sulfide with aqueous alkali appeared to be the formation of sodium polysulfides and the degradation to the disulfide.

Preparation of Ethyl Disulfide.[12]—A mixture of 500 g. of sodium thiosulfate, 220 g. of ethyl bromide, 500 cc. of water and 40 cc. of alcohol was refluxed with stirring for twenty-two and one-half hours. Two hundred and fifty grams of iodine was added in small batches to the hot mixture. After all the iodine was added, the mixture was decolorized with aqueous sodium bisulfite. The crude disulfide was separated. The aqueous layer was extracted with ether and the extract was combined with the crude product. The solution was washed with aqueous sodium bisulfate, dried over calcium chloride, and distilled. The fraction boiling at 145–155° was collected. It weighed 67.7 g. (55.4% yield).

Anal. Calcd. for $(C_2H_5)_2S_2$: S, 52.46. Found: S, 52.84, 52.48.

The ethyl disulfide was redistilled through a small fractionating column, a middle fraction boiling at 152.5–154° being collected. The refractive index of this sample was checked to determine purity: n^{20}D 1.5078; (*cf.* Nasini[11]: n^{20}D 1.50633, b. p. 153–154°; Nekrassow and Melkinow[15]: n^{20}D 1.503).

Preparation of Ethyl Trisulfide.[2,6]—Seventy grams of freshly distilled water-white sulfur monochloride, S_2Cl_2, was dissolved in 50 g. of carbon bisulfide. This solution was slowly added through a reflux condenser to a flask containing a solution of 100 g. of ethyl mercaptan (Eastman Kodak Co.) in 50 g. of carbon bisulfide. The flask was kept cold during the addition. Freshly precipitated calcium carbonate was prepared from soda ash and calcium chloride and dried at 105°. Ten grams was added to the reaction mixture. After the evolution of carbon dioxide had ceased, the mixture was filtered. The carbon bisulfide was distilled from the filtrate on a water-bath in an atmosphere of nitrogen. The reaction products were fractionated under reduced pressure (26 mm.) in a stream of nitrogen, the following fractions being obtained: (1) b. p. 89–100° (26 mm.), n^{13}D 1.5685; (2) b. p. 100–115° (26 mm.), n^{13}D 1.5700; (3) b. p. 123–129° (26 mm.), n^{13}D 1.5888.

Levi and Baroni[6] found for the trisulfide, n^{13}D 1.56890, and b. p. 85° and for the tetrasulfide n^{13}D 1.58436. The middle fraction (2) was redistilled at b. p. 92–97°. Its refractive index was found to be n^{13}D 1.5689. After standing several months this had dropped to 1.5683. Although the boiling point reported here differs appreciably from that reported by Levi and Baroni, it is believed to be correct since it was observed repeatedly. The refractive index coincides with that found by Levi and Baroni, and both boiling point and refractive index are in satisfactory agreement with the values for fraction 2B described in the next paragraph and found by analysis to have the correct composition.

Reaction of Ethyl Disulfide with Sulfur.—A mixture of 15 g. of ethyl disulfide, 7.8 g. of sulfur and 1.5 g. of dibutylamine was heated to 85–90° for three hours and then to 120–128° for five hours. The mixture was cooled, dissolved in ether, and washed with dilute hydrochloric acid and water. After drying the mixture with calcium chloride, the ether was evaporated. The residual liquid was fractionated at a pressure of 16 mm.: (1) b. p. 20–70° (16 mm.), 1.0 g.; (2) b. p. 70–100° (16 mm.), 7.9 g.; (3) b. p. 100–110° (16 mm.), 3.0 g.

Fractions 2 and 3 were combined and redistilled at 18 mm.: (2A) b. p. 83–87° (18 mm.); (3A) b. p. 92–97° (18 mm.).

These were analyzed for sulfur. *Anal.* Calcd. for $(C_2H_5)_2S_3$: S, 62.4. Found: 2A, S, 60.23, 60.08; 2B, S, 62.69, 62.33.

