[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

Sedimentation Equilibrium of Sucrose in the Simplest Opaque Air-Driven Spinning Tops as Ultracentrifuges

BY JAMES W. MCBAIN AND CLAUDIO ALVAREZ-TOSTADO

The present communication is confined to methods of measuring sedimentation equilibrium in any mechanically immobilized liquid.[1]

All that is necessary to obtain sedimentation of any molecule or particle is that convection is avoided. For sedimentation equilibrium, dimensions and amounts are unimportant except from the standpoint of practical expediency in respect to construction and analytical requirements.

In the measurements here described convection has been avoided in two ways. In the first or limited method, the liquid is immobilized by means of insoluble fibers of soap curd. Sedimentation takes place in the interstices, and successive portions of the curd are finally removed for analysis. This method is limited to molecules and smaller particles that are compatible with soap curd. Its use requires only the simplest one-piece top.[2]

The second method is of universal applicability and is more accurate. It requires a two-piece top inside of which is placed a pile of thin washers enclosed in a rubber sac.[3] These annular washers are all of the same outside diameter, but they are alternately narrow and wide. The narrow ones serve as spacing pieces for the wide ones, producing annular spaces in which the liquid does not convect. A cover is sealed on the top to prevent evaporation. The liquid remains inaccessible either to view or to analysis. The simple device for ascertaining its concentration is to place in the rotor enough liquid to supply a mobile supernatant layer lining the hole through the pile of washers. After equilibrium has been obtained this supernatant liquid is of uniform concentration and is of necessity identical with that portion of the immobilized liquid with which it is in immediate contact. Hence, when the rotor is stopped and the supernatant liquid removed, for analysis by any convenient method, it is found to be less concentrated than the original solution placed in the rotor. From the original and final concentration, the dimensions of the rotor and washers, and the speed, the molecular weight or particle size is determined.

For an electrolyte such as barium chloride or for unbuffered charged particles, this is an average molecular weight such as would be obtained by an osmotic or freezing point method.

For polydisperse solutions, a variant enables analysis to be made of the concentration of the liquid at the top and bottom of the sedimentation column. There is no reason why these methods should not be carried out on any desired scale and with any desired number of samplings throughout the sedimentation column, by adopting the well-known elegant expedient of Beams and Pickels of using a large rotor of any desired depth suspended by a driving wire in a vacuum.

Our observations are of importance in confirming the structure of soap curd as consisting of bundles of ultramicroscopic fibers of soap enmeshing and immobilizing a mother liquor, just like a wet wad of cotton wool. Even hemoglobin sediments freely through soap curd, although about ten times more slowly than through water. The boundary moved 0.46 cm. in sixteen hours at 2000 r. p. s., leaving white curd above with a pink transition zone to a very dark red.

I. The Soap Curd Method

In this method the whole of the liquid is immobilized by adding to it while warm a suitable amount of sodium palmitate or sodium stearate. A 0.2 N sodium palmitate is liquid at 60–70°, but solidifies on cooling to form a structure that is sufficiently near the density of water and strong enough that no sedimentation of the curd fibers themselves is observed. Yet the enmeshed mother liquor can be obtained readily by squeezing the curd in a weighing bottle, using a cork impregnated with paraffin as a piston. The rotor is filled with the curd by adding the hot

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[1] McBain and O'Sullivan, THIS JOURNAL, **57**, 780–1 (1935), last paragraph, 4a; conversion of the rotor, containing washers here described, to the measurement of sedimentation velocity, 4b, requires that a disk with a small hole in the center be placed underneath the pile of washers to distribute heavy liquid added during spinning to raise successive radii of immobilized liquid sufficiently to withdraw them for analysis; simpler is the use of a one-piece top with sectorial baffles cut from annular washers.

[2] McBain and Stuewer, *Kolloid-Z.*, **74**, 10–16 (1936).

