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).

Acknowledgment.—The author is indebted to Drs. L. J. Gosting and R. Goldberg for their helpful discussions, to Dr. R. Trautman for the opportunity of seeing his article prior to publication, and to Professor J. W. Williams for his review of the manuscript. This work was supported by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY]

Thermodynamic Measurements of Ultracentrifuge Rotor Temperature

BY AMILCARE BIANCHERIA[1] AND GERSON KEGELES

RECEIVED DECEMBER 21, 1953

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,

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[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).

[7] E. G. Pickels, *Machine Design*, **22**, No. 9, 102 (1950).

[8] J. F. Taylor, *Arch. Biochem. Biophys.*, **36**, 357 (1952).

[9] G. L. Miller and R. H. Golder, *ibid.*, **36**, 249 (1952).

[10] P. G. Ecker, J. Blum and C. W. Hiatt, *Rev. Sci. Instr.*, **20**, 799 (1949).

[11] C. W. Hiatt, *ibid.*, **24**, 182 (1953).

[12] D. F. Waugh and D. A. Yphantis, *ibid.*, **23**, 609 (1952).

[13] T. Svedberg and J. B. Nichols, THIS JOURNAL, **49**, 2920 (1927).

[14] T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, Oxford, 1940, p. 226.

where the freezing point is readily available. An important practical disadvantage of this extrapolation is that as initial melting occurs residual impurities of the preparation will concentrate in the liquid layer, thus lowering the observed melting point well below the independently observed temperature of incipient freezing at one atmosphere. Some evidence for the actual existence of this effect has been reported by Pedersen.[14]

It was desirable to take advantage of the somewhat greater accuracy of observation of the solid–liquid interface under an appreciable layer of liquid, as well as to avoid the inaccuracies of extrapolation just described. Moreover, due to the pressure gradient in the cell, the relative positions of the solid–liquid interface and liquid–air interface in a known gravitational field would, with the aid of requisite thermodynamic data for the effect of pressure on the melting point, provide a thermodynamic thermometer for direct observation of cell temperature in the rotor.[15] In the search for thermodynamic data on diphenyl ether, it was learned that the Bureau of Standards intended this material as a possible substitute for the ice–water system in the Bunsen calorimeter, to study reactions near room temperature.[16] The use of diphenyl ether in the Bunsen calorimeter makes available the Clapeyron coefficient