Fraction 2B was redistilled at 26 mm. Most of it came over at 96–97°. The refractive index was found to be n^{13}D 1.5683.

Desulfurization of Diethyl Trisulfide.—A mixture of 5 g. of ethyl trisulfide, 1.6 g. of sodium hydroxide and 20 cc. of water was stirred one and one-half hours at reflux. After cooling, it was extracted with ether. The extract was dried and the ether evaporated. The residual liquid boiled at 152–154° leaving no residue (n^{20}D 1.5080). The aqueous layer was acidified with dilute sulfuric acid and the gases evolved were passed through an ice-trap. Only a trace of ether was trapped but hydrogen sulfide was evolved. Sulfur precipitated from the solution.

Summary

The preparation of ethyl disulfide and ethyl trisulfide and the conversion of each compound into the other have been described. The mechanisms of the conversions are discussed.

The dielectric constants and densities of dilute benzene solutions of diethyl disulfide and trisulfide have been measured and used to calculate the dipole moments of the molecules. The moment values, in excellent agreement with additional data shortly to be interpreted and published, are 1.99×10^{-18} for diethyl disulfide and 1.64×10^{-18} for diethyl trisulfide.

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[15] Nekrassow and Melkinow, *Ber.*, **62**, 2091 (1929).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

A New Polarographic Diffusion Current Equation

BY JAMES J. LINGANE* AND BRIAN A. LOVERIDGE†

The Ilkovic equation[1,2,3] for the diffusion current observed with the dropping mercury electrode

$$i_{\mathrm{d}} = 607nD^{1/2}Cm^{2/3}t^{1/6} \text{ (at 25°)} \qquad (1)$$

although approximately correct is not completely satisfactory. Lingane and Loveridge[4] demonstrated that the diffusion current "constant," $i_{\mathrm{d}}/Cm^{2/3}t^{1/6}$, of lead ion varies significantly with changing values of $m^{2/3}t^{1/6}$, whereas according to the Ilkovic equation it should be truly constant and independent of the electrode characteristics m and t. Loveridge[5] observed an exactly similar behavior with tetramminozinc ion. The nature and extent of these variations are shown in Fig. 1,

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(1) D. Ilkovic, *Collection Czechoslov. Chem. Commun.*, **6**, 498 (1934); *J. chim. phys.*, **35**, 129 (1938).

(2) D. MacGillavry and E. K. Rideal, *Rec. trav. chim.*, **56**, 1013 (1937).

(3) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1946, Chap. II and Chap. IV.

(4) J. J. Lingane and B. A. Loveridge, THIS JOURNAL, **66**, 1425 (1944); **68**, 395 (1946).

(5) B. A. Loveridge, Ph.D. Thesis, Harvard University, 1947.

in which $i_d/Cm^{2/3}t^{1/6}$ obtained with many different dropping electrode capillaries is plotted against the ratio $t^{1/6}/m^{1/3}$. (The choice of this method of plotting will be evident from the following discussion.) If eq. 1 were strictly correct these plots would be horizontal straight lines.

The rapid increase in the diffusion current "constants" at small values of $t^{1/6}/m^{1/3}$ (small drop times) is caused by stirring produced by the rapidly forming drops, with consequent convective transfer of the reducible ion to the electrode surface, and hence is no real fault of the Ilkovic equation which assumes transfer only by diffusion. However the linear increase of the diffusion current constant at values of $t^{1/6}/m^{1/3}$ greater than about 0.6 (drop time greater than about 1.5 sec.) shows that the Ilkovic equation is significantly inadequate even when the drop time is great enough so that the current presumably is diffusion controlled. The failure of the Ilkovic equation is not great and the total change in the diffusion current constants in Fig. 1 above values of $t^{1/6}/m^{1/3}$ of about 0.6 is only of the order of 5%.

