Molecular Weight: Measurements with Gravity Cells
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Molecular Weight: Measurements with Gravity Cells

Abstract. *A gravity cell can be used to determine the weights of particles greater than 10^8 molecular weight units. The solution is maintained at a temperature which is constant to ± 0.001°C until equilibrium between sedimentation produced by gravity is balanced by back diffusion. The weight-average molecular (particle) weight of tipula iridescent virus was (1.05 ± 0.02) × 10^9.*

The magnetically suspended equilibrium ultracentrifuge (*1*, *2*) has been used successfully for determining the molecular weights of substances over the molecular weight range from the order of 10^2 to 5×10^7. The method is especially useful for solutes of higher molecular weight because of the precise speed control and lack of hunting of the magnetically suspended rotor at all speeds. As the molecular weight increases, the rotor speed must be decreased to prevent sedimentation of the substance on the bottom of the cell. Consequently, the upper limit to the value of the molecular weights which can be measured by this method is determined by the lowest field that can be produced in the centrifuge cell. With the magnetically suspended ultracentrifuge, the rotor speed, and hence the centrifugal field, can be made as small as desired, but sedimentation in the centrifuge cell takes place along the resultant of the centrifugal and gravitational fields. Consequently, the magnitude of the gravitational field essentially sets the limit to the maximum molecular weight that can be determined by equilibrium sedimentation methods. In the molecular weight range above a few million the centrifugal field required is no longer large in comparison to that of gravity, so that the centrifuge cell should be tipped in order that sedimentation can take place along the resultant of the two fields. This angle of tipping becomes quite large for substances with molecular weights much above 5×10^7. In view of this a gravity cell has been used for determining molecular weights of 10^8 and above.

Sedimentation in a gravity cell has been extensively used in a number of different investigations (*3*). We now describe some experiments which show its value for determining the weight-average particle weights of units the size of large viruses.

When equilibrium is obtained in an ideal dilute solution in a gravity cell with a constant horizontal cross section and height h, the molecular weight of a monodisperse substance is given by the relation

$$M = \frac{RT \ln (c_2/c_1)}{(1-\rho\bar{v})gh} \qquad (1)$$

where c_1 and c_2 are the concentrations at the top and bottom of the cell respectively, $\bar{v}$ is the partial specific volume, ρ is the density of the solution, g is the acceleration of gravity, T is the absolute temperature, and R is the gas constant. The weight-average molecular weight M_w is given by

$$M_w = \frac{RT}{(1-\rho\bar{v})gh} \frac{c_2 - c_1}{c_0} \qquad (2)$$

where c_0 is the initial uniform concentration in the cell before sedimentation takes place.

Two identical rectangular gravity cells were carefully machined in a cylinder of Kel-F which is 2 cm in diameter and 1 cm long. The rectangles are 0.200 cm in the direction of sedimentation (vertical), 0.20 cm wide, and 1.00 cm in the direction of the light path. They are situated side by side as shown in cross section in Fig. 1. A single glass window with optically flat parallel faces seals each of the front ends of the two cells, while a similar window seals the rear ends. Each cell is leak tight. The Kel-F cylinder is surrounded by an envelope of brass which weighs 15 lb and contains channels through which liquid at constant temperature is continuously circulated. The brass envelope is enclosed in a housing of "Styrofoam" insulation. Small windows in both the brass and Styrofoam allow light to pass through the cells. The entire apparatus, including the interferometer, was placed in a plywood box, which served to prevent air currents. This assembly was mounted on a heavy table with legs that rest on partially inflated "inner tubes." It was necessary to control the temperature to about 0.001°C.

The solution of the material in which sedimentation took place was injected by a syringe with a long hypodermic needle into one of the cells through a filling tube (Fig. 1). The filling tube was then sealed. The solution was injected into the other cell in the same way and the tube was likewise sealed off.

The values of c_1, c_2 in Eqs. 1 and 2 were determined by the same double-beam interferometer method previously described in detail (*1*, *2*) for the equilibrium ultracentrifuge measurements;

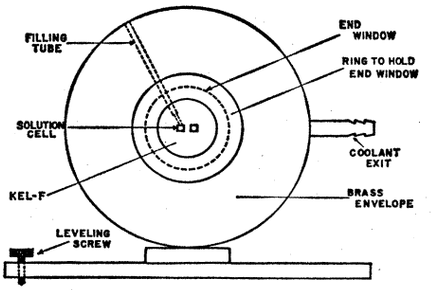

Fig. 1. Cross section of gravity cell (Styrofoam housing and plywood box not shown).

c_0 also was determined by the same method used in the previous experiments. Actually, the interferometer was simplified, for the "chopper slit" was not needed and the exposure time could be greatly reduced.

