atom migration cannot occur, the 2,2-dimethylbutyl radical was generated by the peroxide-catalyzed decomposition of 3,3-dimethylpentanal in the liquid phase at 130°. Again only the unrearranged 2,2-dimethylbutane was isolated.[4] These observations provide additional examples of the stability of saturated free radicals at moderate temperatures and emphasize the special ability of the aromatic nucleus to undergo 1,2-migration in radical systems.

Support of this research by a grant from the Research Corporation is gratefully acknowledged.

Experimental

Cyclohexanecarboxaldehyde.—The acetal prepared by the interaction of cyclohexylmagnesium chloride and ethyl orthoformate was hydrolyzed by stirring with excess 10% hydrochloric acid. After ten minutes the acid was neutralized by the addition of solid sodium bicarbonate, the aldehyde was extracted with ether, and the dried extract was distilled through a 12-inch Vigreux column to yield the desired aldehyde, b.p. 36° (10 mm.), $n^{20}D$ 1.4496.[5]

Decomposition of the aldehyde (6.8 g., 0.061 mole) catalyzed by di-*t*-butyl peroxide (2.9 g., 0.020 mole) for 5.5 hours at 130 ± 5° yielded 93% of the theoretical amount of carbon monoxide. Fractionation of the liquid product, including that condensed in a Dry Ice-cooled trap placed in the gas outlet line, through a Piros–Glover spinning band column at atmospheric pressure yielded no methylcyclopentane, but 0.032 mole of cyclohexane, $n^{20}D$ 1.4260 (lit. $n^{20}D$ 1.4262),[6] was isolated from its azeotrope with the *t*-butyl alcohol produced in the peroxide decomposition. Almost identical results were obtained by decomposition of a 2 *M* solution of the aldehyde in chlorobenzene.

Cyclopentaneacetaldehyde.—Cyclopentaneacetic acid, prepared in 58% yield from cyclopentyl bromide and diethyl malonate, was treated with thionyl chloride to yield the acid chloride, b.p. 55° (10 mm.), amide m.p. 145.5–146.5° (lit. m.p. 143–145°[7]), which was hydrogenated in *p*-cymene at 65–85° over palladized barium sulfate using a sulfur–quinoline inhibitor. The aldehyde was separated as its bisulfite addition compound, which was washed with ether and decomposed by stirring with 10% aqueous sodium carbonate. Steam distillation of the mixture yielded the aldehyde, b.p. of azeotrope 94–96°, which was dried over sodium sulfate and used without further purification. The yield was 33% based on the acid chloride.

Decomposition of the aldehyde (18.4 g., 0.164 mole) catalyzed by di-*t*-butyl peroxide (7.3 g., 0.050 mole) at 132 ± 5° for 4.0 hours yielded 76% of the theoretical amount of carbon monoxide. The entire liquid product was steam distilled to a boiling point of 98°, the organic portion was taken up in toluene, washed with water to remove *t*-butyl alcohol and dried over anhydrous potassium carbonate. Fractionation of this solution through the Piros–Glover column yielded 0.070 mole of methylcyclopentane, b.p. 70° (uncor.), $n^{20}D$ 1.4098, d^{25} 0.740 (lit. b.p. 71.8°, $n^{20}D$ 1.4097, d^{25} 0.744[6]), as the only product of boiling point lower than that of the toluene added as solvent.

3,3-Dimethylpentanal.—This aldehyde, b.p. 132–135°, $n^{20}D$ 1.4299, was prepared by the method of Schmerling.[8]

The peroxide-catalyzed decomposition of the aldehyde as described above gave as the only six-carbon atom product 2,2-dimethylbutane, b.p. 47° (uncor.), $n^{20}D$ 1.3688 (lit. b.p. 49.7°, $n^{20}D$ 1.3688[6]), in 75% yield based on the carbon monoxide evolved.

(4) W. H. Urry and N. Nicolaides, THIS JOURNAL, **74**, 5163 (1952), likewise found only 2,2-dimethylbutane as the product of the cobaltous chloride-catalyzed reaction of ethylmagnesium bromide with 1-chloro-2,2-dimethylbutane.

