

data alone. Hence the heat and entropy of activation have not been computed for this peptide. Similarly for higher peptides, heats and entropies of activation are meaningless unless related to the scission of a specific peptide bond and are therefore not reported. The results for the heat and entropy of activation for diglycine are given in Table II together with similar data from other investigations.

TABLE II  
HEAT AND ENTROPY OF ACTIVATION FOR ACID- AND BASE-CATALYZED HYDROLYSIS OF DIGLYCINE

	Acid hydrolysis	Base hydrolysis
$\Delta H^\ddagger$ (kcal./mole)	19.1	14.1
	20.3 <sup>a</sup>	14.9 <sup>b</sup>
		16.9 <sup>a</sup>
$\Delta S^\ddagger$ (e.u.)	-30.4	-35
	-24.0 <sup>a</sup>	-26.8 <sup>a</sup>

<sup>a</sup> Ref. 3d. <sup>b</sup> Ref. 3b.

It will be seen that the entropy of activation is large and negative in both acid- and base-catalyzed

hydrolysis due to the low probability of formation of an activated complex from two ions (the peptide and the catalyst) and a water molecule. The difference of about 5 kcal./mole in activation energy between acid and base hydrolysis may be related to the fact that in alkaline hydrolysis the formation of the activated complex is favored by the attraction of the negative hydroxyl ion to the positive carbonyl carbon in the peptide bond, whereas in acid hydrolysis the  $H_3O^+$  ion must oppose the positively charged nitrogen atom. This situation is quite analogous to the similarly catalyzed ester hydrolysis.<sup>14</sup>

**Acknowledgment.**—The authors gratefully acknowledge a grant from the American Philosophical Society for partial support of this research.

(14) S. Glasstone, K. J. Laidler and H. Eyring, "Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, p. 451.

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### Diffusion in Sugar Solutions. III. Diffusion of Sucrose in Glucose Solutions as Solvent

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Diffusion experiments have been performed by the Gouy interference method in mixed water-glucose-sucrose systems with sucrose present in small concentrations. Diffusion coefficients of sucrose at infinite dilution in the glucose solutions were calculated by the height-area approximation method. It is shown that independent diffusion of the two solutes does not occur, that large discrepancies exist between the height-area and weight-average diffusion coefficients, that diffusion between two solutions having equal vapor pressures of water approximates the ideal Gaussian pattern, and that the diffusion coefficient of sucrose calculated from the latter experiment agrees well with the data from the other less ideal experiments. The diffusion coefficient of sucrose is greater in the glucose solutions when compared at the same total mole fraction of sugar than in its own solutions. The activation energy for diffusion approaches that for glucose diffusion. A method of performing diffusion experiments on multiple component systems so that the measured average diffusion coefficient will equal that of one component is suggested.

#### I. Introduction

This paper is the third in a series devoted to the study of diffusion in sugar solutions.<sup>1</sup> English and Dole<sup>2</sup> studied sucrose solutions and Gladden and Dole,<sup>3</sup> glucose solutions, all in water as the solvent. The present paper extends the work to the three-component system, water-glucose-sucrose, with the sucrose-glucose ratio in most solutions being very small.

If metals are left out of consideration, investigations on diffusion in three-component systems<sup>4</sup> have not been extensive. However, the new optical methods for the study of diffusion have stimulated efforts to determine diffusion coefficients in mixtures, particularly among the biochemists.<sup>5</sup>

(1) The first two papers were entitled diffusion in "supersaturated" solutions, but as the second and this paper cover the range from low to high concentrations the restricting word "supersaturated" has been omitted from the title.

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

(3) J. K. Gladden and M. Dole, *ibid.*, **75**, 3900 (1953).

(4) The paper of Freundlich and Kruger, *J. Phys. Chem.*, **43**, 981 (1939), on the diffusion of quinone in salt solutions contains references to earlier papers. McBain and co-workers have studied mixtures of ions and ions with glycine or egg albumin: J. W. McBain and C. R. Dawson, *THIS JOURNAL*, **56**, 52, 1021 (1934); J. R. Vinograd and J. W. McBain, *ibid.*, **63**, 2008 (1941).

(5) H. Neurath, *Chem. Revs.*, **30**, 357 (1942).