[3] Commercial caps for bottles, or "culture caps," obtained from the Davol Rubber Company, Providence, R. I., Catalog Number 1276.

liquid while the top spins slowly. The curd solidifies as it cools forming a solid annular ring. The rotor is weighed before and after filling.

Figure 1 shows a sketch of the one-piece hollow steel rotor (protected by twelve coats of Bakelite lacquer, not varnish, baked on[2]). After the rotor has been filled, a circular cover or window, B, is placed on the step, A.

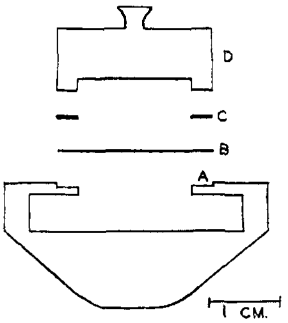

Fig. 1.—One piece hollow steel rotor, A, with cover B, and ring C.

The sealing of the top is accomplished easily and effectively by using a circular disk of commercial moisture-proof Cellophane that has been coated previously with a mixture of equal parts of beeswax and rosin. Alternatively the window may be of platinum, silver, stainless steel, quartz or Bakelite. The adhesive mixture is prepared by melting beeswax and adding an equal weight of rosin. Cellophane is dipped into this molten mixture and allowed to cool with a thin even coating. The aluminum ring, C, is placed on the window. A brass weight, D, is heated and placed on the aluminum ring until the adhesive just melts. The weight is then removed and the adhesive solidifies. Such seals are found to be evaporation proof.

After sealing, the rotor is started at full speed. When the sedimentation equilibrium is attained, the rotor is brought to a stop as smoothly as possible, and the window is removed or cut out. Then the adjustable spoon shown in Fig. 2 (designed by Joseph Grebmeier) is used to cut out successive concentric rings of the curd, each of which is weighed in a stoppered weighing bottle before expressing the mother liquor for analysis.

The distance from the axis of rotation of each of the samples obtained may be calculated as follows. Let

w_i = weight of sample i
W = total weight of curd in the top
v_i = volume of sample i
V = total volume of curd in the rotor
h = height of the rotor
r_i = radius of smaller circle defining the ring for sample i
r_{i+1} = radius of larger circle defining the ring for sample i
R_1 = smaller radius of total curd ring
R_2 = larger radius of total curd ring

Then

$$V = \pi h(R^2_2 - R^2_1)$$
$$v = \pi h(r^2_{i+1} - r^2_i)$$
$$\frac{v_i}{V} = \frac{w_i}{W} = \frac{(r^2_{i+1} - r^2_i)}{(R^2_2 - R^2_1)}$$

or

$$(R^2_2 - R^2_1)\frac{w_i}{W} + r^2_1 = r^2_{i+1}$$

R_1 and R_2 can be measured directly and, since $r_1 = R_1$, we can obtain the two radii defining any given sample. Then, since the rings are fairly thin, we may assume that

$$\bar{r}_i = (r_i + r_{i+1})/2$$

where $\bar{r}_i$ is the point at which the concentration equals the experimentally obtained average concentration of the sample i.

The particle weight is calculated by the familiar formula for binary ideal dilute solutions of nonelectrolytes

$$M = \frac{2RT \ln C_2/C_1}{(1 - \bar{v}d)\omega^2(r^2_2 - r^2_1)}$$

where d is the density of the solution. From the density of the solutions the partial specific volume of the anhydrous solute, $\bar{v}$, may be calculated by any of the methods described by Lewis and Randall.[4] Thus from the concentration at each pair of radii a value is obtained for the molecular weight or particle size. Obviously, results calculated from these consecutive layers are far less accurate than those from a wider range of distance and concentration, although an average of all necessarily minimizes the inaccuracy.[5]

Using the method described above, the sedimentation equilibrium of sucrose solutions was investigated at 20°. The temperature of the rotor is almost exactly that of the slip stream,