(5) O. Wallach and E. Isaac, *Ann.*, **347**, 331 (1906), report $n^{20}D$ 1.4495.

(6) National Bureau of Standards, "Selected Values of Properties of Hydrocarbons," Washington, D. C., 1949.

(7) O. Wallach and K. Fleischer, *Ann.*, **353**, 304 (1907).

(8) L. Schmerling, THIS JOURNAL, **68**, 1650 (1946).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

Variation of the Sedimentation Coefficient with Time during a Single Velocity Ultracentrifuge Experiment[1]

BY ROBERT A. ALBERTY

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In the case of substances for which the sedimentation coefficient (s) depends appreciably upon concentration (c), s varies with time during a single ultracentrifuge experiment as a result of the progressive dilution which occurs in the plateau region of concentration in the sector-shaped cell. Since the decrease in concentration amounts to about 27% per cm. of boundary movement in the present ultracentrifuges the variation of s must be taken into account in order to utilize all of the accuracy with whch velocity ultracentrifuge measurements may be made. By use of the differential equation for the ultracentrifuge, s may be expressed as a power series in time for any particular relation between s and c. Lauffer has given data for tobacco mosaic virus illustrating this effect and his data are used as a test of the theory. The calculation of the sedimentation coefficient corresponding to the concentration of the solution placed in the ultracentrifuge cell is discussed.

Introduction

Svedberg[2] defined the sedimentation constant s by

$$s = \frac{dx}{dt} \Big/ \omega^2 x \qquad (1)$$

where dx/dt is the velocity of transport of a component relative to the centrifuge cell, ω is the angular velocity of the rotor and x is distance from the axis of rotation. Since s may vary considerably with concentration there are advantages in referring to it as the sedimentation coefficient. Although the objective of a velocity ultracentrifuge experiment is to determine the rate of sedimentation of a component in the region of homogeneous solution, the actual calculations are based upon plots of refractive index gradient as a function of position in the cell. Of the several properties of the refractive index gradient curve which might be used in calculating the position of the boundary, Goldberg[3,4] has shown that the correct position for measuring the sedimentation coefficient of the molecules in the plateau region is given by the square root of the

(1) Presented before the Division of Physical Chemistry at the 124th Meeting of the American Chemical Society, Chicago, September 8, 1953.

(2) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford University Press, New York, N. Y., 1940.

(3) R. J. Goldberg, *J. Phys. Chem.*, **57**, 194 (1953).

(4) See also R. Trautman and V. Schomaker, *J. Chem. Phys.*, **22**, 551 (1954).

TABLE I

CONCENTRATION DEPENDENCE OF THE SEDIMENTATION COEFFICIENT (s) AT 20° REPRESENTED BY $s = s_0/(1 + kc)$ AND PERCENTAGE CHANGE OF s PER CM.

Substance	Solvent	pH	s_0 (Svedbergs)	k (100 cc./g.)	Range of c_i (g./100 cc.)	$100\Delta s/s_i^a$ per cm., %
Albumin, bovine plasma[7]	0.15 M NaCl 0.02 M NaAc 0.03 M HAc	4.4	4.27	0.049	0.4 -2.45	2.9
Cellulose nitrate No. 6[9]	Cuprammonium		18.35	5.37	0.05–0.2	16.7
Cotton, raw Georgia[9]	Cuprammonium		12.5	12.15	0.05–0.3	27.1
Fibrinogen, bovine[10]	0.40 M NaCl 0.05μ phos.	6.2	7.80	0.088	0.05–0.4	0.8
Gamma globulin, human[11]	0.1μ phos.	7.0	6.57	0.054	0.23–1.33	1.9
Gelatin[12]	2 M KSCN		2.14	−0.241	0.5 -1.2	−6.6
Hemoglobin, human carbon monoxide[7]	0.0286 M K_2HPO_4 0.0143 M KH_2PO_4	7.07	4.36	0.050	0.12–2.0	2.5
Myosin[13]	0.6 M KCl 0.01 M phos.	6.5	6.17	1.42	0.15–0.75	16.4
Nitrocellulose e.h.[14]	Acetone		17.4	2.82	0.05–0.5	18.5
Ovalbumin[7]	0.15 M NaCl 0.02 M NaAc 0.03 M HAc	4.4	3.66	0.104	0.13–2.17	5.4
Polystyrene No. 7[8]	$CHCl_3$		15.4	4.0	0.01–0.5	17.3
Thymus nucleic acid[15]	0.2 M NaCl	7	12.2	3.05	0.03–0.5	20.0
Virus, Influenza[16] Lee Strain	0.1 M phos.	7.1	80.6	0.0954	0.3 -1.2	2.9
Virus, southern bean mosaic[17]	0.1 M phos.	7.1	115.5	0.0388	0.4 -3.4	3.0
Virus, tobacco mosaic[18]	0.1 M phos.	7	185.2	0.278	0.05–1.8	10.0

