Some Interferometer Techniques for Observing Sedimentation

J. W. Beams

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University of Virginia, Charlottesville, Virginia

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Simple and convenient interferometer methods are described for determining the concentration of a substance in solution at various radial distances in an ultracentrifuge cell. These measurements are necessary for the usual molecular weight determinations by the ultracentrifuge method. The methods are particularly well adapted to equilibrium ultracentrifuge determinations of molecular weight and weight-average molecular weights.

IN the equilibrium centrifuge method for determining molecular weights of substances in solution, the molecular weight M is given by the well-known relation[1]

$$M = \frac{2RT \ln(c_2 f_2 / c_1 f_1)}{4\pi^2 N^2 (1 - \bar{v}\rho)(r_2^2 - r_1^2)}, \tag{1}$$

where T is the absolute temperature, R the gas constant, N the rotor speed in rps, ρ the density of the solution, $\bar{v}$ the partial specific volume, f_1 and f_2 activity coefficients, and c_1 and c_2 the concentrations in the ultracentrifuge cell at the radial distances r_1 and r_2, respectively. In the case of an ideal dilute solution, in a sector-shaped cell, the weight-average molecular weight M_w is given by the formula[1]

$$M_w = \frac{2RT}{4\pi^2 N^2 (1 - \bar{v}\rho)(r_b^2 - r_a^2)} \frac{c_b - c_a}{c_0}, \tag{2}$$

where c_a and c_b are the concentrations at the radial distances r_a of the meniscus and r_b of the periphery of the cell, and c_0 is the initial uniform concentration before centrifuging is started. The quantities f_1, f_2, $(1 - \bar{v}\rho)$, and c_0 usually are measured outside the centrifuge, while all the others are determined during the operation of the centrifuge.

With modern equilibrium ultracentrifuges, the least precisely known quantities which are measured during the

centrifuging are the values c_2/c_1 and $(c_b - c_a)$. Also, a knowledge of the absolute value of the concentration at some point in the cell is important. These quantities usually are determined by an interferometer, and this paper describes an attempt to develop a convenient and more reliable method of making the interferometer measurements. Such a method, adapted from refractometer techniques, has been employed and may be used with almost any two-beam interferometer such as the Rayleigh, Michelson, Mach–Zender, or Jamin types. Its application to a modification of the Jamin–Twyman and Green type interferometer used in our experiments[2] is described here.

Figure 1 shows a schematic diagram of the optical system used for measuring the sedimentation. It essentially is the same as previously described[2] except for the double-cell gas chamber K_1. A mercury arc source and a white light source are situated at equal distances from the lens L_1 which focuses the light on the chopper slit K' mounted on the rotor. The light is next focused by L_2 on a small hole in a diaphragm S_1. A collimating lens L_3 renders the light beam parallel which is limited by the wide slit S_2 before incidence on the first Jamin plate I_1. This plate provides two parallel beams, each of which passes through a separate compartment in K_1 and in K_2. One of the compartments of K_2 (which has been described previously[2]) contains the solvent and the other the solution. The two beams are recombined by the second Jamin plate I_2 and the fringes brought to focus on the photographic plate P,

[1] T. Svedberg and K. O. Pedersen, *The Ultracentrifuge* (Oxford University Press, New York, 1940); H. K. Schachman, *Ultracentrifugation in Biochemistry* (Academic Press Inc., New York, 1959).

[2] J. W. Beams, R. D. Boyle, and P. E. Hexner, Rev. Sci. Instr. 32, 645 (1961).
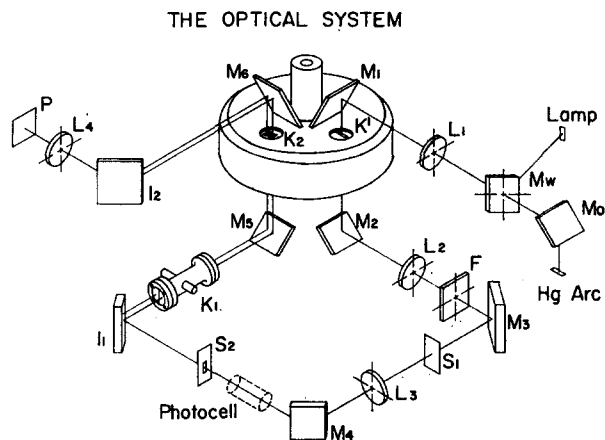
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THE OPTICAL SYSTEM

FIG. 1. Jamin–Twyman and Green type interferometer system showing position of centrifuge rotor and compensating two-compartment gas cell.

or observed in a telescope not shown. The two light sources may be alternated during an experiment by moving M_w in or out of the beam or if M_w is "half-silvered" they may be used simultaneously. The filter F is used with the Hg arc alone for isolating the green line of mercury of wavelength 5460.7 Å. The photocell may be placed in the beam for rotor speed measurements.