Ogston<sup>6</sup> was apparently the first to apply the Gouy interference method<sup>7-9</sup> to mixtures; he studied the diffusion of lactoglobulin in solutions which had been dialyzed against a sodium acetate-acetic acid buffer and to which weighed amounts of sucrose or potassium chloride had been added. Using a rather complicated method of analyzing his data, he obtained good agreement to about 2% between the observed diffusion coefficients of both solutes and their known values.

Gralén<sup>10</sup> suggested the use of the ratio between the diffusion coefficient calculated from the second moment and the diffusion coefficient calculated using the zero'th moment, the height-area diffusion coefficient, as a measure of the polydispersity of the solution. Charlwood<sup>11</sup> recently concluded that use of the fourth moment in studying the polydispersity of the solution was not practical, despite its theoretical advantage in yielding the standard deviation (in conjunction with the second moment),

(6) A. G. Ogston, *Proc. Roy. Soc. (London)*, **196A**, 272 (1949); *Biochem. J.*, **45**, 189 (1949).

(7) G. Kegeles and L. J. Gosting, *THIS JOURNAL*, **69**, 2516 (1947).

(8) L. G. Longworth, *ibid.*, **69**, 2510 (1947).

(9) L. J. Gosting and M. Morris, *ibid.*, **71**, 1998 (1949).

(10) N. Gralén, *Kolloid Z.*, **95**, 188 (1941).

(11) P. A. Charlwood, *J. Phys. Chem.*, **57**, 125 (1953).

and recommended precise determinations of the height-area diffusion coefficient and the diffusion coefficient calculated from the second moment in agreement with the suggestion of Gralén. Some other papers on the problem of the interpretation of diffusion measurements in multi-component systems are those of Bevilacqua, Bevilacqua, Bender and Williams<sup>12</sup> and Beckmann and Rosenberg.<sup>13</sup>

Scheibling<sup>14</sup> used an optical interference technique in measuring the diffusion coefficient of mixtures of sugars in aqueous solutions. He investigated a solution 0.25% in sucrose and 0.25% in galactose and calculated the diffusion coefficients by a unique method. His conclusions, which differ significantly from ours, will be discussed below. Akeley and Gosting<sup>15</sup> have expanded their important studies of the Gouy interference method to three component systems, partly to study the applicability and theory of the method and partly to determine the effect of a third component on the shape of the concentration gradient-distance curve.

## II. Experimental Details

The experimental equipment including the optical system and diffusion cell and the chemical reagents were the same as those used by Gladden and Dole<sup>8</sup> and English and Dole<sup>2</sup> and need not be redescribed here. Three types of solutions were studied; the most common was prepared by making aqueous solutions 5, 30, 50, 60 or 70% in glucose, dividing the solution in half and adding to one of the halves enough sucrose to make the solution approximately 2, 3, 4 or 5% in sucrose. The glucose-sucrose-water solution was then allowed to diffuse into the glucose-water solution. Some solutions were prepared by making up a glucose solution, dividing it in half and adding differing amounts of sucrose to each half. Finally, one solution pair was prepared in which the activity of the water was the same in both halves of the solution as nearly as could be estimated. A 40% glucose solution was prepared, divided in half, enough sucrose added to one half to make it 3% in sucrose and a molecularly equivalent amount of glucose added to the other half. Thus both solutions had the same total mole fraction of solutes. The vapor phase of the two solutions was then allowed to equilibrate at constant temperature in an equilibrater of the type invented by Jones and Kaplan.<sup>16</sup> The rate of transfer of vapor phase between the cells containing the two solutions was about 50 cc. per revolution with 20 revolutions per minute. With approximately 400 g. of water per cell, this gives a time for transfer of half of the water in about 10 days. At the end of three days of

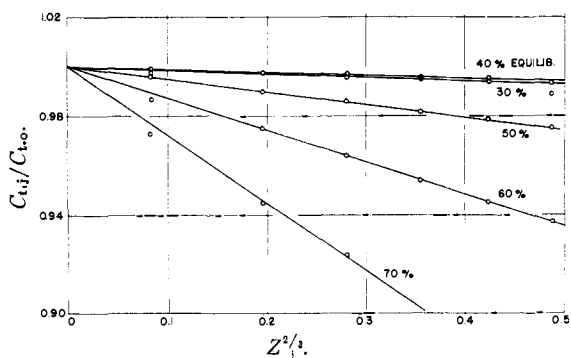


Fig. 1.—Akeley-Gosting method for the extrapolation of  $C_t$  values to zero  $Z_j$ .