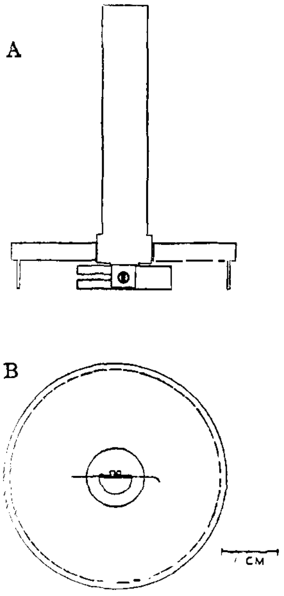

Fig. 2.—Adjustable scraping tool or spoon, for removing concentric samples for analysis: A, longitudinal cross section; B, horizontal cross section.

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[4] Lewis and Randall, "Thermodynamics," McGraw-Hill Book Company, Inc., New York, 1923, p. 36.

[5] The principle is the same as that in a hypothetical attempt to measure a length of 1 cm. which has been divided arbitrarily into ten roughly equal parts. If each such actual length is multiplied by 10, the results might differ very greatly, but the average would be almost exactly 1 cm.

easily determined by a thermocouple. From the formula an error of 1° is only 0.3% in the molecular weight. The angular velocity, with our regulated air supply, also previously described, was constant to within 10 r. p. s. over the whole period of these long runs. The method of analysis was to measure the index of refraction of the solution, using a Zeiss dipping refractometer with an auxiliary prism that makes it possible to determine the concentration of the sucrose solution even when only a small amount of solution is available, even less than 0.03 cc.

When sodium palmitate curd is used as an immobilizing medium, it is necessary to correct the refractometer readings obtained, since a small amount of sodium palmitate is dissolved in the immobilized solution. This correction is obtained by making a reading on the sucrose solution before the curd is made, and one on the liquid obtained by expressing the curd immediately after it is prepared. The difference in the two readings gives the correction that must be applied to other determinations. The use of this correction after sedimentation equilibrium has been reached is quite justified, since it was found that it is the same for a series of solutions of various sucrose concentrations (0 to 4%) but of equal sodium palmitate concentration. The correction, of course, changes with the sodium palmitate concentration, and should be determined for each portion of curd prepared. Three complete experiments are recorded in Table I, to show the practicality of the method. The inside radius of the top, R_1, is 8 mm. and the outside radius, R_2, is 15 mm.

TABLE I

SEDIMENTATION EQUILIBRIUM OF SUCROSE (MOL. WT. 342) IMMOBILIZED WITHIN SOAP CURD

Run 1 Length of run—91 hours. Speed 2000 r, p. s.

Sample	Weight	Refractometer reading Direct	Refractometer reading Corr.	Concn., %	r	M	Mean M
1	0.4289	24.7	23.6	2.24	0.89	424	
2	.4376	26.2	25.1	2.66	1.07	318	
3	1.1926	28.5	27.4	3.24	1.30		
For samples 1 and 3, M = 359							367
Run 2							
1	0.4661	25.3	24.2	2.42	0.93	564	
2	.3432	26.8	25.7	2.82	1.05	181	
3	.3601	27.4	26.3	2.98	1.17	139	
4	.3550	27.9	26.8	3.11	1.28	408	
5	.6233	30.0	28.9	3.66	1.41		
For samples 1 and 3, M = 362; for samples 3 and 5, M = 290; for samples 1 and 5, M = 330							325
Run 3							
1	0.3578	23.8	22.7	2.03	0.88	510	
2	.3090	25.1	24.0	2.37	1.02	490	
3	.3629	26.4	25.3	2.74	1.14	372	
4	.2229	27.3	26.2	3.00	1.23	260	
5	.8244	28.8	27.7	3.37	1.38		
For samples 3 and 5, M = 300; for samples 2 and 5, M = 357.							381

Some preliminary experiments were carried out upon Calgon, commercial Graham's salt or glassy sodium metaphosphate, which showed that this was not monodisperse.