The gas cell K_1 is made of heavy brass with thick optical glass windows. The windows have plane parallel faces and are flat to the order of 0.1 wavelength. The cell has two identical compartments parallel to its length as shown, each of which is air tight. The windows are sealed with rubber gaskets under some pressure. This pressure is adjusted by screws so that the optical paths through the two compartments are equal with air at the same pressure or with a vacuum in each compartment. The gas or air pressure in each compartment can be separately regulated through the two outlets shown in Fig. 1. The air pressure in each compartment is measured by a separate manometer or a single manometer may be used to measure the difference in pressure between the compartments. The temperature of the cell is controlled.

With the rotor at rest and both compartments of K_2 filled with the solvent and both compartments of K_1 filled with air at the same pressure (atmospheric), the interferometer is adjusted so that the central white light fringe is at infinity and uniformly illuminates the image of the cell K_2 at P. One of the compartments of K_2 is next filled with the solution at concentration c_0. The white light fringe now disappears. The compartment of K_1, which is in the same light beam as the compartment in K_2 which contains the solution, next is evacuated until the central white light fringe again appears and the image of K_2 at P is uniformly illuminated. The optical paths of the two beams are now equal provided the dispersion of the gas is the same as that of the solute. This is not exactly true but in most cases, especially where the substance has normal disper-

sion and is transparent in the visible, the error introduced is very small if the concentration c_0 is not greater than about 2%; c_0 is less than 1% in most experiments. In any case, as shown later, the error can be reliably estimated and corrected if necessary. If $(\Delta p)_0$ is the change in pressure in mm Hg, L is the length of K_1, u the index of refraction of the air (or gas) at atmospheric pressure, then assuming Boyle's law and Gladstone–Dale law

$$L[(u-1)/760](\Delta p)_0 = (\Delta n)_0\lambda = c_0 kh, \qquad (3)$$

where $(\Delta n)_0$ is the change in the number of the fringes formed by the monochromatic light, λ the wavelength of the monochromatic light, k a constant, and h the thickness of K_2. The rotor is next started and the centrifuging continued until equilibrium is established in K_2. Due to the concentration gradient in K_2, the central white light fringe is then narrower, is in the same focal plane as the image of K_2, and is located at the so-called "hinge point" where the concentration is c_0. Photographs or photoelectric scannings are then made both with white light and with monochromatic light. The pressure in one of the compartments in K_1 is then changed by an amount $(\Delta p)_1$ until the central white light fringe is very near the meniscus in K_2. Both white light and monochromatic fringe patterns are again photographed or scanned. Since it is possible to extrapolate to the meniscus,

$$L[(u-1)/760](\Delta p)_1 = (\Delta n)_1\lambda = (c_0-c_a)kh. \qquad (4)$$

The pressure is next changed by $(\Delta p)_2$, which brings the central white light fringe to or very near the periphery of the cell and again both the white light and monochromatic fringes are photographed or scanned. This gives

$$L[(u-1)/760](\Delta p)_2 = (\Delta n)_2\lambda = (c_b-c_a)kh. \qquad (5)$$

The pressure in the compartment again is changed by $(\Delta p)_3$ until the central white light fringe is returned to the hinge point and photographs or scannings of both white light and monochromatic fringes taken. Then

$$L[(u-1)/760](\Delta p)_3 = (\Delta n)_3\lambda = (c_b-c_0)kh. \qquad (6)$$

Consequently, from the above equations,

$$\frac{c_b-c_a}{c_0} = \frac{(\Delta n)_2}{(\Delta n)_0} = \frac{(\Delta p)_2}{(\Delta p)_0}, \qquad (7)$$

$$\frac{c_b}{c_a} = \frac{(c_b-c_0)+c_0}{c_0-(c_0-c_a)} = \frac{(\Delta n)_0+(\Delta n)_3}{(\Delta n)_0-(\Delta n)_1} = \frac{(\Delta p)_0+(\Delta p)_3}{(\Delta p)_0-(\Delta p)_1}. \qquad (8)$$

Clearly from Eqs. (7) and (8), it is only necessary to measure the Δp's or the Δn's to obtain the necessary concentration ratios required in Eqs. (1) and (2). This can be done with high precision. Also, since L, u, and h are accurately known or measurable, c_0 can be determined if k is known or

vice versa. It is often convenient to determine k in a separate experiment.

