[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Diffusion in Supersaturated Solutions. II. Glucose Solutions

BY J. K. GLADDEN[1] AND MALCOLM DOLE

RECEIVED DECEMBER 4, 1952

Diffusion coefficients for glucose solutions at 25 and 35° are reported for the concentration range 0 to 80 weight per cent. glucose (the saturated concentration at 25° is 50% glucose). The diffusion coefficients decrease nearly linearly with increase of concentration up to 50% but, at higher concentrations, approach the weight per cent. axis asymptotically. The logarithms of the viscosity and of the diffusion coefficient for both glucose and sucrose solutions are each approximately linear functions of the mole fraction of the solute; the viscosity, however, increases with concentration about 100-fold more rapidly than the diffusion coefficient decreases, illustrating the relative independence of these two factors. The activation energy for diffusion also increases approximately linearly with mole fraction. Over the whole concentration range the logarithm of the diffusion coefficient is proportional to the activation energy which is in turn proportional to the entropy of activation. The data are interpreted in terms of a diffusion mechanism based on the migration of water molecules having activation energies increasing linearly with the mole fraction of the solute.

Introduction

In the first paper of this series, English and Dole[2] published data for the diffusion coefficients of sucrose in concentrated and supersaturated solutions over the concentration range 60 to 74 weight per cent. at 25 and 35°. This paper extends the work to glucose solutions, and covers the entire range of concentrations from 0 to 80% glucose. Friedman and Carpenter[3] have previously investigated diffusion in glucose solutions up to 0.6 mole/l. at 25° by the Northrop[4]–McBain[5] porous disk technique.

Experimental

The optical system previously described[2] in which diffusion coefficients can be calculated from the shift with time of interference fringes by the method worked out by Longsworth,[6] Kegeles and Gosting,[7] and Coulson, Cox, Ogston and Philpot[8] was used with only the slight modification of replacing the plate glass windows of the cell with windows ground optically flat to two wave lengths. Because of the high viscosity of the 80% solution, the solutions were forced into the diffusion cell by air pressure rather than by allowing them to flow under the head of gravity.

Anhydrous glucose, 99.9 or better per cent. pure, from the Corn Products Refining Co. served as the source of glucose. The glucose was weighed out and mixed with distilled water to which alkali had been added in the ratio of 5 ml. 0.1 N NaOH to 595 g. of water. The refractive index of a 20% glucose solution whose pH was slightly below 7 as measured with a glass electrode was followed over a period of 10 hours in order to determine any change with time. We found a maximum change of 0.00007 refractive index units which was considered to be negligible.

The accuracy of the data is probably as good as in the previous work on sucrose[2] except perhaps in the case of the 80% glucose solution. The high viscosity of this solution, approximately 21 poises as measured by us with a rotating disc viscometer[9] made the formation of a sharp clean boundary difficult. The large negative Δt correction was probably due to a prolongation, beyond the moment of starting the diffusion, of the flow used to sharpen the boundary. The C_t values were as constant as in the previous work[2]; fringe numbers 2, 4, 6, 8 and 10 were used in computing the C_t average value. The variation in the slope of the refractive index-concentration plot over the range of concentration differences used in this research was entirely negligible. Viscosity data were taken from Powell[10] who studied glucose up to 50%; values at higher concentrations were our own. Viscosities of sucrose solutions were taken from standard tables.[11]

Results

The results of the work on glucose are collected together in Table I and plotted in Fig. 1. In addition to the glucose solution data we also made a measurement of the diffusion coefficient of a 40% sucrose solution at 25° in order to fill in that part of the concentration range omitted by previous workers, and one at 35° at a low concentration to enable us to calculate activation energies.