II. Immobilization in Known Annular Spaces

(a) Preliminary Experiments with Glass Beads.—Some qualitative observations of the occurrence of sedimentation were carried out by using fine glass beads in a one-piece top to immobilize a portion of the solution for sedimentation equilibrium, leaving some mobile supernatant liquid as already explained for analysis, the amounts being determined by weighing. The glass beads were held tightly in position by a ring of silver gauze. All the results are consistently low, which we found to be due to the fact that after the rotor is stopped in order that the supernatant liquid may be sampled with a minimum of evaporation, the body of liquid is lying within the beads with a wedge shaped distribution of density extending horizontally outward. Hence, since all the spaces intercommunicate, the wedge begins to upset, denser liquid coming out at the bottom and lighter supernatant liquid replacing it at the top. It is therefore an essential of design that no such redistribution can occur.

(b) The Use of Horizontal Washers as Baffles.—If the baffles consist of a pile of thin washers all of the same outside diameter, but alternately wide and narrow, the outside portion is close-packed metal tightly pressed together, whereas the inner portion consists of annular spaces each of thickness equal to a single washer. A rotor consisting of two pieces of steel screwed together is necessary for their assembly, and hence a rubber sac or equivalent seal is necessary for a water-tight assembly.

In the first experiments the results were much too high when calculated for these annular spaces alone and too low if calculated for all the liquid weighed into the bag of washers, although the mean came within 7% of the theoretical value. It was at once discovered that this was due to an appreciable amount of liquid collecting outside the pile of washers when the rubber was pressed tightly by centrifugal force and hydrostatic pressure against the steel case. This defect was eliminated simply by packing the outside surface of the heap of baffles with an indifferent material so that it fitted tightly inside the rubber sac against the steel shell. At first Bakelite lacquer was used, but this is very inconvenient both to apply and to remove. It was found that a simple plastic coating of the beeswax–rosin adhesive mixture, about 0.5 mm. thick, completely served the purpose and confined the liquid within the pile of washers.

(c) **The Final Design.**—The rotor chosen is shown in Figs. 3 and 4, the latter showing the complete assembly. The washers are of 90% silver, 10% copper, all 30 mm. in outside diameter and 0.07 mm. thick. The inner diameter is 20 mm. for the wide ones and 28 mm. for the narrow ones. Fifty-one narrow washers are laid alternately with fifty wide ones. In assembling the rotor, the cone is held by a screw in the cylindrical space left in the bottom, and the upper steel shell is tightened by means of a strap wrench. A disk of German silver is placed upon the rotor cone underneath the rubber sac enclosing the washers, to prevent catching or cutting the rubber.

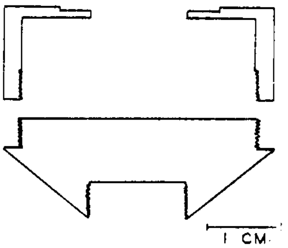

Fig. 3.—Two piece top.

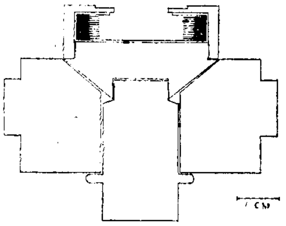

Fig. 4.—Two piece top, complete assembly in holding jig.

It is important that the hole in the upper steel shell as well as in the rubber sac should be of smaller radius than the meniscus of supernatant liquid within the pile of washers, in order that it may directly bear the whole hydrostatic pressure. We had expected to use a soft wire of platinum or silver bent into vertical zigzags to lay inside the pile of washers to check undue convection in stopping the rotor, but it has not proved necessary.