(12) E. M. Bevilacqua, E. B. Bevilacqua, M. M. Bender and J. W. Williams, *Ann. N. Y. Acad. Sci.*, **46**, 309 (1945).

(13) C. O. Beckmann and J. L. Rosenberg, *ibid.*, **46**, 329 (1945).

(14) G. Scheibling, *J. chim. phys.*, **47**, 688 (1950).

(15) D. F. Akeley and L. J. Gosting, *THIS JOURNAL*, **75**, 5685 (1953).

(16) Grinnell Jones and B. B. Kaplan, *ibid.*, **50**, 1600 (1928).

equilibration each of the two cells was separately weighed with no change of weight being detectable. As a result, the conclusion was reached that the vapor pressure of the two solutions was nearly identical. The results of the diffusion experiment using the equilibrated solutions also demonstrated that these solutions were markedly different in their behavior from the other two types of solution mentioned above.

The concentrations of the diffusing solutions in general were determined by measurement of the refractive index of the glucose solutions and by calculation of the concentration from an equation relating refractive index,  $n$ , to concentration.<sup>17</sup> The refractive index measurements were made using a Bausch and Lomb dipping refractometer to about  $\pm 0.00007$  refractive index unit. Knowing the weight of the glucose solution and the weight of the added sucrose, the final weight percentages of both sucrose and glucose were easily calculated. The ratios  $\Delta c_2/\Delta c_1$  were calculated from the known percentages of glucose and sucrose in the solutions. In this paper the subscript 2 will refer to glucose and 1 to sucrose.

As will be pointed out later the ratio  $\Delta n_2/\Delta n_1$  is more significant than  $\Delta c_2/\Delta c_1$  for diffusion measurements by the Gouy interference method. This ratio, which will be denoted by  $\beta$ , was obtained by calculating  $\Delta n_2$  from the known difference in glucose concentration across the boundary,  $\Delta c_2$ , and the known value of  $\partial n/\partial c_2$  for glucose. The value of the latter for pure glucose-water systems was used. The net refractive index increment across the boundary,  $\Delta n$ , was calculated from the expression  $\Delta n = j_m/\lambda a$ , which is discussed below. The difference between  $\Delta n$  and  $\Delta n_2$  gave  $\Delta n_1$  and the ratio  $\Delta n_2/\Delta n_1$ , or  $\beta$ , could then be readily calculated. If  $R_1$  represents  $\partial n/\partial c_1$  and  $R_2$ ,  $\partial n/\partial c_2$ , then the ratio  $R_2/R_1$  should be nearly a constant at any specific glucose concentration. Some estimate of the accuracy of this estimate of  $\beta$  can be gained by an examination of the values of  $R_2/R_1$  given in Table I.

## III. Calculation of the Diffusion Coefficient

In three-component systems the calculation of the diffusion coefficient of either solute is difficult because of the fact that the change of the refractive index gradient with height does not follow a pure Gaussian curve. Akeley and Gosting<sup>15</sup> have demonstrated how to compute the deviations in the position of the interference fringes from the expected Gaussian positions. They have also suggested an extrapolation method for determining  $C_t$  the distance of maximum displacement of light. Knowing  $C_t$  the average diffusion coefficient of the mixture,  $\bar{D}$ , can then be calculated from Kegeles and Gosting's<sup>7</sup> equation applicable to the Gouy interference method

$$\sqrt{\bar{D}'} = \frac{\lambda b j_m}{2 C_t \sqrt{\pi t}} \quad (1)$$

where  $t$  is the time in sec.,  $\lambda$  the wave length of the monochromatic light used,  $b$  the effective optical distance from the center of the cell to the focusing point of the undeviated image and  $j_m$  is  $a\Delta n/\lambda$  where  $\Delta n$  is the total increment in refractive index across the boundary and  $a$  is the distance between the glass windows of the diffusion cell. Akeley and Gosting<sup>15</sup> recommend that  $C_t$  be obtained by calculating  $Y_j/e^{-j^2}$  for different fringe minima,  $j$ , and plotting this ratio as a function of  $Z_j^{2/3}$ . At zero  $Z$ , the extrapolated value is  $C_t$ . Figure 1 illustrates a plot of the function  $Y_j/C_t e^{-j^2}$  as a function of  $Z_j^{2/3}$ . The quantity  $Y_j$  is the distance measured between the undeviated image of the slit source and the minimum in intensity of the  $j$ 'th interference

