screen. These appear at empty cells in the cellular matrix. DIFFUSIONPLOT replaces the empty cells with [-99,-99]. This is done so that the particle tracks remain consistent when using CELL2MAT. If this were not done, CELL2MAT throws away the empty cells and all information about the time interval between particle positions is lost.

Next, DIFFUSIONPLOT calls on a squared displacement computation program we wrote. It runs through each particle track at a given time step and computes the squared displacement in pixels. DIFFUSIONPLOT then computes the average value of these squared displacements ( $\langle x^2 \rangle$ ) in pixels and the standard deviation of the mean ( $\sigma$ ). The values are saved in an array. The squared displacement computation program disregards a computation if either point is [-99,-99]. This is repeated for different time intervals and the result is an array with time interval, mean-squared displacement in pixels, and standard deviation of the mean in pixels.

The  $(t, \langle x^2 \rangle, \sigma)$  arrays are extracted for each particle in each solution. We use IDL to plot  $\langle x^2 \rangle$  versus  $t$  with error bars given by  $\sigma$ . The  $\sigma$  values are bigger for larger values of  $t$  since the standard deviation of the mean goes as  $\sigma_{\bar{x}}/\sqrt{N}$ , where  $N$  is the number of data points. There are more data points for smaller  $t$  values so the uncertainty in the mean is smaller. The values of  $\langle x^2 \rangle$  are converted to meters using the CCD plate scale. Twelve percent is added to  $\sigma$  due to the uncertainty in the pixel to meter conversion and the uncertainty in particle size. A weighted linear-regression is performed on the data using IDL.

The weighted linear-regression is computed as follows. The data is fit to a line of the form  $y = A + Bx$ , where each  $y_i$  has a different uncertainty  $\sigma_i$ . Weights  $w_i = 1/\sigma_i^2$  are introduced. The smaller the uncertainty, the more the point is weighted. The slope of the linear-regression line and

its error is given by

$$\begin{aligned}
 B &= \frac{\sum w \sum wxy - \sum wx \sum wy}{\Delta} \\
 \sigma_B &= \sqrt{\frac{\sum w}{\Delta}} \\
 \Delta &= \sum w \sum wx^2 - (\sum wx)^2
 \end{aligned}$$

This is done for each particle in each solution. The slope of the weighted least squares line yields the diffusion coefficient  $D$ . From the diffusion coefficient, Avogadro's number  $N_A$  is computed from from

$$D = \frac{RT}{6\pi N_A \eta r} \quad (12)$$

using a value of  $T \approx 300$  K for room temperature. The percent error of our experimental values from  $N_A = 6.02 \times 10^{23}$ . The results are listed in Table 3. The plots are shown at the end of the the paper.