$$\mathrm{d}p/\mathrm{d}T = \Delta H/T\Delta V \qquad (1)$$

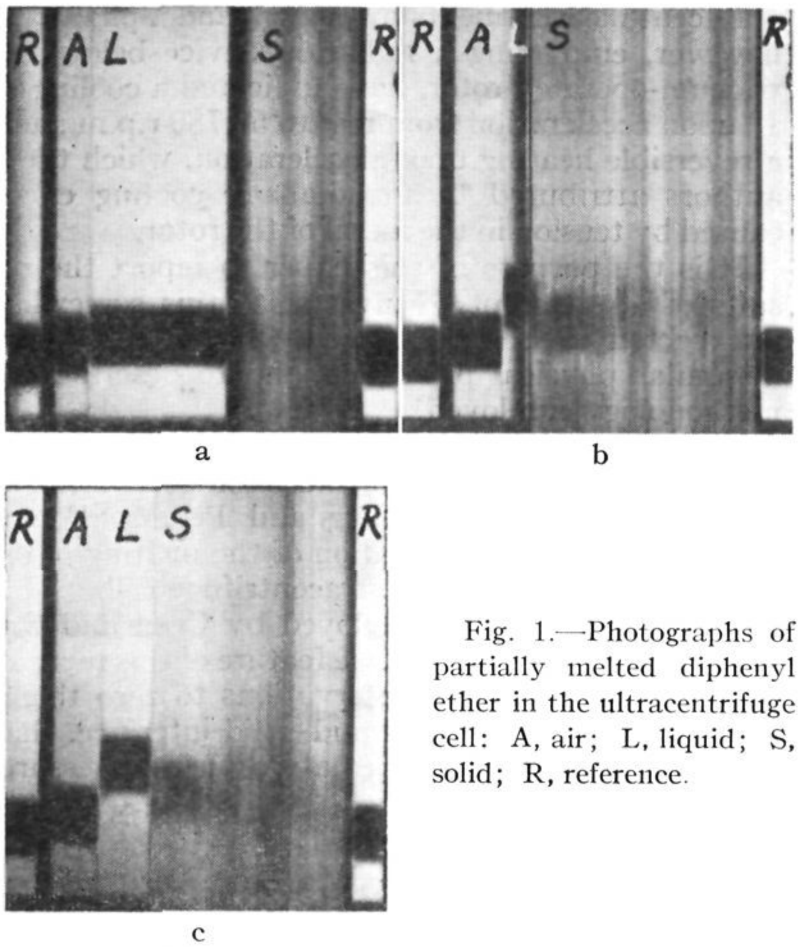
from a single measurement. We are much indebted to Drs. G. Furukawa and R. S. Jessup of the National Bureau of Standards for privately communicated unpublished data for this quantity, indicating an increase of melting point of diphenyl ether of 0.02797×10^{-6}°K. per dyne/cm.^{2} increase in pressure, under a total pressure of 114 cm. of mercury. The increase in pressure above 1 atmosphere, Δp, in dynes/cm.^{2} at the liquid–solid interface, Fig. 1, in the ultracentrifuge cell is given by

$$\Delta p = (^1/_2)\rho\omega^2(x^2 - x^2_0) \qquad (2)$$

where ρ is the average liquid density, 1.0688 g./cm.^{3}, taken at 26.83° and 1 atmosphere,[17] (the effect of pressure on the density being unknown for diphenyl ether, but very small for diethyl ether), ω is the angular velocity of the rotor, and x and x_0 are the radii of rotation of the liquid–solid and liquid–air interfaces, respectively. The centigrade temperature indicated in a particular photograph is then

$$t = 26.83^\circ + 0.02797(10)^{-6}\Delta p \qquad (3)$$

Here 26.83° is the middle of the observed melting range of our diphenyl ether sample, at one atmosphere pressure.



a

b

c

Fig. 1.—Photographs of partially melted diphenyl ether in the ultracentrifuge cell: A, air; L, liquid; S, solid; R, reference.

Experimental

Five pounds of diphenyl ether[18] were fractionally crystallized seven times and dried over phosphorus pentoxide. Approximately half of the material was collected as solid at each crystallization. The final product then had an observed melting point of 26.78 to 26.88°. The thermometer used (Emerson calorimeter, 0.01° graduations) was calibrated against a secondary standard recently calibrated by the National Bureau of Standards.[19] Based on the highest reported melting point of the Bureau of Standards[16] as representing pure diphenyl ether, and using their reported value for the heat of fusion in the Van't Hoff equation for freezing point lowering, our product is computed to be 99.95 mole per cent. pure.

The diphenyl ether was then introduced as a liquid into a standard 2 mm. duraluminum cell of the Spinco ultracentrifuge.[7] It was necessary to use a thin cell, to reduce lag effects caused by poor thermal conductivity of diphenyl ether. The cell was cooled slightly, and the contents seeded in order to produce freezing. To save time, it was found desirable to heat the rotor containing the cell to a temperature just below the melting point of the ether. The technique finally adopted was to warm the rotor slowly by resting it on a heated steel block and observing the temperature by means of the contact "free" thermocouple of the ultracentrifuge. When a temperature approximately 0.1° below the melting point was reached, the rotor was immediately introduced into the instrument and the diphenyl ether was observed at low speeds as soon as possible. To facilitate melting, the rotor was run at 40,000 to 50,000 r.p.m. under a poor vacuum, thereby imparting excessive frictional heating to the rotor. As soon as melting began the rotor speed was lowered and the vacuum dropped to approximately 1 micron. In order to deaerate our diphenyl ether, we continued to lower the speed to a point where a small layer of solid could just be maintained, and the material was then completely frozen by bringing the speed up to 59,780 r.p.m. (a small amount of solid had to be maintained in the cell at all times because the liquid supercools strongly even under pressure). Since the freezing process took place under high pressures, it was assumed that any air present in the sample was squeezed out to the top of the cell. The rotor was allowed to spin at 59,780 r.p.m., usually one hour or more, until a layer of liquid approximately 1 mm. thick could be observed. Then several photographs were taken at 16-min. intervals. As soon as the last photograph was taken, the rotor was decelerated rapidly to 40,000 r.p.m. and then allowed to coast down to 30,000 r.p.m. After 30 to 45 minutes at this speed, a second series of photographs at 16-min. intervals was taken.