(17) *J. Assoc. Offic. Agr. Chem.*, **27**, 295 (1944). The equation is  $n_{20}^D = 1.33299 + 1.42849 \times 10^{-3} c + 4.59415 \times 10^{-6} c^2 + 2.19455 \times 10^{-8} c^3 - 3.7399 \times 10^{-11} c^4$  where  $c$  is the per cent. of glucose by weight.

fringe in the focal plane.  $Z_j$  is approximately  $j + 3/4$  (exact values of  $Z_j$  have been tabulated by Gosting and Morris<sup>9</sup>), and is related to  $f(\xi_j)$  by the equation

$$f(\xi_j) = Z_j/j_m \quad (2)$$

When  $f(\xi_j)$  has been found,  $e^{-\xi_j^2}$  follows from tables prepared by Kegeles.<sup>18</sup>

In eq. 1  $\bar{D}'$  is given a superscript prime to indicate that it varies slightly with the time because of an initial, unavoidable disturbance at the boundary. The diffusion coefficient,  $\bar{D}$ , for infinite time is obtained from an extrapolation based on the relation

$$D' = D \left( 1 + \frac{\Delta t}{l} \right) \quad (3)$$

The diffusion coefficient  $D_1$  of sucrose in glucose-water mixtures as solvent was calculated making use of the following equation first derived by Quensel,<sup>19</sup> where  $D_2$  is the diffusion coefficient of glucose-

$$D_1 = \frac{\bar{D}}{\left[ 1 + \frac{\Delta c_2}{\Delta c_1} \left( 1 - \frac{\sqrt{\bar{D}}}{\sqrt{D_2}} \right) \right]^2} \quad (4)$$

water systems and  $\Delta c_2/\Delta c_1$  is the ratio (negative in our experiments) of the change in glucose concentration across the boundary divided by the change in sucrose concentration across the boundary. Because of the fact that  $\bar{D}$  was obtained experimentally from variations in the refractive index rather than concentration across the boundary, the concentration ratio of eq. 4 must be replaced by  $\beta$ , the refractive index increment ratio. In the work of this research most of the solutions were prepared by adding weighed quantities of sucrose to a known weight of glucose solution; this solution of three components was then allowed to diffuse into the original glucose solution of two components. For a series of measurements using the same glucose solution but different amounts of sucrose, the ratio  $\Delta c_2/\Delta c_1$  was always negative and remained constant. Thus if  $w$  represents grams,  $\bar{v}_i$  the partial specific volume of one component, and the subscripts 0, 1 and 2, water, sucrose and glucose, respectively, then

$$\frac{dc_2}{dc_1} = - \frac{\bar{v}_1 w_2}{\bar{v}_0 w_0 + \bar{v}_2 w_2} \quad (5)$$

The ratio  $\Delta c_2/\Delta c_1$  is, therefore, a constant independent of  $w_1$  the weight of sucrose added, as long as the initial ratio of the weights of glucose and water remained unchanged, and as long as the amount of added sucrose did not become large enough to alter appreciably  $\bar{v}_0$  and  $\bar{v}_2$ . An estimate of the partial molal volumes of glucose and sucrose at 25° in their aqueous solutions showed that over the concentration range 30-70%, the volumes were constant to  $\pm 0.5\%$  at 115.5 and 217 cc., respectively. The small variations in  $\beta$  in Table I at any one glucose concentration are due to the fact that the stock glucose solution was not always the same for each experiment, but had to be made up anew for many of the experiments.