TABLE I

DIFFUSION COEFFICIENTS OF GLUCOSE SOLUTIONS

Wt. per cent. c_1	Wt. per cent. c_2	$c_{av.}$	Mole fraction, N_2	Moles H_2O/mole glucose	Δt, sec.	$D \times 10^7$, cm.^{2}/sec.
		25°				
		0.00				67.5[a]
0.00	1.50	0.75	0.000755	1323	−3.95	66.75
9.56	10.37	9.97	0.01094	90.4	−25.7	57.97
18.23	20.93	19.58	.02377	41.07	198	48.59
28.92	30.88	29.90	.04103	23.37	39.5	39.56
39.07	41.13	40.10	.06274	14.94	4.3	30.16
49.00	51.04	50.02	.09099	9.99	348	21.99
59.61	61.23	60.42	.1325	6.55	284	13.30
69.67	71.20	70.44	.1923	4.20	323	6.29
79.75	80.56	80.16	.2874	2.48	−2,000	1.49
		35°				
		0.00				84.9[a]
0.00	1.675	0.837	0.000843	1184	−13.2	84.00
39.27	41.23	40.25	.06311	14.84	1.6	41.44
59.84	61.67	60.75	.13403	6.46	381	20.28
69.81	70.86	70.33	.19162	4.22	394	10.55
80.39	80.83	80.61	.29365	2.41	−84	2.53
		Sucrose at 25°				
40.52	41.40	40.96	0.0352	27.4	26.8	22.98
		Sucrose at 35°				
0.00	1.478	0.739		2550		65.8

[a] Extrapolated value.

Discussion of the Data

The diffusion coefficients of the glucose solutions at 25° decrease linearly with increase of concentration expressed on a weight per cent. basis up to about 30% glucose; the decrease then proceeds less rapidly with concentration until at 80%

(1) Department of Chemistry, Georgia Institute of Technology, Atlanta, Georgia.

(2) A. C. English and M. Dole, THIS JOURNAL, **72**, 3261 (1950).

(3) L. Friedman and P. G. Carpenter, *ibid.*, **61**, 1745 (1939).

(4) J. H. Northrop and M. L. J. Anson, *J. Gen. Physiol.*, **12**, 543 (1929).

(5) J. W. McBain and T. H. Liu, THIS JOURNAL, **53**, 29 (1931).

(6) L. G. Longsworth, *ibid.*, **69**, 2510 (1947).

(7) G. Kegeles and L. J. Gosting, *ibid.*, **69**, 2516 (1947); Gosting and M. Morris, *ibid.*, **71**, 1998 (1949).

(8) C. A. Coulson, J. T. Cox, A. G. Ogston and J. St. L. Philpot, *Proc. Roy. Soc. (London)*, **A192**, 382 (1948).

(9) A Brookfield Synchro-Lectric Viscometer, Multispeed Model LVF, Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

(10) C. W. R. Powell, *J. Chem. Soc.*, **105**, 1 (1914).

(11) Circular C 440, National Bureau of Standards, Washington D. C.

glucose the curve becomes pronouncedly convex to the concentration axis as can be seen from Fig. 1 where the glucose and sucrose data are compared. The behavior at 35° is similar. The data of Friedman and Carpenter[3] obtained by the porous disc method agree well with ours at infinite dilution, 67.8×10^{-7} as compared to 67.5×10^{-7} cm.²/sec., but at higher concentrations their values rise above ours. In the case of the sucrose data we also found that the porous disc results were higher than ours.

We have been unable to compare the diffusion coefficients for glucose with the values predicted from the empirical equation of Gordon[12] because of unavailability of activity coefficient data of glucose. By analogy with sucrose we would not expect the equation to be applicable; in fact, Gordon[13] has recently pointed out that his equation is strictly valid only if

$$\frac{D_1^0\eta_2^0}{\bar{V}_2} = \frac{D_2^0\eta_1^0}{\bar{V}_2} \qquad (1)$$

"a condition that obviously cannot generally hold."