With sensitive pressure gauges, the pressure may be measured to about 0.01 mm Hg.[3] If then one adjusts L so that $(\Delta p)_0$ is the order of an atmosphere, the pressure ratios are determinable to better than 0.1%. Also, known corrections can be made to Boyle's law if necessary. Dry air or nitrogen is convenient to use in K_1. Consequently, the precision is limited only by the accuracy with which the positions of the central white light and monochromatic fringes, the meniscus, and the periphery of the cell can be obtained. Two methods of increasing the precision of the above measurements are under investigation. The first, and probably most practical, is being used by F. N. Weber, H. G. Kim, R. M. Elton, and R. D. Rose in our laboratory. It consists of an adaptation of the double photomultiplier cell Michigan-type microphotometer[4] for measuring the positions of the fringes on the photographic plate as well as for scanning the fringes directly. For direct scanning, one of the photomultiplier cells is mounted on a comparator behind a slit for scanning the fringes in the plane at P. The second method makes use of the Kennedy scheme[5] for determining the fringe position with great accuracy. It consists of depositing thin transparent strips of film across one of the windows of one compartment of K_2 near the ends of the cell. These films increase the optical path by about 0.1 wavelength. Kennedy and Illingworth[6] were able to determine the position of Michelson interferometer fringes to better than one thousandth of a fringe. Rough preliminary investigations indicate that it may be possible to determine the positions of the order of one hundredth of a fringe in our case since the fringe position can be accurately set by careful control of pressure. This gives ample precision for the purpose, but at present the first method is simpler to carry out. Also, it allows the values in Eqs. (7) and (8) to be determined to three significant figures in most cases. Furthermore, the method is easy to use. It, is of course, clear that the change in optical path in the two interferometer beams may be accomplished by means other than a change in pressure in the gas cell K_1 such as by rotating glass plates in the light beams often used in refractometers.[7,8]

As mentioned above, when the concentration c_0 is well above 1% or when the dispersion of the substance in K_2 in the visible is very different from that of the gas in K_1, it is often necessary to correct for the shift in position of the central white light fringe. This usually can be estimated[7-9] with sufficient precision by the well-known relation $\Delta n' = 2Bh/\lambda^3$, which is the shift of the achromatic fringe obtained with white light with respect to the central fringe obtained with monochromatic light of wavelength λ, B is a constant in the equation $k = \bar{U}_\lambda - 1 = A + B/\lambda^3$, where A is a constant and U_λ is the refractive index of the substance for wavelength λ. B can be evaluated with sufficient approximation by repeating the above measurements [especially Eq. (3)] with each of two monochromatic wavelengths such as two of the fairly widely spaced mercury lines.

In cases where the shift of the white achromatic fringe is large or when the solution absorbs strongly in the visible or when the concentration is well above 1%, then it is simpler to use the method of matched compensating cells previously described.[2] This method is free of errors arising out of uncertainties in the position of the central white light fringe, but the value of c_0 must be obtained by an independent experiment. Another method of eliminating errors due to uncertainties in the identification of the position of the central white light fringe is illustrated by the expanded diagram of a compensating cell containing liquid as shown in Fig. 2. Essentially, it consists of two cells with matched optical glass windows mounted side by side. One cell contains the solvent and the other the solution. The optical paths through the cells may be varied independently or together. They are placed in the position of K_1 (Fig. 1) so that the interferometer beam which passes through the solution in K_2 (Fig. 1) passes through the cell (Fig. 2) containing the solvent and vice versa. In Fig. 2, 15 is a single circular glass plate with plane parallel sides and 10 is a similar glass plate slightly smaller in diameter cut in halves through a diameter. The window 15 is cemented to the cylindrical tube 11 or sealed by an O-ring pressed on the window by the cap 16. The thin partition 13 shown in cross section AA is cemented to the inner face of the window 15. The partition 13 divides the cylindrical tube 11 into two similar liquid-tight compartments which are sealed on the front by window 15. The two semicircular windows 10 are cemented into tubes of the same cross section. The two semicircular tubes 6 mounted side by side fit snugly into the tube 11. The two flat sides of the tubes fit tightly one on each side of the partition 13 shown in cross section at AA and BB. The O-ring 9 seals the circular sliding surfaces and 7, 8, and 9 maintain the alignment. 15 and 10 are the front and back windows of the two cells. One of the back windows 10 can be adjusted by the three screws 5 until the front surfaces of the two windows are in the same plane which in turn is parallel to the surfaces of 15. The flat surfaces of the two cell walls extend back beyond the