Solution/Particle	$D$ ( $\text{m}^2 \text{s}^{-1}$ )	Fractional Error[%]	$N_A$ (atoms $\text{mole}^{-1}$ )	% error
Water/Gold	$(3.37 \pm 0.04) \times 10^{-12}$	1.05	$(5.89 \pm 0.07) \times 10^{23}$	2.16
Water/PS	$(5.68 \pm 0.4) \times 10^{-13}$	3.2	$(3.49 \pm 0.1) \times 10^{23}$	42.03
PVP1/Gold	$(1.17 \pm 0.04) \times 10^{-12}$	31.3	$(6.73 \pm 0.2) \times 10^{23}$	11.79
PVP1/PS	$(1.85 \pm 0.1) \times 10^{-13}$	5.95	$(4.27 \pm 0.2) \times 10^{23}$	29.07
PVP2/Gold	$(1.08 \pm 0.3) \times 10^{-14}$	31.30	$(7.29 \pm 2) \times 10^{23}$	21.10
PVP2/PS	$(2.93 \pm 0.2) \times 10^{-14}$	5.45	$(2.7 \pm 0.1) \times 10^{22}$	95.51
PVP3/Gold	$(1.94 \pm 0.05) \times 10^{-13}$	2.6	$(1.22 \pm 0.03) \times 10^{24}$	102.66
PVP3/PS	$(6.92 \pm 0.3) \times 10^{-14}$	4.69	$(3.43 \pm 0.2) \times 10^{23}$	43.02
GLY1/Gold	$(1.66 \pm 0.05) \times 10^{-12}$	3.26	$(4.77 \pm 0.2) \times 10^{23}$	20.76
GLY1/PS	$(8.67 \pm 0.3) \times 10^{-14}$	2.89	$(9.14 \pm 0.3) \times 10^{23}$	51.83
GLY2/Gold	$(1.64 \pm 0.06) \times 10^{-12}$	3.63	$(4.83 \pm 0.2) \times 10^{21}$	Huge
GLY2/PS	$(1.82 \pm 0.1) \times 10^{-13}$	5.78	$(4.35 \pm 0.2) \times 10^{21}$	Huge
GLY3/Gold	$(2.75 \pm 0.08) \times 10^{-13}$	2.93	$(8.63 \pm 0.3) \times 10^{23}$	43.36
GLY3/PS	$(8.72 \pm 0.3) \times 10^{-14}$	3.55	$(2.72 \pm 0.1) \times 10^{23}$	54.82

Table 3: These are value we computed for the diffusion coefficient for each particle in each solution and the value for  $N_A$  we computed using those values.



## 6. Discussion

### 6.1. Avogadro’s Number

Our experimental values for Avogadro’s number are quite good, with four values that agree with the accept value  $N_A = 6.023 \times 10^{23}$  atoms mole<sup>-1</sup> within approximately 20%. The other values are approximately the correct order of magnitude. The only aberrant value is that of both 100 nm and 1  $\mu$ m in Gly2. We attribute these deviations to be due in large part to human error in mixing the solutions. This is likely because the easiest solution to work with (water) yields the best result for  $N_A$ . There also exists the possibility that there were mistakes made by DOITALL when identifying particle tracks. However, we do not expect this to be a big factor, although a verification of this is needed, because of the large number of data points we have and the fact that most of our movies only contain one or two particles.

### 6.2. Distinguishing Solvent Molecule Size

The diffusion coefficient equation we gave in Eq. (12) does not explicitly show that the diffusion coefficient should depend on the size of the solvent molecule. We test this prediction by plotting the diffusion coefficient versus the viscosity for a single particle size for both PVP and glycerol. These are shown in Figure 1 and Figure 2. However, we notice immediately that the data for glycerol is quite bad. For a given particle size in at a fixed temperature, the relationship between  $D$  and  $\eta$  should be an inverse relationship. The plots for both particle size in glycerol do not indicate this relationship in the slightest. This strengths our conjecture that the error was human. It is possible that the mixed solutions did not have the intended viscosity.