If  $c$  is expressed in weight percentage units as is the case in all the work reported in this paper,

(18) Private communication from Dr. L. J. Gosting. See also Kegeles and Gosting.<sup>7</sup>

(19) As quoted by Gralén<sup>10</sup> from the Dissertation of O. Quensel, Uppsala, 1942.

$\Delta c_2/\Delta c_1$  is simply equal to the weight fraction of glucose in each master solution before the addition of sucrose when the solutions are prepared as described above.

The value of the diffusion coefficient of glucose in the three component system was assumed to be the same as in the two component system water-glucose. As the calculated values of  $D_1$  were extrapolated to zero sucrose concentration in the glucose-water solution, any error in the calculations resulting from the use of  $D_2$  as measured by Gladden and Dole<sup>3</sup> should have been eliminated.

#### IV. Results

All the significant data which were obtained are collected in Table I. Values of  $D_1$  at 30, 50, 60 and 70% glucose at 25° and 60% at 35° are plotted in Fig. 2 as a function of the sucrose concentration. Within the considerable experimental uncertainties the lines at 30 and 50% glucose have the same slope. Using this slope the data at 5, 40, 60 and 70% glucose, where a series of measurements at different sucrose concentrations were not carried out, or

TABLE I  
DIFFUSION COEFFICIENTS IN GLUCOSE-SUCROSE-WATER MIXTURES

Av. wt. %		$-\beta$	$R_2/R_1$	$\bar{D} \times 10^7$ , cm. <sup>2</sup> /sec.	$\Delta t$ , sec.	(a) $D_2$	(b) $D_1$
Glucose	Sucrose						
No sucrose in one solution at 25°							
5.0	0						(47.9)
5.176	0.999	0.0519 <sub>2</sub>	0.998	47.1 <sub>5</sub>	1.3	62.4 <sub>6</sub>	47.8 <sub>0</sub>
30.0	0						(28.8)
29.82	0.600	0.2985	0.995	25.6 <sub>3</sub>	-16	38.7 <sub>9</sub>	28.7 <sub>6</sub>
29.73	0.900	.2978	.993	25.6 <sub>0</sub>	64	38.8 <sub>7</sub>	28.7 <sub>3</sub>
29.55	1.500	.2959	.986	25.5 <sub>2</sub>	-23	39.0 <sub>4</sub>	28.6 <sub>8</sub>
40.0°	0						(21.2)
39.93	1.501	0.6877	0.991	11.2 <sub>7</sub>	91	30.3 <sub>0</sub>	21.0 <sub>5</sub>
50.0	0						(15.5)
49.70	0.601	0.4970	0.996	10.5 <sub>4</sub>	-15	22.2 <sub>5</sub>	14.7 <sub>6</sub>
49.50	1.000	.4941	.988	11.2 <sub>7</sub>	-87	22.4 <sub>1</sub>	15.3 <sub>7</sub>
49.37	1.467	.4957	.989	11.0 <sub>1</sub>	-231	22.5 <sub>1</sub>	15.2 <sub>0</sub>
49.23	1.540	.4956	.991	11.2 <sub>0</sub>	321	22.6 <sub>2</sub>	15.3 <sub>9</sub>
49.06	2.393	.4935	.982	11.1 <sub>1</sub>	-220	22.7 <sub>6</sub>	15.3 <sub>3</sub>
60.0	0						(8.8)
59.48	0.953	0.5931	0.988	5.05 <sub>5</sub>	-157	13.9 <sub>7</sub>	8.6 <sub>7</sub>
59.77	1.520	.5972	.984	4.69 <sub>5</sub>	188	13.7 <sub>7</sub>	8.3 <sub>1</sub>
58.95	1.998	.5909	.982	4.98 <sub>5</sub>	17	14.3 <sub>5</sub>	8.6 <sub>9</sub>
58.29	2.516	.5886	.985	5.05 <sub>0</sub>	159	14.8 <sub>2</sub>	8.8 <sub>6</sub>
70.0	0						(3.5)
70.40	0.966	0.7003	0.985	1.22 <sub>8</sub>	430	6.3 <sub>2</sub>	3.3 <sub>2</sub>
69.99	1.485	.6980	.983	1.29 <sub>9</sub>	236	6.5 <sub>7</sub>	3.4 <sub>6</sub>
at 35°							
60.0	0						(13.8)
59.47	1.004	0.5922	0.988	8.3 <sub>5</sub>	91	21.7 <sub>5</sub>	13.9 <sub>1</sub>
59.51	1.017	.5973	.993	8.0 <sub>5</sub>	35	21.6 <sub>1</sub>	13.0 <sub>7</sub>
59.30	1.512	.5919	.983	7.8 <sub>3</sub>	169	21.8 <sub>3</sub>	13.4 <sub>7</sub>
Sucrose in both solutions at 25°							
Glucose		Sucrose		$-\beta$	$R_2/R_1$	$\bar{D} \times 10^7$ , cm. <sup>2</sup> /sec.	$\Delta t$
$c_1$	$c_2$	$c_1$	$c_2$				
15.19	14.49	23.38	26.94	0.1952	0.984	21.3 <sub>3</sub>	3519
32.96	31.56	33.50	36.89	.4872	.975	3.7 <sub>1</sub>	-144
39.39	38.34	22.28	24.30	.4984	.984	5.4 <sub>8</sub>	79
42.18	40.87	16.51	19.11	.4967	.984	6.7 <sub>2</sub>	111
45.95	44.58	7.82	10.48	.4914	.956	9.0 <sub>1</sub>	-71