[a] Percentage increase in s corresponding to a boundary displacement of one cm. ($x_0 = 5.8$ cm.) for the highest concentration studied.

second moment of the gradient curve. The term "plateau region" is used to refer to the region in which concentration is independent of distance from the axis of the centrifuge.

During a velocity ultracentrifuge experiment the concentration in the plateau region changes with time since the centrifugal field and the cross sectional area of the cell are proportional to distance from the axis. Consequently the sedimentation coefficient of the molecules in this region may increase (or in special cases decrease) appreciably during the experiment, depending upon the relation between s and concentration, c (weight per unit volume). The magnitude of the dilution was first discussed quantitatively by Svedberg and Rinde[2,5] for the case that s is independent of c. Their equation gives the concentration in the plateau region as a function of the boundary position.

$$\left(\frac{c_t}{c_i}\right) = \left(\frac{x_i}{x_t}\right)^2 \qquad (2)$$

where c_i and c_t are the concentrations in the plateau region initially and at time t and x_i and x_t are the corresponding boundary positions. Trautman[4] has shown that this equation is applicable even when s is dependent upon c, provided that the boundary position is calculated in the correct way.[3,4] The decrease in concentration during an experiment with the standard velocity ultracentrifuges is 27% if the boundary moves through a distance of one centimeter (x_i = 5.8 cm., x_t = 6.8 cm.). Thus in the case of substances for which s varies appreciably with c, the change in s during an experiment must be taken into account in order to take full advantage of the accuracy with which velocity ultracentrifuge measurements may be made. In determining the relation between s and c the average value of the concentration in the cell during the experiment rather than the concentration of the initial solution has been used.[6,7]

The variation of s with c may be represented by either of the following power series expansions

$$s = s_0/(1 + kc + k'c^2 + \ldots) \qquad (3)$$

$$s = s_0(1 - lc - l'c^2 + \ldots) \qquad (4)$$

Equation 3 has been considered to be preferable from a theoretical standpoint since this is the form which is obtained if the frictional coefficient is expanded as a power series in concentration.[8] The coefficients in these equations are related by $k = l$ and $k^2 - k' = -l'$. Thus equation 3 with terms in c^2 and higher omitted is equivalent to equation 4 with l^2 as the coefficient of c^2. Equation 3 with two constants (s_0 and k) has probably been most used for representing experimental data, and the parameters for a number of substances are given in

(5) T. Svedberg and H. Rinde, THIS JOURNAL, **46**, 2677 (1924).

(6) I. Jullander, *Arkiv. Kemi, Mineral. Geol.*, **21A**, 61 (1945).

(7) G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951).

(8) S. Newman and F. Eirich, *J. Colloid Sci.*, **5**, 541 (1950).

(9) N. Gralén, Uppsala, Ph.D. Thesis, 1944.

(10) S. Shulman, personal communication.

(11) J. R. Cann, THIS JOURNAL, **75**, 4213 (1953), and personal communication.

(12) E. O. Kraemer, *J. Phys. Chem.*, **45**, 660 (1941).

(13) G. L. Miller and R. H. Golder, *Arch. Biochem. Biophys.*, **41**, 125 (1952).

(14) I. Jullander, Uppsala, Ph.D. Thesis, 1945, p. 55.

(15) R. Cecil and A. G. Ogston, *J. Chem. Soc.*, 1382 (1948).

(16) G. L. Miller, *J. Biol. Chem.*, **169**, 745 (1947).

(17) G. L. Miller and W. C. Price, *Arch. Biochem. Biophys.*, **10**, 467 (1946).