A clue to the inadequacy of the Ilkovic equation is the fact that an equation of identically the same form and differing only in the value of the numerical constant can be derived directly from the diffusion current equation for *linear* diffusion to a *plane* electrode. The equation for an instantaneous "linear diffusion current" is[6]

$$i_t = nFCAD^{1/2}/\sqrt{\pi t} \qquad (2)$$

Assuming that the mercury drops are spherical the area A at any instant t during the drop life is

$$A = (4\pi)^{1/3}3^{2/3}m^{2/3}t^{2/3}/d^{2/3} \qquad (3)$$

where d is the density of mercury and m is the rate of mercury flow. When this relation is substituted into eq. 2, and the various quantities are expressed in the customary units,[3] we obtain at 25°

$$i_t = 464nCD^{1/2}m^{2/3}t^{1/6} \qquad (4)$$

Correspondingly the average current during the life of a drop is

$$i_d = \frac{1}{t}\int_0^t i_t \, dt = 397nCD^{1/2}m^{2/3}t^{1/6} \qquad (5)$$

The only difference between eq. 5 and the Ilkovic (eq. 1) is the value of the numerical constant. If the constant 397 in eq. 5 is multiplied by $\sqrt{7/3}$ the "Ilkovic constant" 607 is obtained. This seemingly arbitrary procedure has theoretical significance because in the derivation of the Ilkovic equation the factor $\sqrt{7/3}$ represents the fact that the expansion of the mercury drops counteracts the decay of the concentration gradient at the electrode surface, and hence increases the diffusion current proportionately. This effect is distinct from the mere increase in the area of the diffusion field.

It seems evident that, although the derivation of the Ilkovic equation begins with the postulate of symmetrical *spherical* diffusion, the simplifications subsequently made in certain of the intermediate mathematical operations are equivalent to a neglect of the curvature of the electrode surface. In other words, the final Ilkovic equation takes into account the enhanced concentration gradient at the electrode surface resulting from the expansion of the mercury drops but it neglects the curvature of the electrode surface. Because the effect of the curvature is a function of the drop size it is understandable that the diffusion current "constant" defined by the Ilkovic equation should vary with the characteristics of the dropping electrode as shown by the right-hand branches of the curves in Fig. 1.

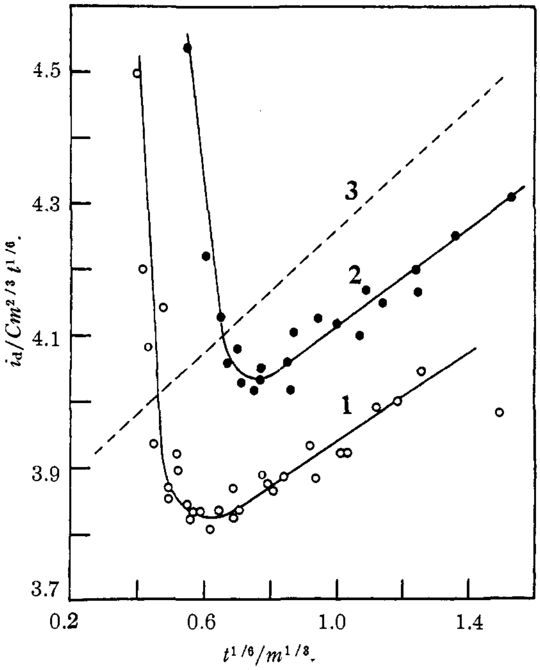

Fig. 1.—Variation of $i_d/Cm^{2/3}t^{1/6}$ with capillary characteristics: (1) lead ion in 1 M potassium chloride containing 0.01% gelatin; (2) tetramminozinc ion in 1 M ammonium chloride-1 M ammonia containing 0.01% gelatine; (3) theoretical line for lead ion according to eq. 11.