(d) **The Method of Calculation.**—The initial concentration obtained experimentally is not the true initial concentration of the immobilized portion of the solution that alone must be considered, since the immobilized solution is enriched by a transfer of solute from the supernatant portion. The corrected initial concentration is given by

$$C_0^c = C_0 + \frac{W_s}{W_i}(C_0 - C_f)$$

where

C_0^c = corrected initial concentration
C_0 = initial concentration of the supernatant solution
W_s = weight of supernatant solution
W_i = weight of immobilized solution
C_f = final concentration of supernatant solution

To calculate the molecular weight of the solute from the initial and final concentrations thus obtained, a suitably modified form of the equilibrium equation must be used.

Proceeding from the familiar equation for sedimentation of uncharged ideal solutes

$$\mathrm{d}c/c = M\frac{(1 - \bar{V}d)}{RT}\omega^2 \cdot r\mathrm{d}r \qquad (1)$$

setting

$$K = (1 - \bar{V}d)\omega^2/2RT \qquad \mathrm{d}c/c = MK2r\mathrm{d}r \qquad (1a)$$

Solving this differential equation for c we find

$$c = c_1e^{MK(r^2-r_1^2)} \qquad (2)$$

where c_1 is the concentration at a point r_1 cm. from the center of rotation.

Now if we let n = total amount of sucrose in the solution in the top, and V the volume of solution

$$n = c_0V$$

But since

$$V = h(r_2^2 - r_1^2) \text{ for an annular segment}$$

$$n = c_0\pi h(r_2^2 - r_1^2) = \int_V c\mathrm{d}V \text{ where } c = \mathrm{f}(r)$$

and

$$V = \mathrm{f}(r) = \pi h(r_2^2 - r_1^2)$$

Thus

$$\mathrm{d}V = 2\pi hr\mathrm{d}r$$

and as shown in (2)

$$c = c_1e^{MK(r^2-r_1^2)}$$

Therefore

$$n = c_0\pi h(r_2^2 - r_1^2) = \int_{r_1}^{r_2} c_1e^{MK(r^2-r_1^2)}2\pi hr\mathrm{d}r \qquad (3)$$

or simplifying

$$c_0(r_2^2 - r_1^2) = \int_{r_1}^{r_2} c_1e^{MK(r^2-r_1^2)}2r\mathrm{d}r$$

or

$$c_0(r_2^2 - r_1^2) = c_1/e^{MK(r^2-r_1^2)}\int_{r_1}^{r_2} e^{MKr^2}\mathrm{d}(r^2) \qquad (3a)$$

Integrating (3a)

$$c_0(r_2^2 - r_1^2) = (c_1/e^{MKr_1^2})(1/MK)(e^{MKr^2})\Big|_{r_1}^{r_2}$$
$$= (c_1/MKe^{MKr_1^2})(e^{MKr_2^2} - e^{MKr_1^2})$$
$$= c_1/MK(e^{MK(r_2^2-r_1^2)} - 1) \qquad (4)$$

Rearranging

$$e^{MK(r_2^2-r_1^2)} - c_0/c_1MK(r_2^2 - r_1^2) - 1 = 0 \qquad (5)$$

This is the basic equation, since it gives M in terms of measurable quantities, but it is extremely difficult to solve directly for M. Equation (5) may, however, be solved by three methods; two are graphical and the third is approximate.

(a) The first graphical method is to plot c_0/c_1 as a function of $MK(r_2^2-r_1^2)$. That is, if we let $MK(r_2^2-r_1^2) = y$ and rearrange (5), we obtain

$$c_0/c_1 = (e^y - 1)/y \qquad (6)$$

We can then take arbitrary values for y and plot $c_0/c_1 = \text{f}(y)$. When c_0/c_1 is then obtained experimentally, we can read off the graph the numerical value of y corresponding to this c_0/c_1 and obtain M easily from the equation

$$M = y/K(r_2^2 - r_1^2)$$

(b) Rearranging equation (5) we obtain

$$e^{MK(r_2^2-r_1^2)} = \frac{c_0}{c_1}MK(r_2^2 - r_1^2) + 1 \qquad (5a)$$

where for any given run, K, r_2, r_1, and c_0/c_1 are known.

