Notes on Experiment 6-10.

This experiment is essentially that of Perrin, who following Einstein's theory of the Brownian motion, corroborated a number of aspects in theoretical statistical physics. One of these was the law of atmospheres applied to a suspension of small particles in a liquid, wherein the density of particles decreases exponentially with height in a manner depending on temperature and Boltzmann's constant. Knowing the latter and the universal gas constant, one can determine Avogadro's number. A second method involves measuring the particle displacements in set intervals of time.

The particles employed in this experiment are small polystyrene spheres of quite uniform diameter prepared in some trick way by Dow Chemical Company. They are about one micron in diameter and have a density of 1.05. Two drops of the prepared "sphere-plus-carrier" liquid in 25 ml of distilled water is what we use here. A thin "pool" of the diluted concentrate is made in the following manner: two thin microscope cover slips (about 170 microns thick) are laid down on a microscope slide coated with a thin layer of viscous vacuum (or other) grease. The slips are about a centimeter square and are separated on the slide by about half a centimeter. The space between the anchored slips is cleaned of grease with a Q-tip and some solvent. The upper surfaces of the two slips are coated thinly with grease and a third slip is laid astraddle (and pressed down on) the two, thus covering the space between the two slips. A medicine dropper is used to inject some of the suspension between the two slips under the covering slip. The enclosed volume is filled and the two open ends are dammed with grease. The suspension is thus confined and will last for several days.

The prepared slide is put under the viewing microscope (45X objective—it's fun to observe visually), focused carefully, and photographs made with the 55 mm camera of the suspended particles at different depths, coming up from the bottom, say in 5 micron steps as indicated by the microscope fine focus dial. Eastman Plus-X film is used; exposure is 1/25 or 1/50 second, aperture just what the microscope exit pupil is; no auxiliary camera lens is used. Eight pictures should be taken. To recover the film, wind it back in the dark without rewinding, the film (which in the dark) with a pair of scissors. It is then developed, fixed, and washed. The developing and fixing is done manually, working the film up and down in the solutions (all in the dark)—hold the two ends of the film and make it into a U, vary the lengths of the two sides of the U by moving the two ends up and down, keeping the loop of the U immersed in solution; it is no great trick.

After drying the film and keeping the order of exposures straight, the several negatives are mounted in slide mounts and are projected onto a screen (P-310 Lab projector). Counts are made of the individual particles in focus at each level. A line grid on the screen helps to break the area up into convenient sections. (Please return the slide mounts.) Illumination for the photography seems to be adequate with simply a 50 watt bulb sitting under the slide and microscope objective, the condenser height adjusted to give optimum contrast in the "scene" as viewed visually.

After the suspension is encapsulated between the cover slips, it is allowed to settle for about a day before photographs are made and after an equilibrium situation has been allowed to develop. Visualy,
it is very pretty to see the jiggling undergone by all the particles. Counts should be made of single particles, avoiding scattered clumps.

The camera back is that of a Kodak Retina. It is fixed so that double exposures are not possible. **When** to advance the film, the release button on the camera top is pressed, which allows the next advance to be made. After each advance the shutter is tripped; the cable use for this is essential to prevent jarring the set up. Twenty exposure rolls are used; this gives enough extra on the leading end to be engaged in the take-up reel for two sets of eight exposures. On the second set (for the next experimenter), the free end of the film is pulled out a bit and trimmed to fit in the slot in the take-up reel. Two or three frames must be advanced to ensure exposures free of room light fog. It helps also to cover up the unused eyepieces during photography.

Finally, leave a note on the back of the camera informing the next user as to the state of film in the camera when you have finished.

Comment on the use of the random walk in another microscope set-up follows on the next two pages.
As is well known in Brownian motion, the square displacement of a particle from some starting position increases linearly with time. It also involves a linear constant and the temperature (in this exact sense) in a linear relationship and the particle radius and viscosity of the medium in inverse relationship. We can also try this approach to Boltzmann's constant and Avogadro's number.

It would save time if the measurements could be made on the suspension of particles after the slide is prepared. No settling will be taken place, however, and the density may be a bit high to keep one's attention fixed on a given particle. It is certainly easier to do it after the exponential distribution of height is established. One can plot out a particle near the top where the density is low and have little difficulty in tracking it.

It would be rather fun to plot the observations of a given particle, say, over a period of half an hour. This does not seem very easy to do. Rather, what we will do is to track the x-displacements of different particles (all assumed to be the same) for 30 seconds intervals. We will use a microscope with a micrometer stage micrometer, and a micrometer eyepiece. The eyepiece setting can be set conveniently on zero and, with the micrometer stage, a selected particle brought to view between the two short lines of the eyepiece micrometer. Then the particle is centered between the two lines, a 30 second interval timer is set on and for thirty seconds the selected particle is followed in the microscope eyepiece, kept the particle centered in between the two movable lines. Then the interval timer sounds off, tracking is stopped, and the microscope reading is recorded. Another particle may be selected and the process is repeated.