Since the Ilkovic equation follows directly from the equation for linear diffusion to a plane electrode by introducing the factor $\sqrt{7/3}$, it is logical to assume that a more nearly correct equation for the diffusion current which will take into account the curvature of the electrode surface can be obtained by an analogous procedure starting with the equation for the diffusion current at a stationary spherical electrode.

The equation for the instantaneous diffusion current at a *stationary* spherical electrode is[7]

$$i_t = nFDCA\left(\frac{1}{r} + \frac{1}{\sqrt{\pi Dt}}\right) \qquad (6)$$

We assume that this relation will apply to the

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[6] Ref. 3, p. 21.

[7] Ref. 3, p. 29.

dropping electrode if the second term in parentheses is multiplied by $\sqrt{7/3}$ to obtain

$$i_t = nFDCA\left(\frac{1}{r} + \sqrt{\frac{7}{3\pi Dt}}\right) \qquad (7)$$

The radius of the mercury drop at any instant is given by[8]

$$r = (3mt/4\pi d)^{1/3} \qquad (8)$$

and eq. 3 expresses its instantaneous area. Introducing these relations into eq. 7 and employing the customary units leads to

$$i_t = 709nD^{1/2}Cm^{2/3}t^{1/6} + 31{,}560nDCm^{1/3}t^{1/3} \qquad (9)$$

for the instantaneous current. Integration over the drop life yields for the average current

$$i_d = 607nD^{1/2}Cm^{2/3}t^{1/6} + 23{,}670nDCm^{1/3}t^{1/3} \qquad (10)$$

or

$$\frac{i_d}{Cm^{2/3}t^{1/6}} = 607nD^{1/2}\left(1 + 39\,\frac{D^{1/2}t^{1/6}}{m^{1/3}}\right) \qquad (11)$$

This new equation for the diffusion current differs from the Ilkovic equation by the term $1 + 39D^{1/2}m^{-1/3}t^{1/6}$ which represents the influence of the curvature of the electrode surface. Eq. 11 accounts for the linear increase of $i_d/Cm^{2/3}t^{1/6}$ with increasing values of $t^{1/6}/m^{1/3}$ above about 0.7 shown by the experimental data in Fig. 1.

The dashed line in Fig. 1 is the theoretical line for lead ion according to eq. 11. Since the exact diffusion coefficient of lead ion in 1 *M* potassium chloride is not known, this theoretical line was drawn by employing the diffusion coefficient of lead ion *at infinite dilution*, which from the equivalent conductance of lead ion at infinite dilution (73 ohm^{-1} cm.^{2}) is 0.98×10^{-5} cm.^{2} sec.$^{-1}$ at 25°. The observed diffusion current of lead ion is about 8% smaller than the ideal infinite dilution value computed from eq. 11. This indicates that the actual diffusion coefficient of lead ion in 1 *M* potassium chloride is about 16% smaller than the ideal infinite dilution value, which is entirely logical. The slope of the line drawn through the experimental points above $t^{1/6}/m^{1/3}$ of 0.7 agrees well with the theoretical slope, and is slightly smaller than the theoretical slope because the actual diffusion coefficient of lead ion is somewhat smaller than the ideal infinite dilution value.

Neither direct measurements of the diffusion coefficient of the tetramminozinc ion under the conditions of Fig. 1 nor its equivalent conductance are available, and hence it is not possible to draw the theoretical line for this ion. However, the diffusion current data themselves show that the diffusion coefficient of the tetramminozinc ion is only slightly larger than that of lead ion under these conditions and therefore the slope of the zinc line would be expected to be only slightly greater than that of the lead line as observed.