Longsworth[14] recently has published values for the diffusion coefficient of a number of amino acids, peptides and sugars at 1° and at one concentration for each. He quotes several empirical equations for the diffusion coefficient in terms of the molecular weight. In order to calculate the molecular weight of the solute Friedman and Carpenter[3] used the Stokes–Einstein relation in the form

$$M = \frac{R^3T^3\rho}{162\pi^3\eta_0^3D_0^3N^2} \qquad (2)$$

where ρ is the density of the solute, N the Avogadro number and the other symbols have their usual meanings. If we use the density of the solid, we find 184 for the molecular weight of glucose from eq. (2) but 409 for sucrose. The value for glucose is close to the correct molecular weight, 180, but the molecular weight of sucrose is considerably too high. Longsworth[14] found that the diffusion coefficient of different solutes could be correlated better with the apparent molal volume than with the molecular weight. In terms of the molal volume, eq. (2) may be written

$$D = \frac{kT}{6\pi\eta_0}\left[\frac{4\pi N}{3V}\right]^{1/3} \qquad (3)$$

Using (3) to calculate the diffusion coefficient of glucose and sucrose at infinite dilution we obtained 69.10 and 56.03 $\times 10^{-7}$ cm.²/sec., respectively, whereas the values extrapolated from the observed data are 67.5 and 52.3 $\times 10^{-7}$ cm.² sec.[15] The differences between the calculated and observed amount to 2.3 and 7.8%.

Another way of testing eq. (2) is to use it between two temperatures to calculate activation energies (assuming that ρ is independent of T), and then to compare the activation energy calculated from (2) with that observed. If we restrict (2) to data for one solvent at infinite dilution, then all solutes should have the same activation energy providing that ρ is independent of the temperature. Between 1 and 25° the calculated energy is 5042 cal./mole. Using the data for glucose and sucrose of Longsworth[14] at 1°, the data for sucrose of English and Dole[2] at 25° and the data for glucose of this paper adjusted to the same concentrations in the two cases as used by Longsworth, we calculate the activation energy for glucose and sucrose to be 5110 and 5160 cal./mole, respectively. These results differ by only 1.4 and 2.3% from those predicted by the Stokes–Einstein equation. Hence eq. (2) can reproduce the activation energy somewhat better than it can reproduce the diffusion coefficient.

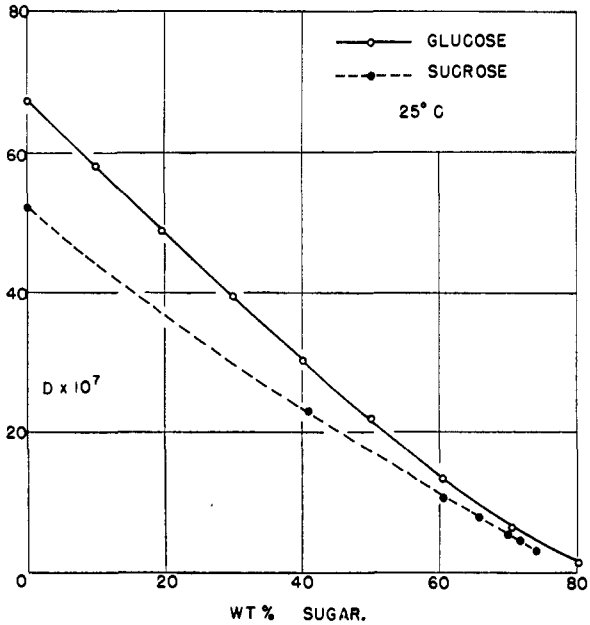


Fig. 1.—Diffusion coefficients of sucrose and glucose at 25°.

In studying the viscosity and diffusion data for sucrose and glucose, we have found that the logarithm of the relative viscosity is a linear function of the mole fraction of the sugar, Fig. 2. The logarithm of the diffusion coefficient ratio, shown in Fig. 2, is also approximately a linear function of the mole fraction although the value for the 80% glucose solution (mole fraction 0.287) is out of line with the data at the lower concentrations. (This logarithmic function is not accurate enough for extrapolation to zero concentration.) Since at the highest concentration the relative viscosity is 100-fold greater than the ratio D_0/D, it is clear that the viscosity increases with concentration far more rapidly than the diffusion coefficient decreases. Apparently, these two phenomena are not related in these highly concentrated solutions. This conclusion is also borne out by the comparison of activation energies for diffusion and viscous flow illustrated in Fig. 4.

