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A Magnetic Suspension Osmometer

(molecular weight estimation)

J. W. BEAMS, M. G. HODGINS, AND D. W. KUPKE*

The Department of Physics and * The Department of Biochemistry, The University of Virginia, Charlottesville, Va. 22903

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ABSTRACT **The magnetic suspension balance is used for the measurement of very small osmotic pressures. The apparatus is essentially the same as that previously used for the measurement of the density and viscosity of protein solutions except that the magnetically suspended buoy is modified to make it pressure sensitive. The method is especially useful for the measurement of osmotic pressure in small samples of dilute solutions (about 10^{-6} M) or of substances with molecular weights greater than 10^6. A height-sensing device has been developed which is not dependent upon the visual precision of the operator.**

Osmotic pressure, while still a very useful equilibrium method for the study of macromolecules, is not sufficiently accurate in routine practice at molar concentrations below 10^{-4}. Because of this limitation, this straight-foreard equilibrium method has not been useful for the study of many of the, now timely, self-association reactions of proteins, nor for the molecular-weight estimation of very large macromolecules ($>10^6$ daltons). Numerous efforts have been made to develop more sensitive, reliable, and convenent methods (1–3). In this paper a new method is described which is designed primarily for the measurement of very small osmotic pressures, whereby molar concentrations at the level of 10^{-6} can be studied with satisfactory precision.

The method uses the servo-controlled magnetic suspension balance which, except for the buoy, essentially is the same as that used previously for the simultaneous determination of the density and viscosity in small volumes (0.2 ml) of solutions (4–6). Figs. 1 and 2 show schematic diagrams of the apparatus. A buoy, B, is suspended in the macromolecular solution contained in the glass cell, C. Across the bottom of the glass cell is fastened a semipermeable membrane, N. Surrounding C is a glass test tube which contains the solvent. The temperature control that surrounds the test tube is not shown. The buoy, B, is a hollow cylinder closed at the top end and open at the bottom and contains trapped air so that it functions like the familiar Cartesian diver. Two types of these cylindrical buoys have been used; one type made of Kel-F with a small permanent magnet or a soft ferromagnetic rod fastened at the top or bottom on its axis and the other a thin-wall [1- to 3-mil (0.0254- to 0.0762-mm) wall] magnetic stainless steel cylinder sealed at the top with a thin, light, nonmagnetic cover. This latter type should be coated with gold or other nonreacting substances. The buoy can be made to float or to sink in the solution, and the magnetic force is used to maintain it very accurately at the desired height in the cell, C, as observed by the microscope, L. In Fig. 1, the buoy B is made lighter than the solution it displaces so the magnetic force is downward; i.e., the permanent magnet A is below the buoy. The magnetic force on the buoy plus the force of gravity balances the buoyant forces on the buoy at its desired height so that

$$M\,\partial H/\partial Z = V_B g(\rho - \rho_B) \qquad [1]$$

where M is the magnetic moment of the buoy, $\partial H/\partial Z$ the vertical gradient of the magnetic field H, V_B the volume of the buoy, ρ_B its average density, ρ the density of the solution surrounding the buoy, and g the acceleration of gravity. The magnetic force is produced by a very hard permanent magnet A (or an air-core solenoid) and the two identical air-core solenoids (gradient coils) S_1 and S_2 which are coaxial with the buoy B. Although the vertical position of A is adjustable, it is maintained at a constant height throughout any set of experiments. The two coils S_1 and S_2, each 15 cm in diameter and containing 600 turns of no. 20 copper wire (about 20 ohms each), are connected in series in such a way that their magnetic fields cancel at the position of the buoy, but their magnetic field gradients $\partial H/\partial Z$ add. This resultant field gradient is linearly proportional to the current I over changes of many orders of magnitude since M remains constant. Hence, the vertical forces on the buoy may be written as

$$F_1 + mg + KI = (V_m + V)\rho g \qquad [2]$$

where F_1 is the vertical magnetic force on the buoy due to the permanent magnet (or air-core solenoid) A, K is a constant,

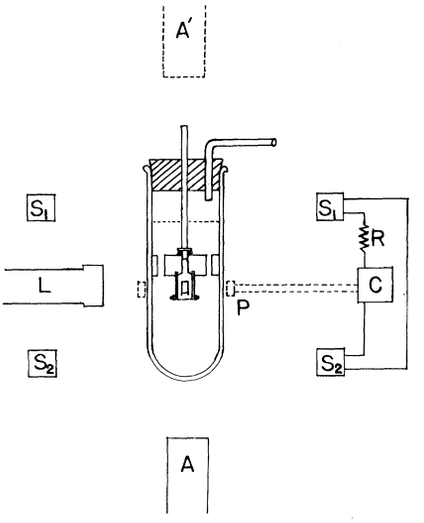

FIG. 1. Schematic diagram of the apparatus.