Additional evidence for the validity of equations 10 and 11 exists in data obtained by Loveridge[5] for the influence of the pressure on the dropping mercury on the diffusion current. According to the Ilkovic equation

$$i_d = kh^{1/2} \qquad (12)$$

where the constant k is a function of the geometry of the dropping electrode capillary, and h is the vertical distance between the mercury level in the reservoir and the tip of the dropping electrode corrected for the back pressure due to the interfacial tension at the mercury–solution interface.[9] This relation follows from the fact, verified by Loveridge,[5] that m is directly proportional to h but t is inversely proportional to h. On the other hand, eq. 11 predicts that $i_d/h^{1/2}$ should not be constant with various values of h for a given capillary but should follow an equation of the form

$$\frac{i_d}{h^{1/2}} = k + \frac{b}{h^{1/2}} \qquad (13)$$

where the constants k and b are characteristic of the particular capillary. Experimental values of $i_d/h^{1/2}$ obtained by Loveridge[5] with lead ion with many different capillaries actually do show a small but reproducible decrease with increasing values of h as predicted by eq. 13. When h is increased above the point (characteristic of the particular capillary) where the drop time decreases to less than about 1.5 sec. the ratio $i_d/h^{1/2}$ ceases to decrease and begins to increase rapidly because of the same stirring effect shown by the left branches of the curves in Fig. 1.

In view of the new equation for the diffusion current the previously recommended[10] use of standardized diffusion current "constants" in practical analyses requires some modification. From eq. 11 it is evident that the true diffusion current constant is

$$\frac{i_d}{Cm^{2/3}t^{1/6}\left(1 + \frac{39D^{1/2}t^{1/6}}{m^{1/3}}\right)} = 607nD^{1/2} = I^0 \qquad (14)$$

rather than $i_d/Cm^{2/3}t^{1/6}$. With capillaries normally used $t^{1/6}/m^{1/3}$ will be between about 0.7 and 1.2 and correspondingly $i_d/Cm^{2/3}t^{1/6}$ will vary over a range of about 4%. Therefore when an accuracy of better than about ±2% is desired the true diffusion current constant defined by eq. 14 should be employed.

The practical use of eq. 14 requires a knowledge of D in order to evaluate the correction term $1 + 39D^{1/2}t^{1/6}m^{-1/3}$. For many ions D can be approximated sufficiently accurately for the purpose of this correction by employing the ideal value computed from the equivalent ionic conductance at infinite dilution. Alternatively, an approximate value of D can first be computed from the observed diffusion current by means of the approximate relation $i_d/Cm^{2/3}t^{1/6} \simeq 607nD^{1/2}$, and introduced into the correction term to compute a more nearly correct value of D from eq. 14.

An equation identical with eq. 11, except that the constant in the second term is 17 instead of 39,

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[8] Ref. 3, p. 33.

[9] Ref. 3, p. 76.

[10] J. J. Lingane, *Ind. Eng. Chem., Anal. Ed.*, **15**, 588 (1943).

has recently been derived independently by Strehlov and Stackelberg.[11] This derivation, which we understand will soon be published, follows the general pattern of Ilkovic's original derivation[1] and that of MacGillavry and Rideal[2] but retains in the intermediate mathematical operations those terms, neglected in the original derivations, which represent the curvature of the electrode surface.

Summary

The following new equation has been derived for the diffusion current observed with the dropping mercury electrode at 25°

$$i_d = 607nD^{1/2}Cm^{2/3}t^{1/6}\left(1 + \frac{39D^{1/2}t^{1/6}}{m^{1/3}}\right)$$

This equation differs from the familiar Ilkovic equation by the term in parentheses, which has a value in the neighborhood of 1.1 with capillaries of the usual characteristics. This term represents the curvature of the electrode surface which the Ilkovic equation neglects. The new equation accounts quantitatively for the experimentally observed linear increase in the diffusion current "constant" $i_d/Cm^{2/3}t^{1/6}$ with increasing values of the ratio $t^{1/6}/m^{1/3}$. From the new equation the true diffusion current constant is $i_d/Cm^{2/3}t^{1/6}(1 + 39D^{1/2}t^{1/6}m^{-1/3})$.

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(11) Private communication from Dr. H. Strehlov and Prof. M. v. Stackelberg received after the conclusions in this paper were formulated.