It is interesting to consider the interpretation of our data in terms of the absolute reaction rate theory of diffusion as developed by Glasstone, Laidler and Eyring.[16] Consider their equation

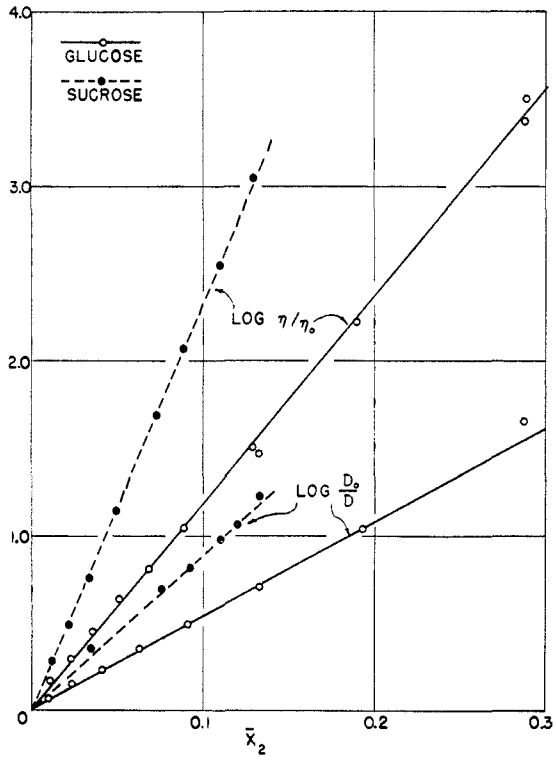
(12) A. R. Gordon, *J. Chem. Phys.*, **5**, 522 (1937).

(13) A. R. Gordon, THIS JOURNAL, **72**, 4840 (1950).

(14) L. G. Longsworth, *ibid.*, **74**, 4155 (1952).

(15) The value for D_0 for sucrose at 25° was taken from the paper by L. J. Gosting and M. Morris, THIS JOURNAL, **71**, 1998 (1949); it also agrees with the extrapolated value estimated from the data of this paper in conjunction with the data of English and Dole.[2]

(16) S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, Chap. IX.



Mole fraction of solute.

Fig. 2.

$$D = \lambda^2 \frac{kT}{h} e^{\Delta S^{\ddagger}/R} e^{-\Delta E^{\ddagger}/RT} \qquad (4)$$

where λ is the distance between equilibrium points in the solution across which the diffusing entity must migrate in surmounting the potential barrier of activation energy $\Delta E^{\ddagger}$. This equation should be applied, probably, only to self-diffusion coefficients,[17] such as the value for glucose solutions extrapolated to infinite dilution. At all other concentrations the measured diffusion coefficient is that of the solution and is probably influenced in part by the diffusion of the water. However, as we have no data for the self-diffusion coefficients of glucose or sucrose in aqueous solutions (which can be determined only by a tracer technique), we shall limit our discussion to the measured diffusion coefficients.

In Fig. 3 we have plotted the logarithm of the measured diffusion coefficient against the activation energy calculated by subtracting RT from ΔE_{Act}. Wang[18] believes that the measured activation energy may be as significant theoretically or more significant than $\Delta E^{\ddagger}$ because of the fact that the calculation of $\Delta E^{\ddagger}$ by subtracting RT from ΔE_{Act} neglects any change of the factor $\lambda^2 e^{\Delta S^{\ddagger}/R}$ with temperature. As a matter of fact the relationships illustrated in this paper are not significantly altered by the substitution of $\Delta E^{\ddagger}$ for ΔE_{Act} or *vice versa*. Glucose solutions at 35° and sucrose solutions at both temperatures follow the relationship expressed in Fig. 3 with great exactness, but the slopes of the lines are not equal to $-RT/2.3$ as we would expect from eq. (4); furthermore the slopes decrease more in passing from 25 to 35° than one would expect from the factor $1/RT$.