Then we can plot the curves

$$Z = \text{f}(M) = e^{MK(r_2^2-r_1^2)}$$
$$Z = \text{f}(M) = \frac{c_0}{c_1}MK(r_2^2 - r_1^2) + 1$$

These two curves will intersect and at the point of intersection $\text{f}(M) = \text{f}(M)$, and thus the value for M at this point will be a solution of equation (5a).

(c) For the approximate method we rearrange equation (6) and obtain

$$e^y - \frac{c_0}{c_1}y - 1 = 0 \qquad (6a)$$

But

$$e^y = 1 + y + \frac{y^2}{2!} + \frac{y^3}{3!} + \dots$$

we may substitute this series in (6a) neglecting terms for which the power of y is greater than 3. (6a) becomes

$$1 + y + \frac{y^2}{2} + \frac{y^3}{6} - \frac{c_0}{c_1}y - 1 = 0$$

or collecting and simplifying

$$y^3 + 3y^2 + 6(1 - c_0/c_1)y = 0 = y^2 + 3y + 6 - 6c_0/c_1 \qquad (6b)$$

solving (6b) for y, we obtain

$$y = \frac{-3 \pm \sqrt{9 + 24c_0/c_1 - 24}}{2} = \frac{-3 \pm \sqrt{24c_0/c_1 - 15}}{2} \qquad (7)$$

But since $y = MK(r_2^2 - r_1^2)$, we can solve for M and obtain

$$M = \frac{-3 + \sqrt{24c_0/c_1 - 15}}{2K(r_2^2 - r_1^2)}$$

Methods (a) and (b) give an accurate solution of equation (5), but (a) is by far the more convenient since only one curve is required for all experiments. Method (b) requires that two curves be drawn for every run made. Method (c) affords a good approximation, but only when $y<1$. For larger values of y its accuracy decreases rapidly. It is satisfactory for the experiments on sucrose here described. Measurement of the difference of radius of the wide and narrow washers, r_2-r_1, is made to any desired accuracy with a micrometer. The washers are made simultaneously, pressed together in a jig, turned on the outside like a solid cylinder, and then bored before the whole pile is released.

(e) **Experimental Results for Sucrose.**—In Table II are given the complete data for three runs with 3.11% aqueous sucrose. The theoretical molecular weight is obtained within an error of 3.2, 0.9, and 3.2%, respectively. It is evident that the method is successful and that it is of general applicability for monodisperse solutions or particles of whatever size up to the largest viruses and colloidal particles.

TABLE II

MEASUREMENT OF THE MOLECULAR WEIGHT OF SUCROSE BY SEDIMENTATION EQUILIBRIUM IN THE OPAQUE STEEL TWO-PIECE TOP CONTAINING A PILE OF THIN WASHERS

	Run 1	Run 2	Run 3
Wt. of immobilized soln.	0.4370	0.4976	0.6385
Wt. of supernatant soln.	.0895	.1855	.0900
Refractometer reading—initial	26.8	26.8	25.8
Initial concentration	3.11	3.11	2.92
Refractometer reading—final	25.8	25.9	24.2
Final concentration	2.84	2.87	2.55
Corrected initial concn.	3.16	3.20	2.97
Ratio of concns., c_0/c_1	1.112	1.115	1.164
Rotor speed, r. p. s.	1800	1800	1800
Length of run, hours	96	117	98
Molecular weight, known	342	342	342
Molecular weight obtained	331	339	353

Summary

A general method of measuring sedimentation equilibrium of solutions or monodisperse particles in a simple opaque two-piece steel rotor, where the contents are directly accessible for chemical, physical or biological analysis, has been developed.

Sedimentation occurs normally within soap curd, confirming that the curd fibers enmesh the continuous aqueous mother liquor.