[3] *Dictionary of Applied Physics*, edited by R. Glazebrook (Macmillan and Company, Ltd., London, England, 1923), Vol. 3.

[4] H. H. Grossman, E. W. Peterson, J. L. Saunderson, and V. J. Caldecourt, J. Opt. Soc. Am. **39**, 261 (1949).

[5] W. J. Kennedy, Proc. Natl. Acad. Sci. U. S. **12**, 621 (1926).

[6] K. K. Illingworth, Phys. Rev. **30**, 692 (1927).

[7] C. Chandler, *Modern Interferometry* (Hilger and Watts Ltd., University Press, Glasgow, 1951).

[8] E. W. Williams, *Applications of Interferometry* (Methuen and Company, Ltd., London, 1936).

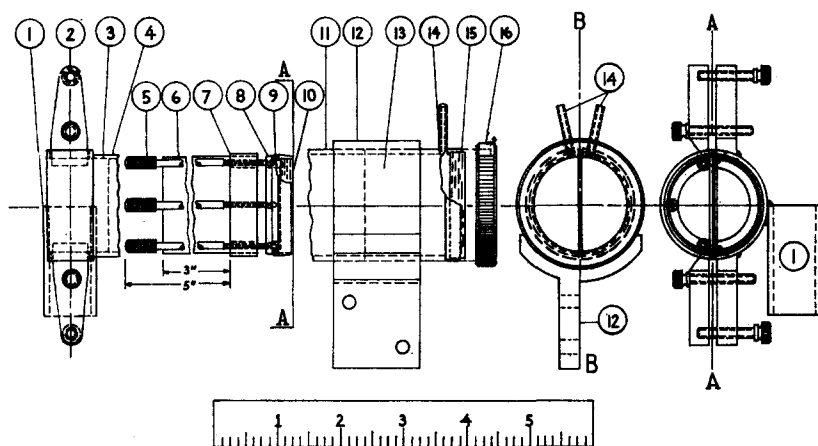[9] R. W. Wood, *Physical Optics* (The Macmillan Company, New York, 1934).

FIG. 2. Expanded diagram of two-compartment compensating cell which contains liquids.

semicircular surfaces and are fastened together by the clamp 2. By means of a screw drive (not shown) connecting the two supports 1 and 12, the windows 10 are then moved backward or forward at the same rate so that the lengths of the two cells formed are equal at all times. At the same time, solvent flows through a tubular connection 14 into one cell and the solution through a similar tubular connection into the other cell. Since the optical path through the solvent is different from that through the solution for the same length of cells, the optical paths in the two beams can be changed slowly with respect to each other and fringe shifts can be measured. This allows the same type of measurements as shown in Eqs. (3)–(8) to be carried out. Here it is necessary to count fringes instead of reading pressures. Since the dispersion in the compensating cell in Fig. 2 is essentially the same as that in K_2 (Fig. 1), no appreciable error is introduced by a shift of the achromatic white light fringe. The concentration in the compensator can be made practically the same as that in K_2, so that any small change in dispersion with concentration can be eliminated.

Also, changes in the dispersion of the solvent and solution due to pressure variations in the cell K_2 usually are negligible, especially with solutions of macromolecules, in the equilibrium ultracentrifuge where the pressures are relatively low. Except for the glass windows the entire compensating cell is made of metal covered on the inside by special fluorinated oils which serve both to seal the sliding parts and to protect the metal from corrosion. When working near room temperature the temperature is maintained sufficiently constant by surrounding the apparatus except for holes for the light beams with a metal box whose temperature is regulated to the order of 0.001°C.

While the compensating cell of Fig. 2 may be used even with concentrated solutions which have absorption bands in the visible, it is much more difficult to adjust and use than the gas cell. As a result, we have employed the gas cell whenever possible in our experiments.

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