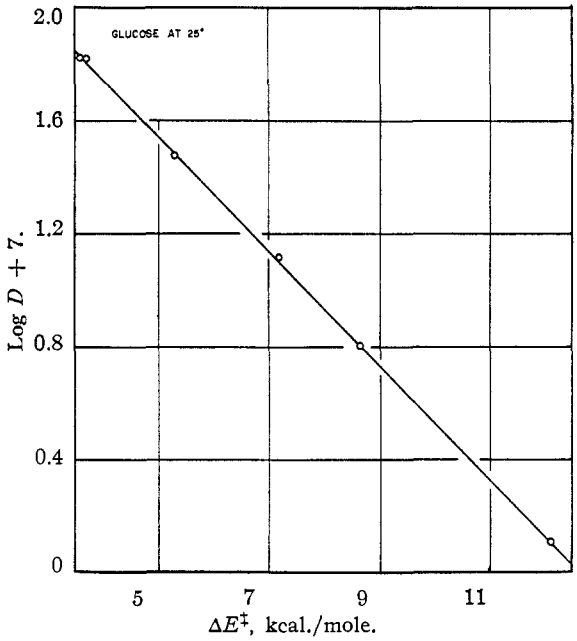


Fig. 3.—Relationship between the logarithm of the diffusion coefficient and the activation energy.

From the linear relationship between log D and $\Delta E^{\ddagger}$ it might be expected that the entropy of activation would be constant over the concentration range studied, but this is not the case. As a consequence the term log $[\lambda^2 e^{\Delta S^{\ddagger}/R}]$ must be linearly proportional to $\Delta E^{\ddagger}$ as has been found to be true. In Table II we list the calculated values of the entropies and energies of activation.

TABLE II

ENERGIES AND ENTROPIES OF ACTIVATION FOR DIFFUSION, 25 TO 35°

Mole fraction	Wt. %	ΔE_{Act}, k. cal.	$[\lambda^2 e^{\Delta S^{\ddagger}/R}]^{1/2}$, Å.	$\Delta S^{\ddagger}$, e.u.
		Sucrose		
0	0	4.40	2.23	1.50
0.0791	62	7.20	10.6	7.68
.0856	64	7.33	11.2	7.89
.1006	68	7.76	13.9	8.77
.1192	72	8.61	23.2	10.80
.1303	74	9.51	43.0	13.25
		Glucose		
0	0	4.20	2.19	1.41
0.0631	40.25	5.88	6.06	5.46
.1340	60.75	7.78	19.87	10.18
.1916	70.33	9.24	47.71	13.66
.2933	80.61	12.73	403.5	22.15

If the entropy of activation is equal to zero, then the square root of $\lambda^2 e^{\Delta S^{\ddagger}/R}$ should be of the order of magnitude of an Ångström unit. From the data of Table II it is evident that this is correct at low concentrations; however, at 80% glucose the value rises as high as 403.5 Å., which is obviously ridiculous. The entropy of activation must be

(17) This was kindly pointed out to us by Professor A. W. Adamson of the University of Southern California.

(18) L. H. Wang, THIS JOURNAL, **74**, 1614 (1952); **75**, 466 (1953), and private communication.

considered, but before doing so let us discuss a possible mechanism of diffusion.