<sup>a</sup> The  $D_2$  values for glucose in this column were calculated at the glucose concentration given, assuming no sucrose in the solution. <sup>b</sup> The data in parentheses represent the values extrapolated to zero sucrose concentration. <sup>c</sup> The data at 40% glucose were obtained using the solutions equilibrated to equal water vapor pressure.

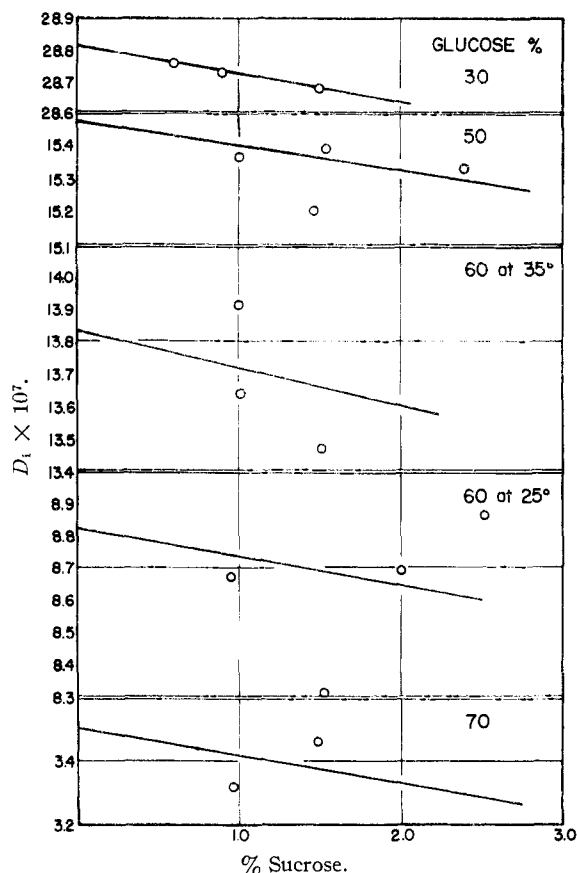


Fig. 2.—Calculated diffusion coefficients of the sucrose component,  $D_1$ , as a function of the average weight percentage of sucrose in the interdiffusing solutions.

where the data were too scattered to determine the slope, were extrapolated to zero sucrose concentration. The  $D_2$  values of Table I were calculated at the specific glucose concentrations using an empirical equation fitted to the data of Gladden and Dole.<sup>3</sup>

## V. Discussion

1. **Validity of the Calculations for the Three-component Systems.**—First, let us consider the validity of the data. As mentioned above in the case of a three-component system the net refractive index gradient at any point in the boundary does not follow a Gaussian curve. As a result the  $C_t$  values calculated for different fringe minima by the method valid for two component systems<sup>7</sup> are not constant. However, the height of two combined Gaussian curves, each of which represents the diffusion of one solute, is equal to the height of the sum of the two Gaussian curves. Also the area of the combined curve has to be equal to the sum of the areas of the two single curves. Thus, it seems reasonable to calculate  $C_t$  in the case of the mixed solutes from the distance of maximum displacement of light. This value of  $C_t$  corresponds to the maximum value of  $dn/dh$  at the point  $h$  equals zero where  $h$  is height or distance across the diffusion boundary measured from the initial boundary position (there was no observable net displacement of the boundary during the diffusion experiments).