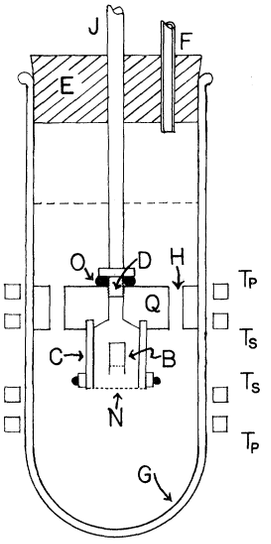

FIG. 2. Schematic diagram of the osmotic cell assembly.

V_m is the volume of the buoy material, V is the volume, p is the pressure of the trapped air in the buoy, and m is the mass of the material in the buoy plus the mass of the entrapped air. The very small changes in ρ with the small changes in pressure are negligible. Also, since A is very hard magnetically and in practice is a distance of about the diameter of S_2 below S_2, its moment is not appreciably altered by the resultant fields of S_1 and S_2. Furthermore, the magnetic fields produced by S_1 and S_2 are in practice relatively small. It should be noted that when the buoy has a permanent magnetic moment, stable support is obtained in the complete absence of the permanent magnet A. However, usually this is not practical except when ρ_B and ρ are almost equal. If we assume Boyle's law for the entrapped air and isothermal conditions, then $\Delta I/\Delta p = \text{const}/p^2$. It is clear that by measuring I for a few values of applied pressure to the solution at constant temperature, a usable relation should be found between I and p and a calibration curve of the instrument obtained. The sensitivity of the apparatus can be estimated by rewriting Eq. 2 as follows

$$\frac{K\Delta I}{F_1 + mg + KI} = \frac{\Delta p}{p} \qquad [3]$$

and observing that in practice $F_1 + mg$ may be made the order of 10^2 times KI and that $\Delta I/I$ may be the order of 10^{-6}. Consequently, from theory $\Delta p/p$ can be determined to about one part in 10^8; i.e., if p is the order of 1 atm, a Δp (osmotic pressure) of 10^{-2} dynes/cm^2 (about 10^{-5} cm of water pressure) should ultimately be observed.

The glass cell C which contains the solution (0.2–0.5 ml) consists of a cylindrical glass tube with its lower end covered by a semipermeable membrane. The membrane, while, wet is sealed to the glass tube by means of an O-ring as shown in Fig. 2. The upper end of C is cemented into a nylon plug Q which contains an axial channel for filling. The upper end of the channel is sealed by a short nylon screw and neoprene O-ring rigidly cemented to a glass rod J which extends out of the rubber cork E. Q fits tightly into a 15 × 1.8-cm glass test tube G and contains a number of vertical holes H parallel to the axis which permit free movement of the solvent. The assembled cell C is pushed into the position shown in Fig. 2. It is then filled with the solution by means of a hypodermic syringe. Special care is taken to avoid air bubbles in C. The test tube is next filled (free of air bubbles) to the dotted line; the rubber cork E is quickly inserted and the screw D slowly tightened by rotating J until the O-ring seals the channel in C. Care is taken to avoid excessive pressure change in C during the sealing procedure. The test tube is attached to a manometer and two 1-liter sealed glass flasks which may be opened or closed to the atmosphere at F. It is necessary to seal the test tube and flasks during the experiment because the small continuous changes in the atmospheric pressure which take place may prevent equilibrium from being established. Usually the pressure at the buoy is measured just before and just after the cell is closed and then from time to time until the pressure stops changing. The cell C is then opened and the pressure drop immediately measured. As a result, any effects due to possible absorption by the solution of the trapped air in B are negligible. Also, the effect of temperature variations are much reduced. Next, a calibration curve of applied pressure against current I is quickly taken.