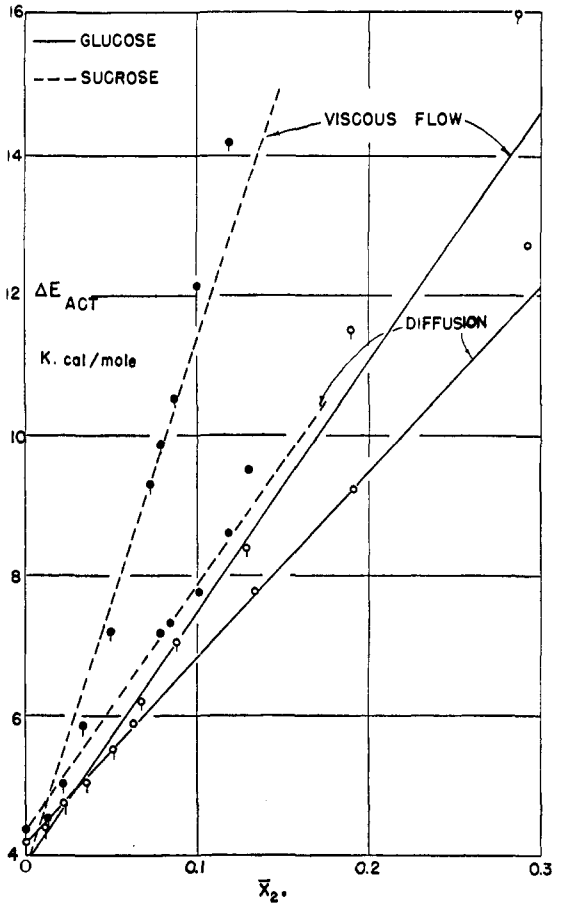
At zero concentration the activation energies for diffusion of both sucrose and glucose solutions are equal within the experimental uncertainties to that for the self-diffusion of water, 4.4 ± 0.3 kcal./mole, as determined by Wang, Robinson and Edelman[19] using O^{18} as tracer. This suggests that the hypothesis given by Glasstone, Laidler and Eyring, which postulates that for solutes of large size it is the solvent molecule whose jumps between equilibrium points in the liquid determines the activation energy for diffusion, may be correct. If we adopt a value of 1.53 Å. for λ as calculated by Wang, Robinson and Edelman[19] from dielectric relaxation data of water, it becomes possible to estimate the entropy of activation. The results of this calculation are included in Table II where it can be seen that at zero concentration the value is about 1.5 e.u. If we make a similar calculation from the diffusion data for the self-diffusion of water, we obtain 5.59 e.u. Thus, the high value of the diffusion coefficient of water in liquid water at 25°, 283 × 10^{-7} cm.^{2}/sec., as compared to sucrose or glucose at infinite dilution is seen to be due to a larger entropy of activation.

At high concentrations the entropies and energies of activation, which are proportional to each other, rise to very high values. At 80% glucose they are 22.1 e.u. and 12.1 kcal./mole, respectively. This entropy value is greater than the gain in entropy when ice melts, 5.2 e.u., and greater than the rotational entropy of a gaseous water molecule at 25°, 10.46 e.u. as calculated using the moments of inertia given by Herzberg.[20] The energy of activation is greater than the vaporization energy of water although it may be no greater than the adsorption energy of a water molecule in the first adsorption layer on solid glucose (which would be interesting to measure).

The activation energy for diffusion for both glucose and sucrose increases approximately linearly with the mole fraction of the solute as illustrated in Fig. 4. The activation energies in the case of the sucrose solutions rise more rapidly with concentration than do those of glucose solutions. If the postulate with respect to the transitions of water molecules between equilibrium points as the rate determining factor in diffusion is correct, then the increasing activation energy with increasing mole fraction of the solute must be the result of the participation in the diffusion process, at the high concentrations, of water molecules bound with considerable hydration energy to the solute molecules. This hydration energy must be added to the normal activation energy for such water molecules to surmount the potential barrier. The average activation energy at any one concentration could then be expressed by the equation

$$\overline{E^{\ddagger}} = E_1^{\ddagger} N_1 + E_h^{\ddagger} h p N_2 \qquad (5)$$

where $E_1^{\ddagger}$ is the activation energy in pure water,



Mole fraction of solute.

Fig. 4.