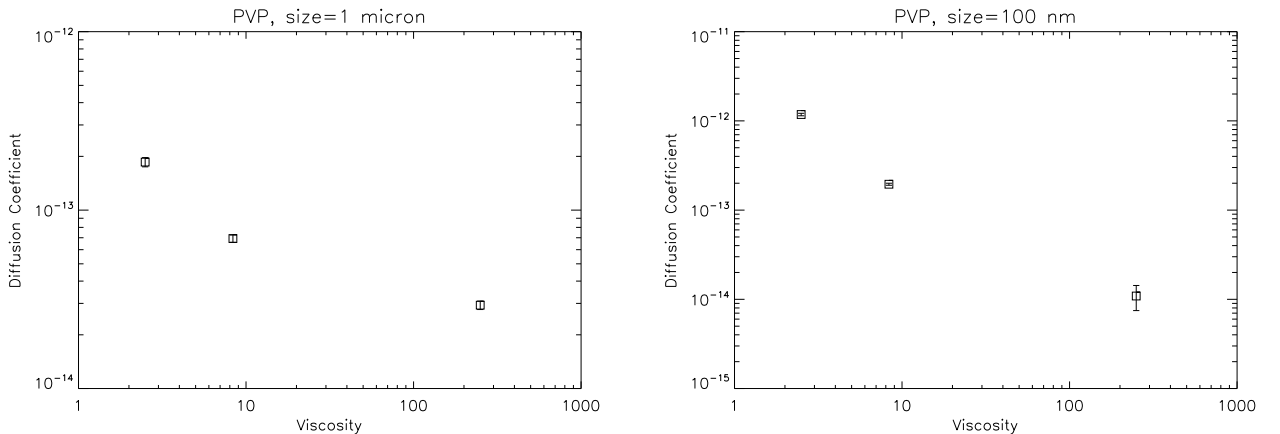


Fig. 1.—  $D$  versus  $\eta$  for the PVP solution.

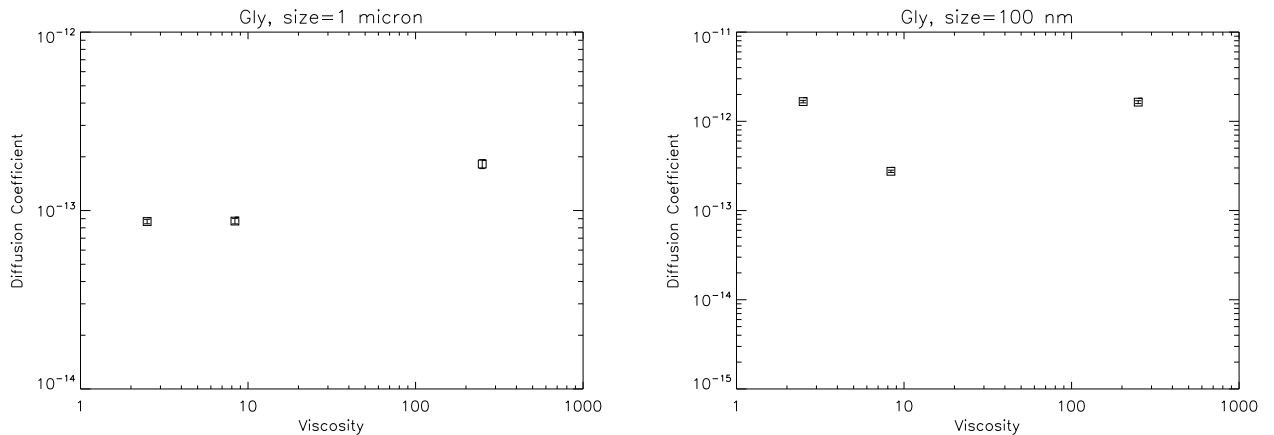


Fig. 2.—  $D$  vs.  $\eta$  for the glycerol solution.

## 7. Conclusion

This experiment was intended to reproduce Perrin’s experiment and to test Einstein’s prediction about the atomic nature of matter. Our results indicate that the Brownian motion of particles is dictated by the equation Einstein set forth in Eq. (1) i.e. Einstein was right! After performing this experiment, we have gained an appreciation of the perspicacity of Einstein’s reasoning. We have even obtained a feeling of the power and reach of Einstein’s result after viewing an onion cell under the microscope. We could see the millions of tiny microtubules transporting molecules within the cell. It is simply amazing that cells can do anything when their underlying motion is random.

## 8. Acknowledgements

I would first and foremost like to thank Scott Woody for hacking being a super potions master and hacking away at the Matlab code. I would also like to thank Steve for giving us invaluable guidance during the entire duration of the experiment.

