
A High Resolving Power Ultracentrifuge

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rise, even when this serum was equivalent in amount to the injection of 500 cc into a 150-pound man.

Animals twice vaccinated with crude chick embryo vaccine have in all but one instance been completely protected against massive doses of intranasal virus. This vaccination has produced antibodies which were present in high titre after eastern, in lower titre after western vaccine injection. Such titres were not appre-

ciably enhanced by the subsequent test dose of virus. Clarified and deformalinized embryo vaccines have now been made which have been effective in guinea pigs and which should be more suitable for any human use that may in the future be needed.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A HIGH RESOLVING POWER ULTRACENTRIFUGE

IN the case of most large molecular weight substances the molecular weights and sizes are determined by measuring the rates with which they settle out in an ultracentrifuge, together with their diffusion constants.¹ Usually the material is enclosed in a sector-shaped cell with transparent windows so that the rate of sedimentation can be observed optically. Since the diffusion constants are very small for large molecular weight substances, the sedimentation boundaries remain comparatively sharp for long periods of time provided the substance is homogeneous. If the substance contains two or more molecular species, each forms a separate sedimenting boundary. The ability of an ultracentrifuge to separate a mixture of molecular species is proportional to $\omega^2 r h$, where ω is the angular velocity of the centrifuge, r the distance from the axis of rotation and h the length of the column of solution which may be observed.¹ In the modern ultracentrifuges the strength of the rotor materials sets an upper limit to ω , r and the height of the cell, h , that can be used.^{1,2} The purpose of this note is to describe briefly a method which, in effect, increases the length of the column of solution under observation without increasing the length of the cell. The method consists in forcing the solution through the transparent cell along the radius in the opposite direction to the motion of the sedimentation boundary. Consequently, if the rate of flow of the solution is equal to the rate of sedimentation, the sedimentation boundary remains stationary in the cell for long periods of time. This not only allows the sedimentation constants to be measured with precision but also makes it possible to determine the sedimentation constants for two or more molecular species in a mixture with very small differences in molecular weight.

Fig. 1 shows a cross-section of the ultracentrifuge rotor which was used to make a preliminary test of the method. This rotor was spun inside an evacuated chamber by an air-supported air-driven turbine (not shown but described previously^{2,3}) situated above the

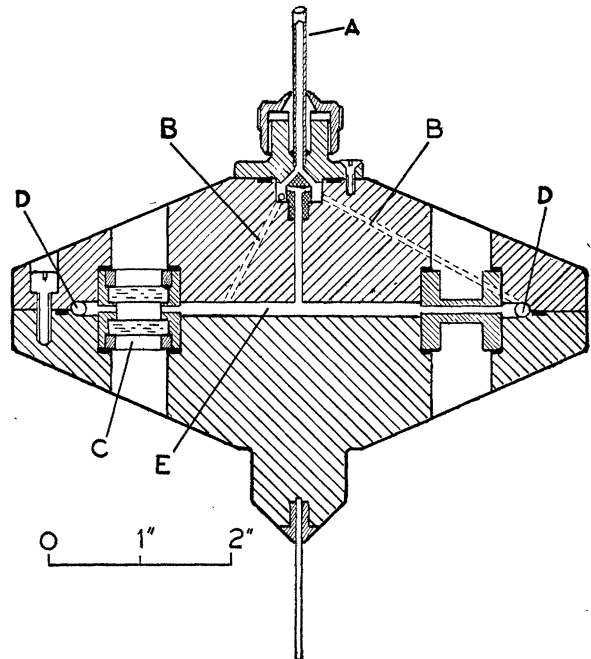


FIG. 1

vacuum chamber. The shaft A is a stainless steel tube which connects with the tubes B. B connects with a ring-shaped tube D, which in turn communicates with the periphery of the cell C. The cell C is sector-shaped with crystal quartz windows for viewing the sedimentation; it is 12 mm long and its center is 45 mm from the axis of rotation. Short channels connect the ends of the cell C and the cylindrical collecting chamber E. To operate the apparatus the rotor is accelerated to the desired speed, and the material to be centrifuged is injected into A with a hypodermic syringe. The material flows through B, D, and into the cell C. In this way the cell is filled about two thirds. Then as the sedimenting boundaries move outward toward the periphery, more material is injected into A at the proper rate to maintain the boundaries near the center of the cell. The solvent is collected in the chamber E. In most of these preliminary tests hemoglobin (kindly furnished by Professor A. Chanu-