It is worthy to note that if the proper amount of time was not allowed at a specific speed before photographing, equilibrium was not attained and a very poor and obscure liquid–solid meniscus resulted, Fig. 1a. Further, when accelerat-

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[15] This possibility was suggested several years ago by Dr. J. B. Bateman (private communication).

[16] G. Furukawa, D. C. Ginnings, R. E. McCoskey and R. A. Nelson, *Bur. Standards J. Research*, **46**, 195 (1951).

[17] C. Dodd and Hu Pak Mi, *Proc. Phys. Soc.* (*London*), **62B**, 454 (1949).

[18] Dow Chemical Co., Midland, Mich. (perfume grade).

[19] We are indebted to Frederick J. Gutter of the National Cancer Institute, Bethesda, Md., for this comparison.

ing from 30,000 to 59,780 r.p.m., a double meniscus formed due to a crust of solid frozen at the air–liquid meniscus, Fig. 1b.[20] A long period of time elapsed before this crust melted down and a single sharp meniscus formed, Fig. 1c. Due to this fact and to the high heating rate at 59,780 r.p.m., the effect of Waugh and Yphantis could not be measured properly during acceleration. However, in one of our earlier experiments, a good photograph was obtained after acceleration to 59,780 r.p.m., which indicated reversibility of the temperature shift. When any appreciable quantity of liquid is observed at 59,780 r.p.m., it is not possible to retain any solid at speeds close to zero. In order to cover the entire range of speed, 59,780 r.p.m. to 0, resort was therefore made to the contact "free" thermocouple as a means of observing the temperature of the rotor while at rest.

In our first experiment made during the winter, an unexpected over-all cooling effect at low speeds was detected. We believed this to be due partially to the conductance of heat from the rotor through the supporting piano wire which is in indirect contact with the water cooling system of the bearings, and partially to radiation of heat from the rotor to the blackened radiator shield supplied in the rotor chamber. In an effort to minimize the radiation effect, the chamber sides and bottom were lined with aluminum foil, with the shiny side facing the rotor.

Results

In Table I are shown the data from experiments where the rotor was decelerated from 59,780 r.p.m. to approximately 30,000 r.p.m. The first experiment in this table shows the reversible heating and cooling effect mentioned in the experimental section. The photographs indicated that the rotor heated up by 0.58° upon decelerating from 59,780 to 29,500 r.p.m. and then cooled by 0.46° upon acceleration. The cooling effect during acceleration appears to be less than the heating effect because of the temperature rise which takes place while waiting for equilibrium at 59,780 r.p.m. The average of the deceleration data in Table I is 0.59° ± 0.01°. Since the effect is proportional to the square of the speed, this corresponds to a calculated total effect, covering the entire range of speed from 59,780 r.p.m. to rest, of 0.79 ± 0.02° as compared to 1° reported by Waugh and Yphantis.[12]

TABLE I

Temp., °C. at 59,780 r.p.m.	Temp., °C. at 29,500 r.p.m.	ΔT
27.33	27.91	+0.58
27.45	27.91	−0.46[b]
27.33	27.90	+0.57
28.63	29.20[a]	+0.57
27.26	27.85	+0.59
28.68	29.28[a]	+0.60
28.25	28.85	+0.60

[a] Speed, 31,410 r.p.m. [b] Temp. decrease during acceleration from 29,500 to 59,780 r.p.m.