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[CONTRIBUTION FROM THE INSTITUTE OF INORGANIC CHEMISTRY TECHNICAL UNIVERSITY OF NORWAY]

Fusion Point, Vapor Pressure and Heat of Evaporation of Vanadium Oxytrichloride

BY H. FLOOD, J. GØRRISSEN AND R. VEIMO

In connection with investigations relating to the formation of vanadium oxytrichloride during chloridizing roasting, the authors attempted to determine the fusion point, vapor pressure and heat of evaporation of this compound.

Vanadium oxytrichloride was prepared by conducting chlorine over vanadium pentoxide and cleaning the product in a current of nitrogen followed by a fractional distillation.

Fusion Point.—The fusion point of vanadium oxytrichloride has not been determined previously. H. E. Roscoe[1] (1870) states that the compound does not solidify when cooled down to −15°, while F. E. Brown and F. A. Griffitts[2] (1934) only verify that it is still liquid at −77°.

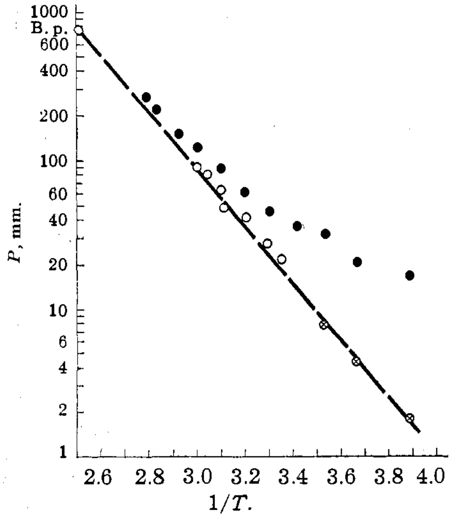

Fig. 1.—●, F. E. Brown, F. A. Griffitts; ○, ⊗, H. Flood, J. Gørrissen and K. Veimo.

By cooling our preparation with liquid air in a vessel surrounded by ethyl alcohol, a well-defined freezing point was recorded at −79.5° by means of a calibrated pentane thermometer.

Vapor Pressure.—The vapor pressure was determined by two methods, *viz.*, by comparative measurements based on saturated water vapor in the temperature range of 20–60° and for the range of −15 to +15° by conducting dry nitrogen gas over the preparation and determining by analysis the content of vanadium oxytrichloride per liter of gas. The analysis was made by reduction with hydrogen sulfide and titration by potassium permanganate.

The weakness of the first method is due to the ease with which the mercury of the manometer is attacked by the vanadium oxytrichloride, which produces errors on account of capillary depression and also possibly by the formation of volatile products of reaction.

In Fig. 1 are the observed vapor pressures plotted in a logarithmic scale in relation to the reciprocal of the absolute temperature ($1/T$), the open circles representing the values obtained by comparative measurements against saturated water vapor, while the crossed circles were established from tests in the nitrogen current.

After the authors had commenced their investigations, their attention was called to a communication from F. E. Brown and F. A. Griffitts[2] where data on vapor pressure of vanadium oxytrichloride are given for the temperature range of −77 to +80°. These data also have been included in Fig. 1 and are represented with full circles.

As will be seen from the plotting on Fig. 1 there is a considerable discrepancy between the latter data and those of the authors.

As the tensions determined by Brown and Griffitts show an abnormal variation with temperature (the heat of evaporation *decreases* rapidly with falling temperature) it is likely that their measurements are obscured with errors due to attack of manometer mercury by vanadium oxytrichloride.

Heat of Evaporation.—If a straight line is drawn through the plotted tension–temperature

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[1] H. E. Roscoe, from J. W. Mellor, "Inorganic and Theoretical Chemistry," Vol. IX, Longmans, Green & Co., New York, N. Y., 1929, p. 807.

[2] F. E. Brown and F. A. Griffitts, *Iowa State Coll. J. Sci.*, **9**, 89 (1934).