¹ Svedberg, *Ind. Eng. Chem., Analytical Ed.*, 10: 113, 1938; *Proc. Roy. Soc.*, A 170: 40, 1939.

² Beams, *Rev. Mod. Phys.*, 10: 245, 1938.

³ Beams, Linke and Sommer, *Rev. Sci. Inst.*, 9: 248, 1938.

tin) was used. It was found that the sedimentation could be observed for practically as long as desired, the limiting factor apparently being only a blurring by diffusion (a dilution due to the field gradient also was noticeable). The sedimentation of colloidal particles very much larger than the hemoglobin molecules also was observed until E was filled. The preliminary tests indicate, in accord with theory, that the resolving power of the ultracentrifuge can be much increased, at least in some cases. A new and stronger rotor with the cell further from the axis is under construction with which further tests will be made.

The writer is greatly indebted to Messrs. Fritz Linke and Philipp Sommer, instrument makers, who constructed the apparatus, and to the Rockefeller Foundation for a grant in support of the development of the ultracentrifuge.

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A SMALL HYGROMETER

THE construction of a hygrometer that was much smaller and more sensitive than the usual commercial equipment became a necessity during the course of an experiment in which the relative humidity of a long narrow sealed tube had to be determined without changing its humidity by opening it.

This instrument is shown in Fig. 1. It consists of

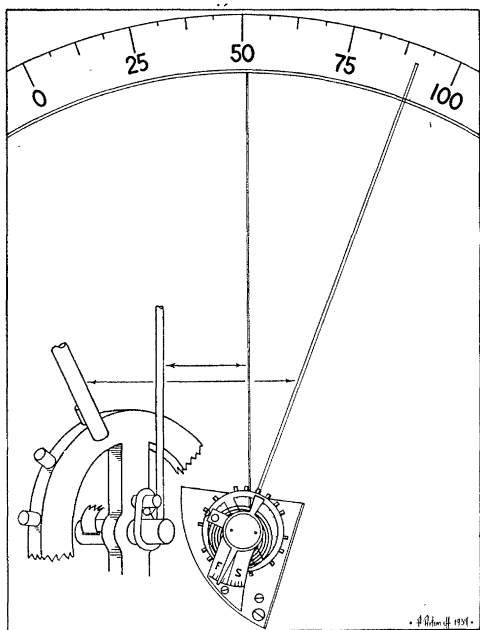


FIG. 1

the balance wheel, hair spring and small portions of the bearing plates of a small jeweled wrist watch attached to a glass plate. To the escape nub on the arm of the balance wheel is attached a very fine human

hair carefully washed in ether and alcohol. It then bends over the shaft of the balance wheel and is attached at the opposite end of the glass plate under slight tension produced by the hair spring. A very fine glass rod attached to the circumference of the balance wheel indicates the motion produced by the elongation or contraction of the hair with humidity changes. A more sensitive measurement may be obtained by attaching small mirrors to the rim of the balance wheel and the regulator arm and using the distance between the spots of light reflected as an indicator of the motion produced by humidity changes.

The instrument was calibrated and checked by alternately placing it in a closed chamber first over water and then over concentrated H_2SO_4 . All readings were within 2 per cent. R. H. When placed above salt solutions in closed containers, the instrument checked to about 1 per cent. in every instance. Under these conditions equilibrium was reached within 15 minutes.

The advantages of this instrument are threefold. (1) It is quite accurate and may be easily read without altering the humidity in the chamber. (2) As the small size makes its use in very small spaces possible without changing the humidity, humidity of microclimates may be obtained. (3) It is inexpensive and easy to construct.

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