Sedimentation experiments were first carried out with polystyrene latex spheres of known density and size as tests of the method. Tipula iridescent virus (TIV) (*4*) was then placed in the cell, and photographs were made of the fringe pattern at daily intervals for 18 days although no appreciable change in the pattern occurred after about 12 days. The temperature was maintained at 4.500° ± 0.001°C. With $c_0 = 0.085$ percent, $\rho = 1.0034$ g/ml, and $\bar{v} = 0.719$ ml/g (*5*), M_w for the particle weight of this sample of TIV was (1.05 ± 0.02) × 10^9. Measurements on another preparation of TIV have confirmed this value. Figure 2 shows a plot of ln c versus the distance from the top of the cell for a typical experiment. This plot is almost a straight line, which indicates that the TIV is quite uniform in size, and the solution apparently is ideal at all concentrations along the cell. The above value is in substantial

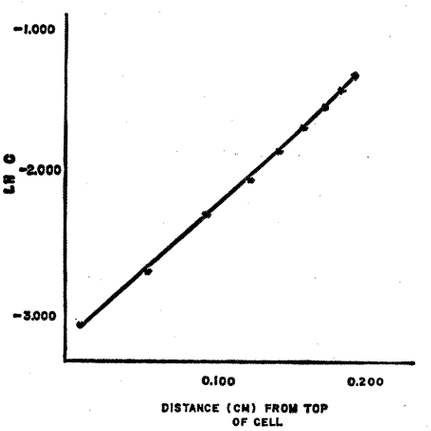

Fig. 2. Logarithm of concentration of tipula iridescent virus versus distance from top of cell at equilibrium.

agreement with that recently found by the counting method (*5*).

For the TIV experiments the virus was suspended and centrifuged down several times at low centrifugal force in 0.075*M* KCl containing potassium phosphate to give a final ionic strength of 0.1 and a *p*H of 7.0. The washed virus was resuspended and dialyzed against this solvent for 24 hours before insertion into the gravity cell. The concentration was estimated from refractive measurements in a differential refractometer at 20°C with the 5461 Å Hg line. Dry-weight determinations were carried out on both solution and equilibrated solvent from which a value of 0.199 ml/g was found for the specific refractive increment. The nitrogen content of this sample was 16.0 percent. No extraneous material nor aggregates were observed in schlieren photographs during velocity sedimentation in a double sector cell; the sedimentation coefficient, s_{20}, was 22×10^2 S.

The principal drawback to the gravity cell is the long time required for equilibrium to be established. Unfortunately, the equilibrium time cannot be shortened by some of the simple devices used in ultracentrifuge experiments (*2*, *6*), but short cells can be used and layering techniques could be employed. However, the cell requires virtually no care once it has been set up. The time required for equilibrium to be established can be estimated by the theory of Mason and Weaver (*7*). Also the effective range over which the particle weights can be determined is limited by the precision with which $(c_2 - c_1)$ can be determined and upon sedimentation of the solute on the bottom of the cell. While experimental tests for the latter should always be carried out, its effects can be estimated from the relation of the velocity of sedimentation to the velocity of back diffusion (*3*). In general the apparatus should be quite useful in the molecular weight range from 10^8 to 10^{10}, depending somewhat upon the partial specific volume and density (*8*).

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References and Notes

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Potency of Conditioned Reinforcers Based on Food and on Food and Punishment

Abstract. *Pigeons peck more frequently on the left key of two keys when food is presented more frequently during the stimulus that reinforces pecks on that key than during the stimulus that reinforces pecks on the right key. This preference can be annulled and reversed by punishing each peck on the left key during the stimulus that reinforces pecks on the left key.*

Much has been learned recently about the effects of punishment on an organism's tendency to emit a single reinforced response (*1*). In this experiment (*2*), punishment alters the pigeon's tendency to emit each of two responses, each of which is reinforced with presentations of a stimulus associated with a different frequency of intermittent positive reinforcement.

Two pigeons at 80 percent of their free-feeding weights were reinforced daily on two, concurrent, chained schedules of reinforcement, a procedure studied extensively by Autor (*3*). The experimental chamber contained two response keys. One chain composed of two links was programmed on each key. Pecks on a key during the first link of the chain were reinforced on a 3-minute, variable-interval (VI) schedule with the presentation of a second color of key signaling the start of the second link. Pecks on the key in the second link were reinforced with 4 seconds of access to mixed grain, according to a variable-interval schedule of a different value for each key. The first links of the chains were programmed concurrently; the left key was lighted orange and the right key was simultaneously lighted green. However, during the second link on a key, only that key was lighted; either the left key was red and the right dark, or the right key was yellow and the left dark. Each second link lasted for 30 seconds. When the second link ended, both keys were again lighted with the colors appropriate to the first links.

Pecking during the concurrent first links of both chains was maintained by a conditioned reinforcer, the presentation of the second color, on which pecks were reinforced with grain. A preference for pecking the left key in the first link was established by reinforcing 72 pecks per hour (on the average) in the second link on the left key and only 30 pecks per hour in the second link on the right key.

Punishment was introduced in order to determine how much current following each peck during the second link on the left key would annul and reverse the preference for pecking the left key during the first link, when both keys were lighted. Each peck on the left key during the second link passed an electric current through the pigeon for approximately 30 msec (*4*). The current was varied between 0 and 2.9 ma by adjusting a resistance in series with the pigeon and the source (120 volt a-c). Each intensity was in effect for a minimum of seven sessions and until the responding did not change systematically from session to session. After the intensity of punishment that annulled the preference based on 72 and 30 reinforcements per hour was determined, the experiment was repeated with 43 and 18 reinforcements per hour.

Figure 1 shows the rate of pecking during the two links of the chain on each key as a function of the intensity of the punishment following each peck during the second link on the left key. The upper graphs show the rates of pecking during the concurrent first links. The lower graphs show the rates during the second links. Circles and triangles represent different pigeons. Filled points and solid lines show the rates of pecking maintained by 72 and 30 reinforcements per hour. Unfilled points and dashed lines show the rates maintained by 43 and 18 reinforce-