## 9. References

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 A. Einstein, *On the Motion of Small Particles Suspended in Liquids*  
 R. Newburgh, J. Peidle, *Einstein, Perrin and the reality of atoms*

## 10. Appendix

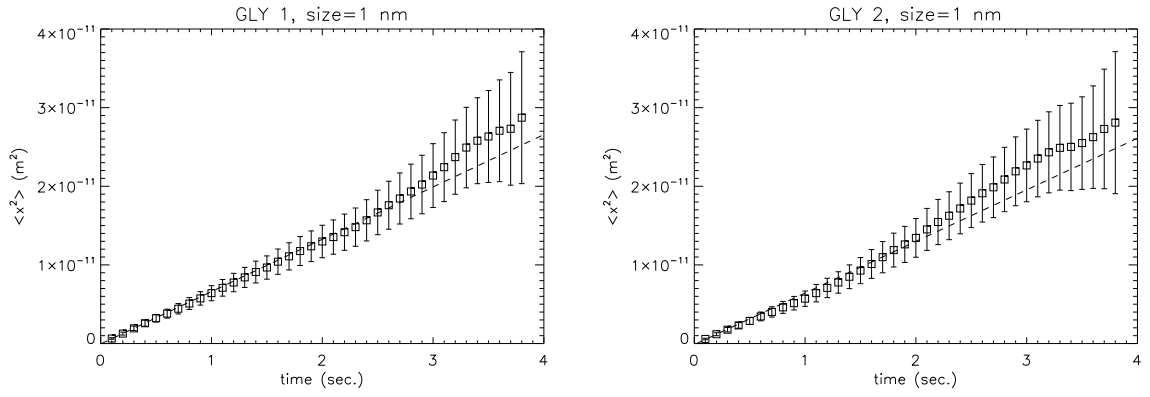


Fig. 3.— 100 nm particles in Gly1 and Gly2.

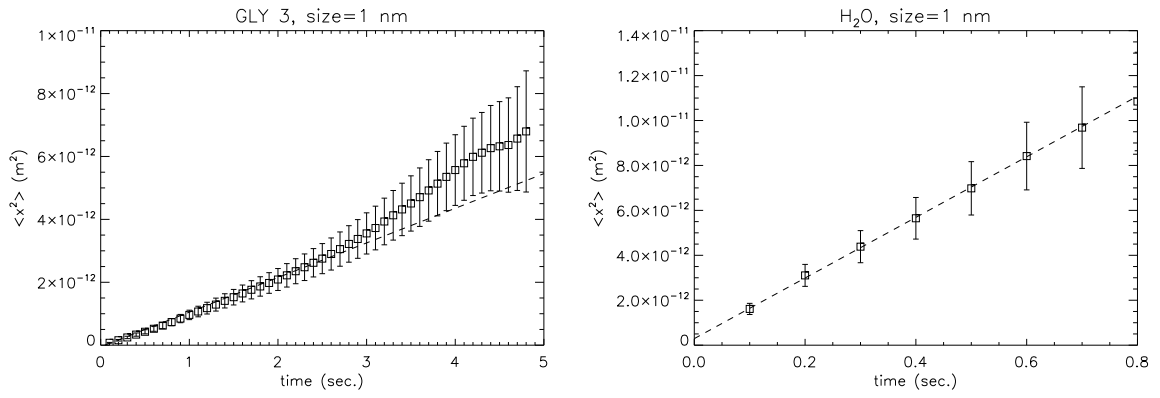


Fig. 4.— 100 nm particles in Gly3 and water.