$E_h^{\ddagger}$ the activation energy of water molecules in the hydration shell, h the number of the water molecules in such shell, p the relative frequency of hydration-shell molecules making the diffusion transitions as compared to normal molecules and N_1 and N_2 the mole fractions of solvent and solute, respectively. Equation (5) rearranges to

$$\overline{E^{\ddagger}} = E_1^{\ddagger} + [E_h^{\ddagger} h p - E_1^{\ddagger}] N_2 \qquad (6)$$

At very high concentrations where there is an insufficient number of water molecules to hydrate completely the solute molecule (at 80% glucose there are only 2.4 water molecules per glucose molecule), the average activation energy should be given by the equation

$$\begin{aligned} \overline{E^{\ddagger}} &= E_h^{\ddagger} p_s N_1 + E_s^{\ddagger} N_2 \\ &= E_h^{\ddagger} p_s + [E_s^{\ddagger} - E_h^{\ddagger} p_s] N_2 \end{aligned} \qquad (7)$$

Here p_s is the relative probability that a water molecule having an activation energy $E_h^{\ddagger}$ makes diffusional transitions as compared to a sugar molecule whose activation energy is $E_s^{\ddagger}$. As in this case all the water molecules are in the hydration shell, we do not need to include the factor h. If this formulation of the activation energy is correct, we would expect the slope of the activation energy–mole fraction curve to increase at high concentrations. There is some indication from Fig. 4 that this is correct.

(19) J. H. Wang, C. V. Robinson and I. S. Edelman, THIS JOURNAL, 75, 466 (1953).

(20) G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand Co., Inc., New York, N. Y., 1945, p. 488.

Because of the fact that the activation energy for diffusion rises even above the heat of vaporization of water, we are inclined to believe that the mechanism of diffusion involves the simultaneous breaking of several hydrogen bonds, as many as three in the case of the most concentrated glucose solution. If diffusion takes place by a rotation of a sugar molecule in such a way as to carry a water molecule forward as previously suggested by English and Dole,[3] several hydrogen bonds would have to be broken in as much as the sugar molecule in question would be hydrogen bonded to a number of water molecules which would in turn be partially bonded to other sugar molecules.

The more hydrogen bonds that are broken in the diffusion process, the greater the activation energy and the greater the increase in entropy of the activated state. Referring to eq. (4) it is clear that if $\Delta S^{\ddagger}$ is the same at similar values of $\Delta E^{\ddagger}$ for both glucose and sucrose, the diffusion coefficient of the two substances should be the same at the same $\Delta E^{\ddagger}$. This is not quite the case as the diffusional entropy for the sucrose solutions is less than the entropy of the glucose solutions at the same activation energy.

Acknowledgments.—We wish to acknowledge gratefully the financial support and gift of materials from E. J. Brach and Sons, Inc., Chicago. We continue to be indebted to Dr. K. A. Strand, Director of the Dearborn Observatory at Northwestern University for the use of his dividing engine.

EVANSTON, ILLINOIS

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

Iron–Thioglycolate Complexes

BY D. L. LEUSSING AND I. M. KOLTHOFF

RECEIVED MARCH 14, 1953

In solutions with a *p*H between about 9 and 11, two complexes composed of iron(II) and thioglycolic acid are found: $Fe(OH)(RS)^-$(yellow), and $Fe(RS)_2^=$(red), in which RS denotes $^-OOC—CH_2S^-$. The equilibrium contants $K_{Fe(II)_1}$ and $K_{Fe(II)_2}$ of the reactions: $Fe(OH)(RS)^- + 2H^+ \rightleftarrows Fe^{++} + RSH^- + H_2O$ and $Fe(RS)_2^= + 2H^+ \rightleftarrows Fe^{++} + 2RSH^-$ were found from solubility data to be equal to 1.5×10^{12} and 1.5×10^{10}, respectively, at 25°. The ratio of the two constants was also determined spectrophotometrically and found equal to 0.009 in good agreement with the value found from solubility data. In weakly acid medium slightly soluble FeRS is formed. The activity constant $S_{FeRS} = a_{Fe^{++}}a_{RSH^-}/a_{H^+}$ was found equal to 7. The intensely colored complex of iron and thioglycolate in alkaline medium is derived from ferric iron and does not contain ferrous iron. Ferric iron catalyzes the air oxidation of thioglycolate to the disulfide. From solubility data of ferric hydroxide in ammoniacal thioglycolate solutions, extrapolated to zero time when no reduction of ferric iron by thioglycolate has occurred, it was postulated that the complex has the composition $FeOH(RS)_2^=$. The formation of the complex is given by: $Fe(OH)_3(s) = 2RSH^- \rightleftarrows Fe(OH)(RS)_2^= + 2H_2O$, with an equilibrium constant at 25° $a^2_{RSH}/a_{Fe(OH)(RS)_2^=}$ of 0.020.