In Table II are summarized the results of direct measurements over the entire speed range, employing diphenyl ether at 59,780 r.p.m. and the "free" thermocouple at rest. These data average to a total temperature rise of $0.9_4 \pm 0.08°$.

The somewhat larger variation in Table II as compared to Table I is due to the difficulty in obtaining accurate temperature readings with the "free" thermocouple. The results in Tables I and II are felt to verify the existence of the temperature shift discovered by Waugh and Yphantis.

TABLE II

Temp., °C. at 59,780 r.p.m.	Temp., °C. at rest[a]	ΔT
29.06	29.88	+0.82
27.92	28.92	+1.00
28.81	29.79	+0.98
33.38	34.23	+0.85
30.23	31.27	+1.04

[a] As read by external "free" thermocouple after deceleration.

Discussion

Since one must wait about a half hour at each speed before photographing, there is an error due to frictional heating of the rotor. When accelerating, this heating lessens the observed cooling effect; when decelerating, it increases the observed heating effect. The amount of heating at 29,500 r.p.m. for a newly installed drive was found to be approximately 0.25° per hr., compared to approximately 1° per hr. at 59,780 r.p.m. A more recent experiment showed the heating at 59,780 r.p.m. to be approximately 0.6° per hr., suggesting an overestimate of about 0.1° in the last column figures in Table I.

Because of the possibility of variations of $\mathrm{d}T/\mathrm{d}p$ with pressure, it is necessary to investigate the errors in equation 3, due to this effect. In general, the total differential of $\mathrm{d}T/\mathrm{d}p$ may be written as

$$\mathrm{d}\left(\frac{\mathrm{d}T}{\mathrm{d}p}\right) = \mathrm{d}\left(\frac{\Delta V}{\Delta S}\right) = \left\{\frac{\partial\left(\frac{\Delta V}{\Delta S}\right)}{\partial T}\right\}_p \mathrm{d}T + \left\{\frac{\partial\left(\frac{\Delta V}{\Delta S}\right)}{\partial p}\right\}_T \mathrm{d}p$$

from which we obtain

$$\frac{\mathrm{d}\left(\frac{\mathrm{d}T}{\mathrm{d}p}\right)}{\frac{\mathrm{d}T}{\mathrm{d}p}} = \frac{1}{\Delta V}\left[\frac{\mathrm{d}T}{\mathrm{d}p}\left\{2\left(\frac{\partial \Delta V}{\partial T}\right)_p - \frac{\Delta C_p}{T}\frac{\mathrm{d}T}{\mathrm{d}p}\right\} + \left(\frac{\partial \Delta V}{\partial p}\right)_T\right]\mathrm{d}p$$

Subject to $(\partial T/\partial p)_V \approx \mathrm{d}T/\mathrm{d}p$ for phase equilibrium

$$\left(\frac{\partial \Delta V}{\partial p}\right)_T \approx -\left(\frac{\mathrm{d}T}{\mathrm{d}p}\right)\left(\frac{\partial \Delta V}{\partial T}\right)_p$$

and substitution of this equation into the preceding expression and collection of terms results in

$$\frac{\mathrm{d}\left(\frac{\mathrm{d}T}{\mathrm{d}p}\right)}{\frac{\mathrm{d}T}{\mathrm{d}p}} \approx \frac{1}{\Delta V}\left[\frac{\mathrm{d}T}{\mathrm{d}p}\left\{\left(\frac{\partial \Delta V}{\partial T}\right)_p - \frac{\Delta C_p}{T}\frac{\mathrm{d}T}{\mathrm{d}p}\right\}\right]\mathrm{d}p$$