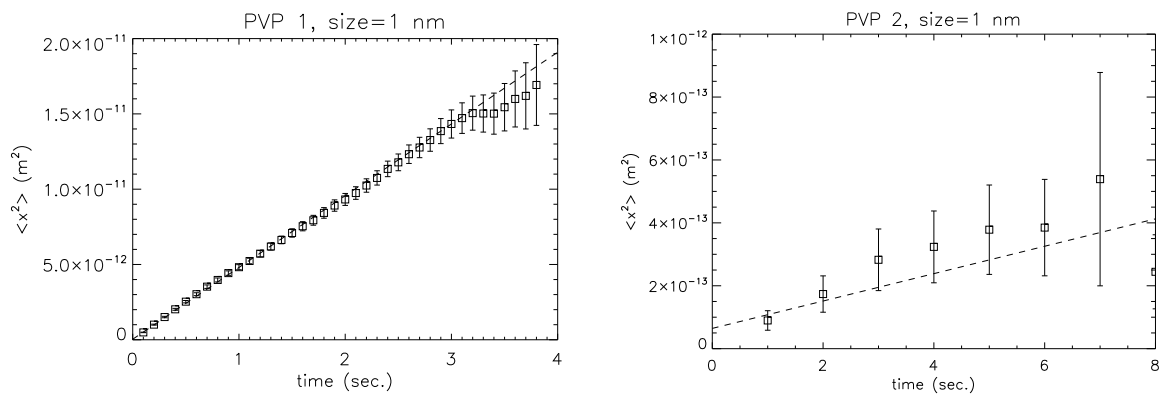


Fig. 5.— 100 nm particles in PVP2 and PVP3.

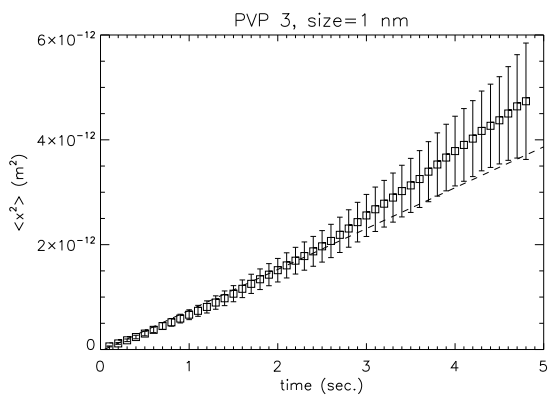


Fig. 6.— 100 nm particles in PVP3.

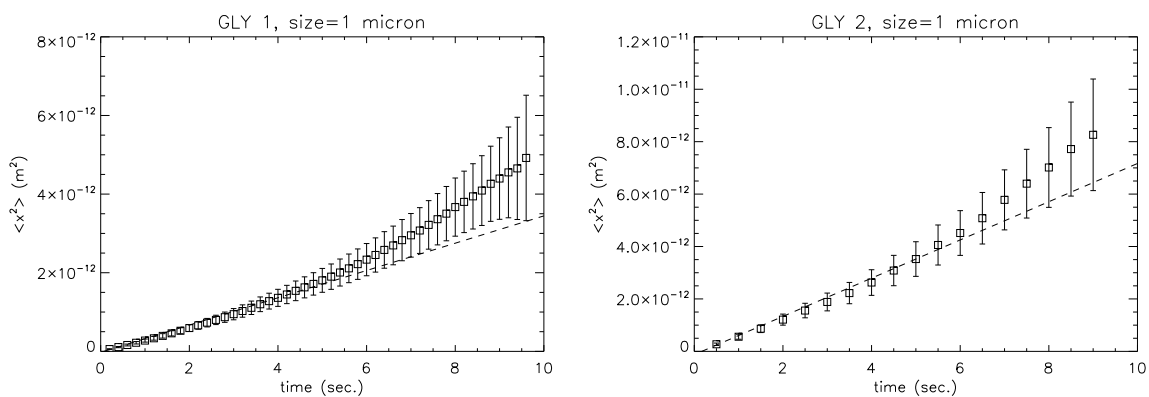


Fig. 7.— 1  $\mu m$  particles in Gly1 and Gly2.