[CONTRIBUTION FROM THE HARVARD MEDICAL SCHOOL]

Preparation and Properties of Serum and Plasma Proteins. XXIII. Hydrogen Ion Equilibria in Native and Modified Human Serum Albumins[1a,1b]

BY CHARLES TANFORD[1c,1d]

Introduction

The use of electromotive force measurements to obtain information about the acid- and base-binding groups of proteins dates back to 1898,[2] and the first measurements on bovine serum albumin were made in 1913.[3,4] The study on human serum albumin reported in this paper was undertaken because it is now possible to interpret such electromotive force measurements with far greater certainty than ever before, for the following reasons: (1) Human serum albumin preparations of high purity and consistent properties are now available. (2) The work of Linderstrøm-Lang and Scatchard has provided a theoretical basis for an understanding of the binding of small ions by proteins in terms of fundamental equilibrium constants. (3) Interaction studies on ions other than hydrogen or hydroxyl ions have been made, and their effect on the hydrogen ion equilibria can be evaluated. (4) Well-defined chemical modifications of human serum albumin have been prepared in this Laboratory, the hydrogen ion titration curves of which should differ in a predictable manner from native albumin.

Materials and Methods

Human Serum Albumin.—Two preparations of human serum albumin were used, one crystallized from decanol (preparation decanol-10),[5] the other first crystallized as the mercury dimer, from which mercury has been subsequently removed.[6] The latter preparation, which has one free sulfhydryl group per molecule, has been given the name mercaptalbumin. Stock solutions of the protein were prepared by dissolving crystals in water. The solutions were exhaustively electrodialyzed, and the protein concentrations (in grams per kg. of water) were measured by heating at 110° to constant weight. The molecular weight of human serum albumin (of both preparations) is known to be 69,000.[7]

Guanidinated Albumin.—Preparation U of Hughes, Saroff and Carney, made from decanol-crystallized albumin[8] has been used. The maximum number of free amino groups are guanidinated in this preparation. Stock solutions of this protein could not be electrodialyzed because it is insoluble at its isoionic point. They were instead dialyzed against 0.15 *M* sodium chloride, and protein concentrations were measured as the difference between the dry weights of the protein solution and the dialyzing solution. The molecular weight of the guanidinated albumin, calculated from the extent of guanidination, is 71,500.

Iodinated Albumin.—A preparation of iodinated albumin prepared from decanol crystals, and containing 17 atoms of iodine per mole, was obtained from Drs.

[1a] This paper is Number 80 in the series "Studies on the Plasma Proteins" from the Harvard Medical School, Boston, Massachusetts, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross.

[1b] This work was originally supported by grants from the Rockefeller Foundation and from funds of Harvard University. It was aided early in 1941 by grants from the Committee on Medicine of the National Research Council which included a grant from the American College of Physicians. From August, 1941, to July, 1946, it was carried out under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University. Since then it has been aided by a grant recommended by the Panel on Hematology of the National Institutes of Health.

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[1d] Some of the initial experiments leading up to this work were performed while the author held a Lalor Foundation Post-doctoral Fellowship (1947–1948) at Harvard Medical School.

(2) S. Bugarszky and L. Liebermann, *Pflügers Arch.*, **72**, 51 (1898).

(3) K. Manabe and J. Matula, *Biochem. Z.*, **52**, 369 (1913).

(4) A complete review of early work in this field is given by E. J. Cohn, *Physiol. Rev.*, **5**, 349 (1925); see also ref. 17.

(5) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, THIS JOURNAL, **69**, 1753 (1947).

(6) W. L. Hughes, Jr., *ibid.*, **69**, 1836 (1947).

(7) J. L. Oncley, G. Scatchard and A. Brown, *J. Phys. and Coll. Chem.*, **51**, 184 (1947).

(8) W. L. Hughes, Jr., H. A. Saroff and A. L. Carney, THIS JOURNAL, **71**, 2476 (1949).