All terms on the right hand side of this equation, except $(\partial \Delta V/\partial T)_p$ are accurately known or may be calculated with the aid of the thermodynamic data from reference 16. Since the data for the temperature coefficient of expansion of the solid were insufficient, a maximum value for $(\partial \Delta V/\partial T)_p$ was obtained by using only the coefficient of expansion of the liquid.[17] Assuming a pressure of 100 atmospheres (a maximum value), we calculate

$$\frac{\mathrm{d}(\mathrm{d}T/\mathrm{d}p)}{\mathrm{d}T/\mathrm{d}p} \approx 0.014$$

which represents a probable maximum error of 1.4% in $\mathrm{d}T/\mathrm{d}p$. An extreme case which must be considered in the above expression is the possibility of $(\partial \Delta V/\partial T)_p$ being approximately zero. This would give a negative correction to $\mathrm{d}T/\mathrm{d}p$ and cause us to observe an overestimate of the reversible temperature effect. However, the negative term

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[20] This observation is interpreted as furnishing evidence for the existence of a large temperature gradient with respect to radius of rotation in the rotor during acceleration.

calculates to a maximum error of 0.8% in dT/dp, which means a correction of approximately 0.03° for 100 atmospheres increase in pressure. In the determination of the adiabatic temperature *shift*, these errors would not exceed approximately 0.002° in an over-all shift of 0.6°.

As a check on the possibility of allotropic forms of diphenyl ether existing under the pressures used, the data for a heating experiment made at 59,780 r.p.m. were plotted on a temperature *vs.* time basis. Now, since the heating rate is essentially constant with time a single line should result. However, if there were a change in the solid phase of diphenyl ether, and consequently in the value of dT/dp, there should be a sudden break in the plot, producing two separate lines. No such sudden break was detected in our curve which was plotted from data for an experiment where the diphenyl ether was subjected to pressures from 24 to 235 atm.

Another source of error in our system stems from the melting point range caused by small amounts of impurity in the sample. A correction for this can be made by assuming that during the freezing process only pure solid separates out and all the residual impurities are concentrated in the liquid.[21] Then the freezing point depression at any time during freezing can be calculated according to

$$\Delta T = AN_2 \qquad (4)$$

where ΔT is the freezing point depression caused by the mole fraction of impurity N_2 and A is the cryoscopic constant $-RT^2/\Delta H$. By using this simplification and by estimating the ratio of total sample to liquid at the time temperature readings were recorded during freezing, approximations of the freezing point depression of nearly pure diphenyl ether[16] and numerous other compounds[22,23] have been obtained, as well as approximations for the mole per cent. of impurity. As separate freezing point studies were made on our diphenyl ether, small corrections could be applied to the melting point at one atmosphere for the various stages of freezing in the ultracentrifuge. However, since the above simplification is not entirely valid, as otherwise pure solid would be obtained by one crystallization, the corrections would have the tendency to give an overestimate of the temperature effect already observed; if they are not applied, an underestimate of the effect results. Since we felt the data at hand were already sufficient to verify the findings of Waugh and Yphantis, we preferred not to make these somewhat ambiguous corrections.