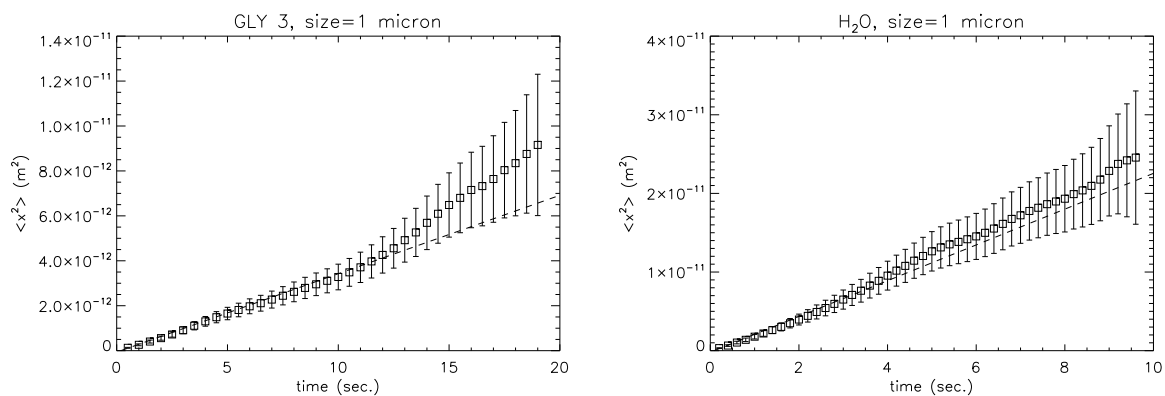


Fig. 8.—  $1 \mu\text{m}$  particles in Gly3 and water.

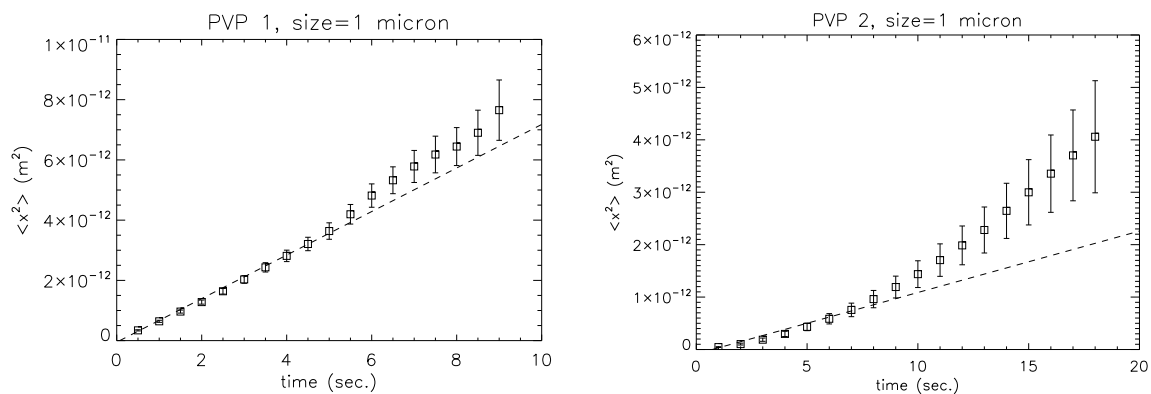


Fig. 9.—  $1 \mu\text{m}$  particles in PVP1 and PVP2.

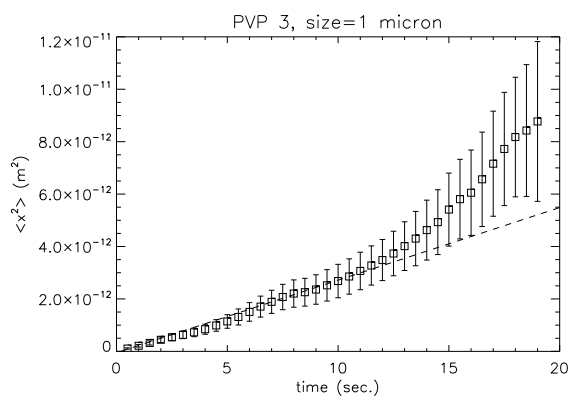


Fig. 10.—  $1 \mu\text{m}$  particles in PVP3.