In most of the experiments, the sensing of the buoy and the servo-control circuit were the same as described in detail previously (4–6). While this circuit is very satisfactory and has been used in most of our work, an alternate method has been developed in which the sensing coils are outside the solvent

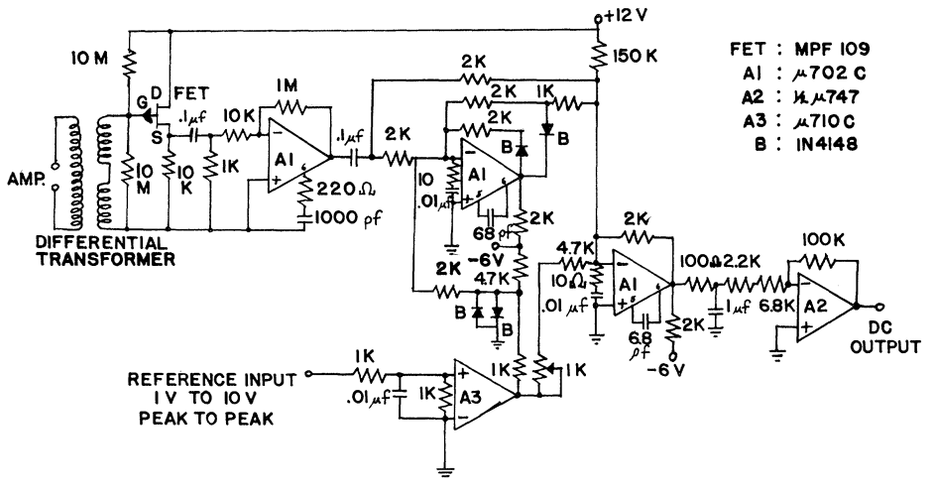

FIG. 3. Height-sensing system.

and solution under investigation. The height of the buoy is sensed by a differential transformer operating at 10^5 Hz, the output of which is amplified, demodulated, and filtered. The demodulator is similar to one designed by J. R. Nielson (7). Fig. 3 shows a circuit diagram of the control system. Essentially, this circuit detects a change in phase in the secondary T_S of the differential transformer produced by a change in the vertical position of the buoy B, (Fig. 2); i.e., as the height of the buoy changes, the dc output changes accordingly. This output (sometimes amplified) actuates a commercial bipolar controlled amplifier power supply, which in turn furnishes the current for the coils S_1 and S_2. The current, I usually is measured by the potential drop across a standard resistor as determined by a high-resistance digital voltmeter or recording potentiometer. The bipolar power supply permits stable support of the buoy with a comparatively small current ($\pm I$), which in turn reduces the power loss in S_1 and S_2 and the standard resistor R (about 10^{-6} W). However, much larger currents are often used. It will be observed that the potential across the dc output of the height-sensing circuit of Fig. 3 is an accurate measure of the vertical position of the buoy provided the characteristics of the circuit do not change with time. It is found that this output potential, when measured with a sensitive high-impedance multirange microvoltmeter, is a more convenient and precise means of maintaining the height of the buoy constant than by use of the microscope L. The method also can be used with opaque solutions. On the other hand, L is still used for occasional checks. The test tube G fits loosely inside of the rigid mounting of the transformer windings T_pT_s so that G can be slipped in or out of its mounting without disturbing the other parts of the apparatus. This facilitates the measurements, since the osmotic pressure in a series of different complete osmotic cell assemblies can be measured in rapid succession. Fortunately, these assemblies, consisting of the buoy and the other parts contained in G, are inexpensive or easy to construct. In practice, two osmotic cell assemblies are filled with the same or different solutions at the desired concentrations and sealed at the same time. They are then allowed to reach osmotic equilibrium before measurement.

In order to test the above method, the osmotic pressures of a series of dilute solutions of isoionic bovine albumin (0.05–5 mg/ml) in 0.15 M NaCl were determined at 25° C and pH 5.35. The albumin (K & K Laboratories, Plainview, N.Y., microbiological grade) was deionized, purified, and assayed for concentration as reported (6). Velocity sedimentation patterns exhibited essentially a single sedimenting boundary. Extrapolation of the osmotic data to infinite dilution yielded a molecular weight between 6.6×10^4 and 6.7×10^4. The equilibrium pressures for the lowest albumin concentrations used (about 7.5×10^{-7} M, total pressure 0.019 cm of H_2O) could be repeated within a few percent. With concentrations above 0.5 mg/ml, approximately 1% variation was observed when samples of the same solution in two different osmometer assemblies were allowed to equilibrate side by side for 24 hr before being inserted into the thermostated, magnetic sensing apparatus for measurement.

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