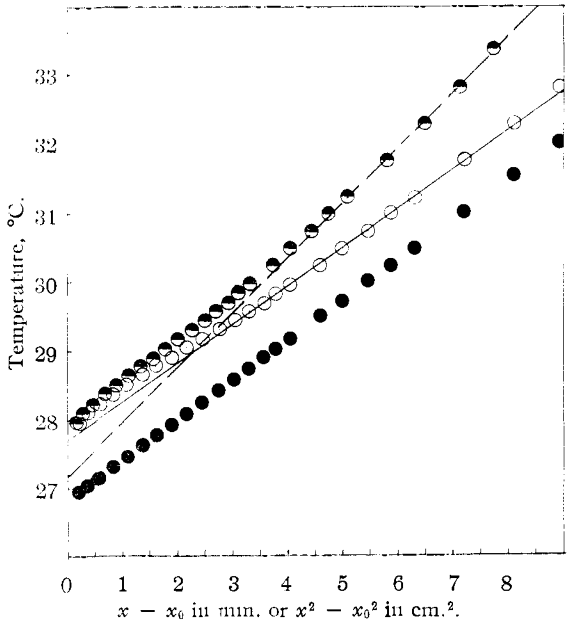

Fig. 2.—Extrapolations at 59,780 r.p.m. Half inked circles are interpolated temperature *versus* $(x - x_0)$. Empty circles are interpolated temperature *versus* $x^2 - x_0^2$. The full inked circles are calculated temperature (equation 3) *versus* $x^2 - x_0^2$.

Although the simplification is not entirely valid, it is none the less obvious that as freezing progresses, impurities are continually being concentrated in the liquid and, in accordance with equation 4, the equilibrium temperature is continually dropping. This effect is negligible when thick layers of liquid are used but, as thin layers of liquid are approached, the effect becomes more significant and the plots of temperature *vs.* time or pressure will begin to deviate from a straight line. When temperatures are calculated, assuming pure diphenyl ether, directly from the liquid layers, the plots will become concave upward at early times, or thin liquid layers. When the temperatures are obtained by an external means such as the thermocouple, the plots of thermocouple temperature *vs.* pressure will become concave downward at low pressures.

Since the first liquid produced upon melting a normal two-component solid mixture will be observed at the eutectic composition and temperature, these same considerations also apply to observations of extremely thin layers of liquid produced during melting in the ultracentrifuge of externally pre-frozen samples. This effect was observed for such experiments by Pedersen[14] in 1932, and his suggestion was to neglect the data obtained from very thin layers of liquid.

There is, on the other hand, a possible danger of incorrect extrapolation when using thick liquid layers, if the plot is made against the thickness of liquid layer,[6] rather than against the pressure.[3] This is illustrated in Fig. 2, a record of an experiment in which externally pre-frozen diphenyl ether was allowed to melt in the ultracentrifuge at 59,780 r.p.m.

The interpolated temperature data from an external contact thermocouple have been plotted as half-inked circles against the thickness of the liquid layer, $x - x_0$, and the dashed straight line, which fits this plot in the range 4 to 8 mm. thickness of liquid layer, extrapolates to a temperature of about 27° at one atmosphere pressure. On the other hand, the identical interpolated thermocouple data plotted against x^2 or x_0^2 (open circles and solid line), extrapolate to a temperature of 27.73°. As the plot against x or x_0 should be curved, and

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[21] W. P. White, *J. Phys. Chem.*, **24**, 392 (1920).

[22] F. W. Schwab and E. Wickers in "Temperature, Its Measurement and Control in Science and Industry," Reinhold Publ. Corp., New York, N. Y., 1941, pp. 256.

[23] B. J. Mair, A. R. Glasgow, Jr., and F. D. Rossini, *Bur. Standards J. Research*, **26**, 591 (1941).

that against x^2 or x_0^2 should be straight (equations 2 and 3), deductions from the latter plot are taken as correct. This would indicate that the rotor had cooled by 0.9° during acceleration from rest to 59,780 r.p.m.—this value of 0.9° depending on the external thermocouple readings. As a final check, the thermodynamically calculated temperatures were plotted on the same graph in Fig. 2 (full-inked circles), and these lie on a straight line approximately 0.88° below the interpolated thermocouple temperatures, the precision of this value again being limited by our ability to read temperatures with the external thermocouple.

Conclusions and Summary

The data in Tables I and II, obtained by a completely independent physical method, are felt to verify the reversible temperature effect discovered by Waugh and Yphantis. The extrapolation and the comparison of thermocouple temperatures with calculated thermodynamic temperatures discussed in the previous section, lend this further verification. We therefore suggest that all previous published Spinco data on sedimentation be corrected to account for this effect. Further, we suggest that the thermocouple calibrations of the oil-turbine ultracentrifuge be re-examined and, if necessary, new calibrations be made, exercising care that only sufficiently thick layers of liquid are used for the extrapolation. It is worthy to note that our method could be employed to advantage in these calibrations, since cell temperatures can be calculated from each photograph and compared directly with the thermocouple temperatures.