(18) M. A. Lauffer, THIS JOURNAL, **66**, 1195 (1944).

Table I along with the percentage increases in s per centimeter of boundary movement at the highest concentration studied.

Lauffer[18] has given data for an experiment with tobacco mosaic virus which clearly demonstrates the change of s with time. In an experiment at 11,100 r.p.m. with a solution containing 1.335 g. virus/100 ml. the values of s calculated from successive photographs at 5 minute intervals could be represented by

$$s = 144.3 + 0.00169t \quad (5)$$

where t is time in seconds and s in Svedbergs. The temperature of the rotor was 25.3° at the beginning and 25.7° at the end of the experiment.

The objective of the following theoretical development is to express the time dependence of s during a single experiment in terms of the relation between s and c.[19] Kinell[20] has discussed the effect of the dilution during a single experiment upon the measured sedimentation coefficient, but his equations are based on an incorrect form of the dilution law and are not in agreement with the relations described here. Golder[21] has given one method for obtaining information concerning the variation of s with c from the data of a single ultracentrifuge experiment.

Theory

The sedimentation coefficient at time t may be represented by means of the following power series in t in which s_i is the sedimentation coefficient corresponding to the concentration of the initial solution.

$$s_t = s_i(1 + at + bt^2 + \ldots) \quad (6)$$

The objective of the following calculations is to obtain a and b in terms of the coefficients of equations 3 or 4, the initial concentration, and the speed of the ultracentrifuge.

If s is a known function of c, it is only necessary to be able to express c in the plateau region as a function of time in order to obtain s as a function of time. This may be done by use of the differential equation[22] for velocity sedimentation in a sector-shaped cell which reduces to the following simple form in a region in the cell where there are no concentration gradients

$$\frac{\partial c}{\partial t} = -2\omega^2 sc \quad (7)$$

If $s = s_0/(1 + kc)$, integration of equation 7 from c_i at zero time to c_t yields

$$\ln \frac{c_t}{c_i} + k(c_t - c_i) = -2s_0\omega^2 t \quad (8)$$

If $k = 0$ this equation represents an exponential decrease in concentration in the plateau region with time while, if k is very large, the concentration approaches a linear function of time. If flotation occurs rather than sedimentation, the concentration in the plateau region increases with time. Since for the usual velocity ultracentrifuges $1 > c_t/c_i > 0.7$, the logarithmic term may be represented quite well by a power series and the following expression for $\Delta c = (c_t - c_i)$ is obtained from equation 8 if only terms in t and t^2 are retained.

$$\Delta c = -2c_i s_i \omega^2 t(1 - s_i\omega^2 t/s_0 + \ldots) \quad (9)$$

For low concentrations the same relation is obtained by introducing $s = s_0(1 - lc)$ into (7) and integrating, as is expected. The inclusion of the c^2 term in equation 3 simply introduces an additional factor of $(1 - k'c_i^2)$ in the second term of equation 9.

In order to express s as a function of Δc, $s = s_0/(1 + kc)$ is expanded as a Taylor series about c_i.

$$s = s_i[1 - s_i k(\Delta c)/s_0 + s_i^2 k^2(\Delta c)^2/s_0^2 - \ldots] \quad (10)$$

Introducing Δc from equation 9 yields s as a power series in t, and it is found that the coefficients in equation 6 are given by

$$a = 2kc_i s_i^2\omega^2/s_0 \quad (11)$$

$$b = -as_i^2\omega^2(1 - 2kc_i)/s_0 \quad (12)$$

The same expressions are obtained for $s = s_0(1 - lc)$ except the s_i^2 in equations 11 and 12 are replaced by s_0^2. If the term in c^2 in equation 3 is retained the value of k in the expression for a must be replaced by $(k + 2k'c)$, and b is also altered.[23] Of course, the same values for a and b will be obtained whether equation 3 or equation 4 is used to represent the experimental data, provided that an adequate number of terms is used.