This would be done for several particles. It takes a little practice to get everything going at once but then it isn't so bad. The mean square displacement is going to be something like a few microns, with the 40x objective and the eyepiece micrometer, about 1 micron, but this will be checked by the student using the knife-edge scale on glass slide. One has to be quite careful focusing the microscope on this or, indeed, on his own slide carrying the suspension under investigation.

The particle visibility is greatly enhanced by closing the aperture down in the illuminating tube leading the condenser. During a 30 second tracking operation, one hand is on the line focus, while the other is on the micrometer eyepiece dial. Bringing this back to zero after each tracking simply means that only one reading has to be recorded, giving the x displacement directly. Four trials ought to give a reasonable mean square value, but it is not to be presumed that the method is going to give high precision—probably not so good as the particle density with weight determination will provide.

The illumination is simply that of a 25 watt bulb sitting at the back end of the microscope at the opening of the "illumination tube". The magnification is quite high, so one can not lean too much on the eyepieces used to bring a selected particle centered in between the two short vertical black lines. Of course, the particle also undergoes y-displacements but in thirty seconds these will not be great enough
to take the particle out beyond the ends of the two lines if, at the start, the particle is moved so that it is somewhere near MAX 75 along the length of the short lines. This will all be obvious after one looks in the microtome eyepiece. One of the regular microscope eyepieces is removed and the micrometer eyepiece is put in its stead, clamping it to the eyepiece tube with the small knurled set screw opposite the micrometer dial. PLEASE NOTICE THIS THING CAREFULLY—DO NOT DISASSEMBLE ITS INNERWORKS. IF IT GETS WACKED, AS Mine TO MIX IT. You will of course have to see the reticle sharply, the eye lens time, moving it in and out for optimum focus. But take it easy. Fine focus on the particles in obtained with the knob at the back near the base of the instrument. Once only one eye will be involved in the tracking operation, it may be of some help in the contrast to cover the unused eyepiece with an old film can. At least on the microscope used in the photography, light entering in the unused eyepiece weakens the contrast in the used eyepiece. But it isn't bad even leaving it open to the room light.

Interestingly enough, accidental it must be, the viscosity of the water at 20°C (that's 68°F) is awfully close to unity, in centipoises. Our particle diameters are said by the manufacturer to be 1.01 microns.

In principle here, one could also get the $z$-displacements, since he keeps the particle in focus with the fine focus knob of the microscope. It just seems too difficult to know the $z$-coordinate at the start of a timing run; furthermore the depth of focus is not as critical as probably would be necessary. At any rate, this has not been tried as of this writing.
Variance in a random walk: The probability distribution of the value of a particle's position at a time $t$, given that it had a position 0 at time 0, is

$$b(x,t) = \frac{1}{\sqrt{4\piDt}} e^{-x^2/4Dt}$$

where $D$ is the diffusion constant.

Since $t$ is always going to be fixed and equal to 0, we can rewrite this equation as

$$b(x) = \frac{1}{\sqrt{4\pi D t}} e^{-x^2/(4Dt)}$$

Then $b' b(x,t) = \sqrt{\frac{D}{t}} e^{-x^2/(4Dt)}.$

If we make $N$ observations of $e$ and get values $x_1, x_2, \ldots, x_N$. Then, how do we estimate $\lambda$? The joint probability of getting $x_1, x_2, \ldots, x_N$ is

$$P(x_1, x_2, \ldots, x_N) \propto \left(\frac{\lambda}{N}\right)^n e^{-\lambda x_1 - \lambda x_2 - \cdots - \lambda x_N}$$

Let us define

$$\tilde{M} = \frac{\sum x_i}{N}.$$

Then the probability distribution of the parameter $\lambda$ is

$$b(\lambda) \propto C \left(\frac{\lambda}{N}\right)^{n/2} e^{-\lambda \tilde{M}}$$

and $C$ is a normalizing constant.

$$\int_0^\infty b(\lambda) \, d\lambda = \frac{C}{\Gamma(n/2)} \int_0^\infty \lambda^{n/2} e^{-\lambda \tilde{M}} \, d\lambda = \frac{C}{\Gamma(n/2)} \frac{\Gamma(n/2 + 1)}{\tilde{M}^{n/2 + 1}}.$$
Hence the distribution for $X$ is very important in planning a
screw. From the $X$-Edo the same can be done very easily.

This is not all surprising, since from the photon distribution
foremost, we obtain

$$x^2 = \frac{\lambda x}{2}$$

and

$$x = \frac{2N}{N-2}$$

so that for which

$$\lambda$$

is a maximum.

So the best estimator is $\hat{\lambda} = \frac{1}{N-2} \sum_{i=1}^{N} x_i$.

$$\int_{0}^{\infty} \frac{\lambda x}{2} e^{-\lambda x} dx = 2(2^{1/2})$$

Thus is not a factor.
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Practical Tips

1. An electric tumbler is provided to create an evenly distributed colloidal suspension.