Complex formation[1] between iron and thioglycolate has been made of analytical use in the colorimetric determination of iron[2–4] and in the separation of iron from other metals.[5,6] In air-saturated ammoniacal medium a deep purple-red complex is formed regardless, whether the iron was present initially in the ferrous or ferric state.

In alkaline solutions iron(III) oxidizes thioglycolate to dithiodiglycolate, $-O_2CCH_2SSCH_2CO_2-$, with formation of iron(II). Oxygen very rapidly oxidizes the iron(II) in alkaline thioglycolate solutions so the net result is the catalytic oxidation of thioglycolate by oxygen.[7–10] The rate of reduction of iron(III) by thioglycolate increases with the acidity of the solution and in acid solutions is very rapid to form a colorless solution which does not give the deep red color in the presence of oxygen.[9]

Little has been reported on the composition of the ferrous and ferric thioglycolate complexes existing in aqueous solutions. Indeed, even the valence state of iron is in doubt in the deep purple-red complex. Lyons[2] postulates that it is a ferrous complex, Dubsky and Sindelar[11] claim that it is a ferric complex and Mayr and Gebauer[12] state that the highly colored complex contains both ferrous and ferric iron and has the composition KFe(III)$[Fe(II)(SCH_2CO_2)_2]_2$.

Schubert[10] and Mayr and Gebauer[12] have prepared solid ferrous–thioglycolate salts corresponding to the composition $Fe(SCH_2CO_2)$ (yellow) and $M_2Fe(SCH_2CO_2)_2$ (red), where M is an alkali metal. $Fe(SCH_2CO_2)$ is reported to be insoluble but $M_2Fe(SCH_2CO_2)_2$ dissolves in air-free water to form light yellow solutions at low concentrations and raspberry red solutions at higher concentrations. The intensity of the color of these latter solutions is much less than that of air-saturated alkaline solutions containing the same amount of iron.

Michaelis and Schubert[13] have attempted to prepare solid salts of ferric iron and thioglycolate but were unsuccessful because of the reduction of the iron to ferrous. They were able to prepare solid Co(III)–thioglycolate compounds which existed as dimers.

(1) R. Andreasch, *Ber.*, **12**, 1391 (1879).
(2) E. Lyons, THIS JOURNAL, **49**, 1916 (1927).
(3) H. W. Swank and M. G. Mellon, *Ind. Eng. Chem., Anal. Ed.*, **10**, 7 (1938).
(4) E. B. Sandell, "Colorimetric Determination of Traces of Metals," 2nd ed., Interscience Publishers, Inc., New York, N. Y., 1952, p. 378.
(5) C. Mayr and A. Gebauer, *Z. anal. Chem.*, **113**, 189 (1938).
(6) R. A. Hummel and E. B. Sandell, *Anal. Chim. Acta*, **7**, 308 (1952).
(7) P. Claesson, *Ber.*, **14**, 409 (1881).
(8) L. Michaelis, *J. Biol. Chem.*, **84**, 777 (1929).
(9) B. K. Cannan and G. N. Richardson, *Biochem. J.*, **23**, 1242 (1929).
(10) M. P. Schubert, THIS JOURNAL, **54**, 4077 (1932).
(11) J. V. Dubsky and V. Sindelar, *Microchim. Acta*, **3**, 258 (1938).
(12) C. Mayr and A. Gebauer, *Z. anal. Chem.*, **116**, 225 (1939).
(13) L. Michaelis and M. P. Schubert, THIS JOURNAL, **52**, 4418 (1930).