Since Lauffer[1] has determined the change in s with t for an experiment with tobacco mosaic virus, it is of interest to compare his experimental result with that expected from the values of s_0 and k. The value of s_0 for the conditions of Lauffer's experiment (25.3°, 0.1 M phosphate buffer, pH 7) may be calculated in the usual way[24] employing the viscosity of the solvent and using $(s_0)_{20,w} = 185.2$ Svedbergs in water at 20°[18] and a partial specific volume of 0.728 cm.^{3} g.$^{-1}$, the average value[25] in the temperature range 26.8–28.7°. The resulting value of $(s_0)_{25.3,phos.} = 201$ S together with $c = 1.335$ g./100 ml. and $k = 0.278$[18] yields for equation[6]

$$s = 146 + 0.00157t - 8.5 \times 10^{-6}t^2 \quad (13)$$

where t is in seconds and s in Svedbergs. Since the experiment was completed in 160 minutes the t^2 term is negligible.

The agreement between theory and experiment can be improved by taking into consideration the effect of the 0.4° rise in temperature during the experiment. The effect of a small temperature change is largely due to the change in viscosity of the solvent. In the neighborhood of 25° the relative viscosity of water increases 2.3% per degree. Assuming the temperature rises 0.40° in 160 minutes at a constant rate, s_0 may be represented as a function of time by

$$s_0 = 201(1 + 9.59 \times 10^{-7}t) \quad (14)$$

(19) Dr. R. L. Baldwin has called my attention to the fact that since viscosity of the medium, specific volume of the protein and density of the solution vary with pressure, and therefore with distance in the cell, the sedimentation coefficient of the protein will also vary with position in the cell. For aqueous solutions, these effects are small (*cf.* Svedberg and Pedersen, p. 38) and they will not be discussed here.

(20) P. Kinell, *J. chim. phys.*, **44**, 53 (1947).

(21) R. H. Golder, THIS JOURNAL, **75**, 1739 (1953).

(22) O. Lamm, *Arkiv Math. Astron. Fysik*, **21B**, No. 2 (1929); ref. 1, p. 20.

(23) Values of k and k' for nitrocellulose in ethyl acetate have been given by S. Newman, L. Loeb and C. M. Conrad, *J. Polymer Sci.*, **10**, 463 (1953), who also describe a convenient method for determining these constants.

(24) Reference 1, equation (63), p. 36.

(25) M. A. Lauffer, THIS JOURNAL, **66**, 1188 (1944).

The use of equation 14 for s_0 in equation 6 yields

$$s = 146 + 0.00171t \qquad (15)$$

The coefficient of t resulting from this calculation may be compared with the value of 0.00169 obtained experimentally by Lauffer. Thus the computed variation of s with time is in excellent agreement with that obtained experimentally.

Table II gives the predicted rates of increase of s with t for experiments at 25° with tobacco mosaic virus at other concentrations. It is of interest to note that the absolute and relative rates of increase of s with t are smaller at lower concentrations although the slope of the s *versus* c plot is greater under these conditions.

TABLE II

EFFECT OF CONCENTRATION OF TOBACCO MOSAIC VIRUS ON THE VARIATION OF SEDIMENTATION COEFFICIENT DURING A SINGLE EXPERIMENT (25°)

c_i (g./100 ml.)	$as_i \times 10^{13}$, (at ω = 1160 sec.$^{-1}$)	$100\Delta s/s_i$ per cm., %
0.1	0.00028	0.5
0.2	.00052	1.4
0.5	.00102	3.5
1.0	.00145	6.2
1.5	.00160	8.5
1.8	.00162	10.0

Discussion

In calculating the sedimentation coefficient corresponding to the concentration of the initial solution it is desirable to make use of the data for the entire experiment. If b is small enough so that the t^2 term in equation 6 may be neglected the following equation is obtained by introducing equation 1 into equation 7 and integrating from x_i (meniscus position) at $t = 0$ to x at time t.

$$\frac{1}{\omega^2 t} \ln (x/x_i) = s_i + (a/2)t \qquad (16)$$

Since a plot of $\ln (x/x_i)/\omega^2 t$ *versus* t is expected to be linear, the extrapolation back to $t = 0$ is guided. If the variation of s with time is sufficiently great it is necessary to use the effective time of centrifuging at speed ω rather than simply the time at full speed. The time measured from the time the centrifuge reaches full speed may be corrected by plotting log x for the first few photographs *versus* t and extrapolating to log x_i to find the effective time of centrifuging before reaching full speed.