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WORCESTER, MASS.

[CONTRIBUTION FROM FRICK CHEMICAL LABORATORY, AND THE LOS ALAMOS SCIENTIFIC LABORATORY, UNIVERSITY OF CALIFORNIA]

Physicochemical Studies of the Simpler Polypeptides. III. The Acid- and Base-catalyzed Hydrolysis of Di-, Tri-, Tetra-, Penta- and Hexaglycine

BY EDWARD F. HAMMEL, JR.,[1] AND SAMUEL GLASSTONE[2]

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The specific rates for the acid (2 N HCl at 65 and 75°) and base (2 N NaOH at 20 and 30°) hydrolysis of di-, tri-, tetra-, penta- and hexaglycine have been determined. It is shown that for the tetra- and higher polyglycines these rates increase approximately linearly with the number of glycine residues in the chain. The specific rate for the fission of the central peptide bond in tetraglycine has been derived from the data. Heats and entropies of activation for the acid and base catalyzed hydrolysis of diglycine are reported.

Several studies have been made of the hydrolysis of the simpler polypeptides,[3] but the only systematic investigation of a series of polyglycines was that made by Abderhalden and Suzuki.[3a] These workers reported their results in the form of segmented curves and made no mention of their experimental procedure or of the method used for analyzing the hydrolysate. It may be noted that in a previous paper,[4] dealing with the hydrolysis of other polypeptides, both the Van Slyke[5] and Sörensen[6] methods for the determination of amino acids were employed. The former, however, yields accurate results with polyglycines only when the modification of Kendrick and Hanke,[7] which was not published at the time, is employed, and in the experience of the present authors the Sörensen titration is not reliable unless carried out potentiometrically. An examination of Abderhalden's curves revealed two marked discrepancies: In the first place, the rate of hydrolysis of hexaglycine in acid solution appeared to be little more than twice as great as that of diglycine whereas a much larger ratio is to be expected. and, in the second place, the heats of activation for both acid and alkaline hydrolysis were calculated to lie between 3.5 and 5.5 kcal. in all cases but one. It is improbable that the reactions under consideration will have such low activation energies, and this view is confirmed by values of the order of 15 kcal. found in connection with the hydrolysis of esters, a process quite analogous to that involved in the splitting of polypeptides.

The foregoing results are probably to be attributed to difficulties in the analysis of the mixtures of glycine and polyglycines obtained in the course of the hydrolytic reaction. The unexpected observations reported by Yaichnikov and Spiridonova[8] are probably due to the same cause. They reported that the acid (1 N HCl) hydrolysis of diglycine (0.1 N) is complete in 30 hours at 95° but subsequently there is a decrease of amino nitrogen, suggesting a recombination, cyclization or some other reaction which causes glycine to disappear. Repe-

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(3) (a) E. Abderhalden and H. Suzuki, *Z. physiol. Chem.*, **170**, 158 (1927); **173**, 250 (1928); (b) W. Kuhn, C. C. Molster and K. Freudenberg, *Ber.*, **65B**, 1179 (1922); (c) P. A. Levene, *J. Biol. Chem.*, **82**, 167 (1929); (d) L. Lawrence and W. J. Moore, THIS JOURNAL, **73**, 3793 (1951).

(4) E. Abderhalden and H. Sickel, *Z. physiol. Chem.*, **170**, 134 (1927).

(5) D. D. Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929).

(6) S. P. L. Sörensen, *Biochem. Z.*, **7**, 45 (1907).

(7) A. B. Kendrick and M. E. Hanke, *J. Biol. Chem.*, **117**, 161 (1937).

(8) I. S. Yaichnikov and A. S. Spiridonova, *J. Gen. Chem. (USSR)*, **4**, 1286 (1934).