
Centrifuging of Liquids

Author(s): J. W. Beams

Source: *Science*, New Series, Vol. 88, No. 2280 (Sep. 9, 1938), pp. 243-244

Published by: American Association for the Advancement of Science

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THE ISOLATION OF A CRYSTALLINE COMPOUND WITH VITAMIN K ACTIVITY

AN oil, very active in Vitamin K properties, has been obtained by the fractionation of a petroleum ether extract of alfalfa leaf meal. This oil, containing crystals in suspension, was dissolved in a mixture of benzene and absolute alcohol and allowed to stand at -5° C. The crystalline material which separated was recrystallized from a mixture of benzene and absolute alcohol, then from absolute alcohol, then from acetone and finally from absolute alcohol. The crystals, M.P. 69° C., were small, well-formed, transparent, colorless plates. The chick unit as determined by the procedure developed in this laboratory¹ is approximately 0.6 microgram.

Although it is possible that the activity of these crystals may be due to (1) formation of mixed crystals of the vitamin and an inert substance or (2) adsorption

of the vitamin on the crystals, we believe that the crystals are really Vitamin K for the following reasons. These crystals have a greater potency than any other fraction that we have obtained, including the oil from which the crystals were separated. Moreover, the crystals were recrystallized from a variety of solvents without loss of potency.

We have also obtained certain indications that more than one substance possessing Vitamin K activity may be present in our extracts.

Our work is being continued, and the detailed data will be published in a subsequent contribution.

S. A. THAYER
D. W. MACCORQUODALE
S. B. BINKLEY
EDWARD A. DOISY

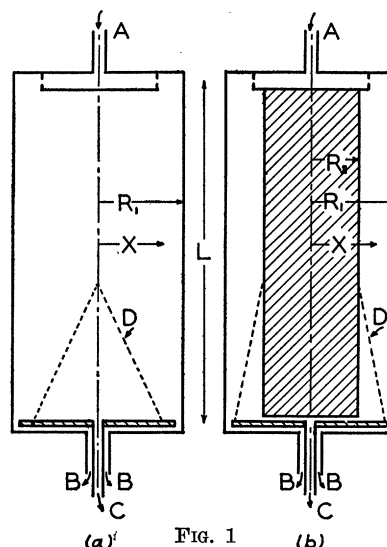
LABORATORY OF BIOLOGICAL CHEMISTRY,
ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE

SCIENTIFIC APPARATUS AND LABORATORY METHODS

CENTRIFUGING OF LIQUIDS

IN a previous article¹ a tubular vacuum-type centrifuge was described which has proven to be a very useful tool for the separation of comparatively large quantities of substances. Hence it may be of interest to mention briefly a rather simple change in the design of the spinning tube which has considerably increased its efficiency. The apparatus consists of a tube that is spun to a high rotational speed inside an evacuated chamber. The vacuum around the rotor avoids heating due to air friction and insures absence of troublesome temperature gradients that cause stirring. Fig. 1a shows a schematic diagram of the tubular rotor. For the method of mounting and spinning this rotor, reference should be made to the previous article.¹ The material to be centrifuged entered the spinning hollow tube at A at a continuous rate and the lighter and heavier fractions were collected at C and B, respectively. The separation took place as the material flowed down the length L of the spinning tube. It can easily be seen that for a given material and rotational speed the amount of separation increases with increasing radius of the tube, increasing L, and decreasing rate of flow of the material; *i.e.*, the radius of the tube and the time the material is in the centrifuge. By definition² of the sedimentation constant *s*, the rate of settling of the

heavier substance toward the periphery $\frac{dx}{dt} = w^2sx$ where *w* is the angular velocity of the centrifuge and *x* the distance of the material from the axis of the tube. Hence $\frac{dx}{dt} = 0$ when *x* = 0, and in the absence of diffusion the separation would be very small near



the center. To overcome this we have been using for some time a solid cylinder in the center of the tube as shown shaded in Fig. 1b. *R*₁-*R*₂ is made comparatively small and *D* is a wire cloth filter to prevent possible remixing at the exits. With this arrangement the material is always in a strong centrifugal field and the

¹ S. A. Thayer, D. W. MacCorquodale, R. W. McKee and E. A. Doisy, *Jour. Biol. Chem.*, 123: cxx, 1938.

² Beams, Linke and Skarstrom, *SCIENCE*, 86: 293, 1937; Beams, *Phys. Rev.*, 53: 850, 1938.

³ Svedberg, "Colloid Chemistry."

separation is much faster. The volume of the centrifuge is, of course, decreased, which decreases the time that the material is in the centrifuge, but a simple calculation shows that this decrease in time is much more than compensated for by the increase of the average centrifugal field. Experimental tests also show a large increase in efficiency.

In addition to the purification of materials by the continuous flow vacuum-type tubular centrifuge, it is possible at the same time to determine the sedimentation constant s from a knowledge of the dimensions of the centrifuge, rate of flow of material, the ratio of the amounts of the light and heavy fractions and the ratio of the concentrations of the original material and the light and heavy fractions. From a knowledge of s and the diffusion constant, the molecular weight may be obtained.² As yet extremely high accuracy has not been obtained, but the results are reproducible with precision. Since the molecular weights of substances from about 40 up to 20,000,000 have previously been measured with precision principally by Svedberg and his collaborators,³ these materials with known molecular weights can be used to calibrate a particular vacuum-type tubular centrifuge. After once calibrated, the apparatus should give comparatively precise results, especially for high molecular weight substances.

It is a pleasure to record my indebtedness to the Division of Natural Sciences of the Rockefeller Foundation for a grant that has made possible the development of the tubular vacuum-type centrifuge.

J. W. BEAMS

THE VELOCITY OF SOUND

THE velocity of sound has usually been measured over long distances (one to twenty miles) by means of guns and stop watches. With the invention of the oscilloscope, it is possible accurately to measure intervals of time as short as one millionth of a second. Therefore, the velocity of sound may be measured with a baseline less than one foot. As a base line so short can not be determined accurately, a longer base line should be used. Since the standard current alternates sixty times per second, one complete cycle of the current represents one sixtieth of a second. The horizontal plates of a standard oscilloscope are connected to the alternating current, which at the same time causes a loud speaker to emit sixty pulses per second. These sound pulses are received on a microphone which is connected through an amplifier to the vertical deflection plates of the same oscilloscope. The electric impulses received by all four plates must be in synchronism; hence a single line appears on the oscilloscopic screen.

² Svedberg, *Ind. and Eng. Chem.*, 10: 113, 1938; *Nature*, 139, 1051, 1937.

If the microphone is moved through approximately twenty feet, the line on the oscilloscope will move through one cycle or one sixtieth of a second. The product of 20 and 60 gives 1,200 feet for the velocity. Careful measurements give the actual velocity 331.57 meters per second at 0° C.

This method may be improved by receiving the impulses alternately upon two microphones through the use of a double pole switch. This obviates the necessity of moving a single microphone twenty feet for each observation.

Accurate measurements can be made over shorter distances by using a continuous sound source instead of a succession of impulses. The sound received in the microphone then sets up a series of sinusoidal waves upon the oscilloscope screen. The microphone is moved until one wave passes across the reference line. In this way both the wave-length and the velocity are determined at the same time.

R. C. COLWELL
A. W. FRIEND

DEPARTMENT OF PHYSICS,
WEST VIRGINIA UNIVERSITY

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