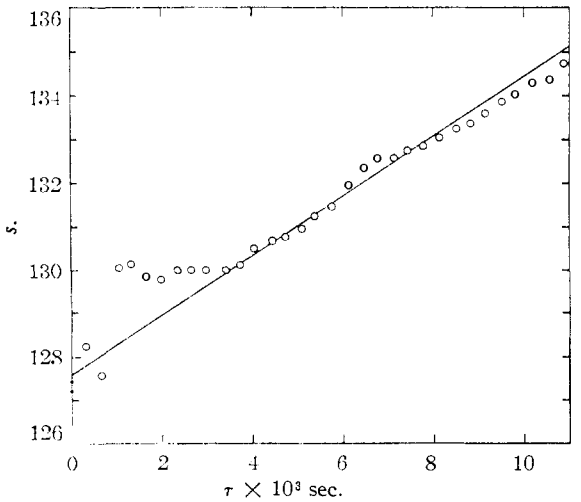

Fig. 1.—Calculation of s_i for Lauffer's experiment with tobacco mosaic virus using viscosity corrected time to allow for a linear rise in temperature. The line through the experimental points has been drawn with a slope $(0.00157/2)$ $(\eta_{25.3}/\eta_{20})$ as computed in equation 13.

If the temperature variation during a velocity ultracentrifuge experiment is negligible considering the accuracy which is desired, equation (16) may be used, but if the temperature variation is greater its effect on the viscosity must be taken into consideration. Oncley[26] recommended a method for correcting elapsed time for viscosity, and Cecil and Ogston[27] have described a similar method for doing this which amounts to correcting increments of time, Δt, for the average viscosity of the solvent during the interval. A similar device may be used in the present case since equation 6 may be written in terms of a sedimentation coefficient corrected to 20° and the ratio of viscosities of water at 20° and the cell temperature T.

$$\frac{\partial c}{\partial t} = -2\omega^2 c s_{20}(\eta_{20}/\eta_T) \qquad (17)$$

When this equation is integrated for any particular relation between s_{20} and c, t may be replaced by $\int_0^t (\eta_{20}/\eta_T)dt$, which may be evaluated experimentally from

$$\tau = \sum_{t=0}^{t} (\eta_{20}/\eta_T)\Delta t \qquad (18)$$

Thus all the earlier equations in this article may be written in terms of τ rather than t if the temperature is not constant. When this is done equation 16 yields s_i at 20°, but this value still has to be corrected for density factors.

Figure 1 gives an example of such a calculation. In the absence of exact information as to the meniscus position in this experiment, time has simply been measured from the first photograph so that s_i corresponds to the concentration in the plateau region at that time. The smooth fluctuations of this plot suggest that there were fluctuations in temperature or rotor speed which produced errors larger than the errors in the measurement of boundary position. Recent thermopile[28] and thermodynamic[29] measurements indicate that the actual rotor temperature in the Spinco ultracentrifuge at 60,000 r.p.m. is about 1.0° below that calculated from the rotor temperatures before and after the experiment.

It is felt that the use of methods such as the one described in this article will increase the precision with which the variation of sedimentation coefficients with concentration may be measured, a subject which is of increasing theoretical interest.[21,30] While this article has been solely concerned with sedimentation in solutions containing a single high molecular weight component, it may be mentioned

(26) J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941).
(27) R. Cecil and A. G. Ogston, *Biochem. J.*, **43**, 592 (1948).
(28) D. F. Waugh and D. A. Yphantis, *Rev. Sci. Instr.*, **23**, 609 (1952).
(29) G. Kegeles, personal communication.
(30) M. Wales and K. Van Holde, *J. Polymer Sci.*, in press.

that the variation of s with t for the slower component in an experiment with a mixture such as BSV and TMV,[31] in which the boundary does not follow the square law because of the Johnston–Ogston[32] effect, would not be expected to follow the present equations.

(31) W. F. Harrington and H. K. Schachman, THIS JOURNAL, **75**, 3533 (1953).

(32) J. P. Johnston and A. G. Ogston, *Trans. Faraday Soc.*, **42**, 789 (1946).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY]

Thermodynamic Measurements of Ultracentrifuge Rotor Temperature

BY AMILCARE BIANCHERIA[1] AND GERSON KEGELES

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Recent studies in hydrogen- and vacuum-operated ultracentrifuges disagree with earlier results from oil turbine instruments, presumably due to uncertainties in the rotor temperature during the earlier measurements. However, Waugh and Yphantis, using a radiation thermocouple, report that the vacuum-operated rotor cools 1° upon acceleration to 60,000 r.p.m., a reversible effect which had not been taken into account, and which has been contradicted in other recent investigations. In our study, seven times crystallized diphenyl ether (m.p. 26.83°) was frozen in a clean cell and melted slightly at 60,000 r.p.m. A sharp meniscus was formed at the liquid–solid interface. From photographs, the hydrostatic pressure at this melting surface was calculated. With privately communicated Bureau of Standards data for the effect of pressure on the melting point the temperature of the cell was readily determined. An average heating effect of 0.59° was found when the rotor decelerated from 60,000 to 30,000 r.p.m. and 0.9° when the rotor was decelerated from 60,000 r.p.m. to rest. Our results, based on an independent physical method, therefore agree with those of Waugh and Yphantis.

Introduction

Recent studies of proteins in oil turbine and high vacuum ultracentrifuges have revealed unexpectedly large discrepancies in measured sedimentation constants.[2–6] These discrepancies have been attributable in part to insufficient accuracy in the calibration of the rotor temperature in the oil-turbine instruments.[2,3,5] Such calibrations are difficult to obtain because the rotor, operated in a low pressure hydrogen atmosphere, reaches a steady state of temperature above that of the chamber, whereas any radiation thermocouple employed to measure rotor temperature is affected by the conductivity of the hydrogen as well as bombardment by gas molecules leaving the surface of the rotor. Thus the thermocouple calibration becomes very sensitive to its position, as well as to the hydrogen pressure[3] and it should also be very sensitive to contamination of the chamber atmosphere with gaseous impurities having a relatively large influence on the viscosity and conductivity of hydrogen.

It was believed that for these reasons, the temperature of a rotor isolated in a high vacuum would be more reliably known. With the advent of the vacuum-operated electrically driven Spinco ultracentrifuge,[7] it became standard practice to measure the rotor temperature before and after the experiment, and to employ a straight line interpolation procedure for intermediate times.[4,8,9] The implicit supposition that the vacuum-operated rotor could at most heat, due to peripheral friction with residual gas, or to bearing friction, appeared to be verified by direct measurements employing a thermistor imbedded in the rotor.[10,11]

Recent measurements by Waugh and Yphantis,[12] however, employing a radiation device below the vacuum-operated rotor, have indicated a cooling of 1° upon acceleration from rest to 59,780 r.p.m., and a reversible heating upon deceleration, which these authors attributed to an adiabatic cooling effect caused by tension in the metal of the rotor.

It is the purpose of this paper to report the results of an attempt to investigate this somewhat unexpected effect by a method based upon quite dissimilar physical principles, and to describe the methodology employed.

Method.—In order to calibrate radiation thermocouples in the oil-turbine ultracentrifuges, Svedberg and Nichols[13] and Svedberg and Pedersen[14] employed the optical observation of the melting of organic compounds in the ultracentrifuge cell. This method was carefully employed by Cecil and Ogston[3] and by Shulman.[6] One feature of this method is the extrapolation of observations to zero thickness of liquid above the liquid–solid interface, that is, to approximately one atmosphere pressure,

(1) Presented by Amilcare Biancheria to the Faculty of Clark University in partial fulfillment of the requirement for the degree of Master of Arts.

(2) K. O. Pedersen, Dissertation Univ. of Upsala, 1945.

(3) R. Cecil and A. G. Ogston, *Biochem. J.*, **43**, 592 (1948).

(4) G. Kegeles and F. J. Gutter, THIS JOURNAL, **73**, 3770 (1951).

(5) V. L. Koenig and K. O. Pedersen, *Arch. Biochem.*, **25**, 97 (1950).

(6) S. Shulman, *Arch. Biochem. Biophys.*, **44**, 230 (1953).

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