By using for  $j_m$  in eq. 1 the maximum number of fringes as measured by the methods normally used in the case of two component systems, effectively the total area of the combined Gaussian curves is used. Hence, this method of computing  $\bar{D}$  is called the "height-area" method.

There are other methods by which the  $C_t$  values calculated from the position of each fringe minimum could have been extrapolated to zero  $Z$  instead of the method used above. One such method investigated in this research, called the "adjusted  $j_m$  method," consisted in arbitrarily varying  $j_m$  until  $C_t$  calculated from the 10 lowest fringes became constant or nearly constant. The average values of the diffusion coefficient so calculated, denoted by the closed circles of Fig. 3, had the remarkable property of coinciding with the diffusion coefficients of the two-component water-sucrose system at the same total mole fraction of sugar above about 0.09 mole fraction, see Fig. 3. This property of the adjusted  $j_m \bar{D}$  values makes possible an estimate of the diffusion coefficient of pure sucrose solutions at concentrations higher than it was possible to measure them (dotted line of Fig. 3).

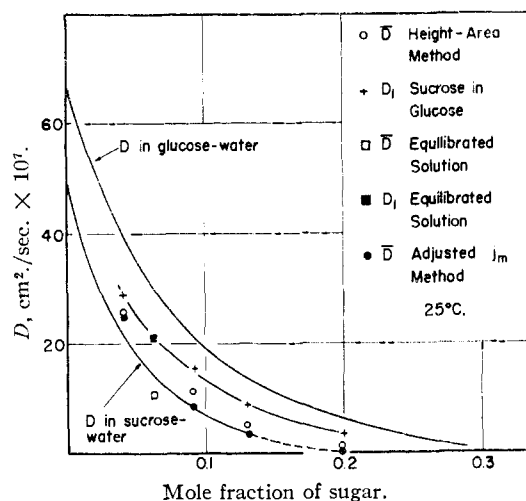


Fig. 3.—Comparison of diffusion coefficients at the same total mole fraction of solute. Top and bottom curves represent diffusion coefficients of glucose and sucrose in their single solute solutions. The dotted extension of the lower curve is not an extrapolation, but has been drawn to coincide with the calculated average "adjusted  $j_m$ " diffusion coefficients. The middle solid curve represents  $D_1$ , the diffusion coefficients of sucrose at infinite dilution in glucose solutions.

The  $D_1$  values calculated from a modified form of eq. 4 (modified to correct for the arbitrarily selected values of  $j_m$ ) agreed to about 0.3% with the  $D_1$  values of Table I and will not be given here.

It is interesting to consider the results of the diffusion experiment at 40% glucose in which the vapor pressure of water of the two interdiffusing solutions was equilibrated to equality before diffusion took place. The  $\bar{D}$  value was only  $11.27 \times 10^{-7}$  cm.<sup>2</sup>/sec., whereas a value of about  $19 \times 10^{-7}$  had been expected on the basis of the data at 30 and 50% glucose. The low value of  $\bar{D}$  arose from the relatively large value of  $\beta$  required for the experiment. Despite this fact, it is reassuring to note

that the calculated  $D_1$  diffusion coefficient fell on the same curve of  $D_1$  versus mole fraction of glucose as the other  $D_1$  values, Fig. 3.

The equilibrated solution experiment is also interesting because the diffusion appeared to take place more nearly according to a pure Gaussian behavior than diffusion in the other solutions. Thus, in Fig. 1 the slope of the curve representing the equilibrated solution experiment, 40% glucose, is more nearly zero than the slope of the curves for any of the other solutions. An exactly Gaussian diffusion for the mixture would have given rise to a constant value of  $C_t$ , independent of fringe number; in other words, to a zero slope of the straight lines<sup>15</sup> of Fig. 1.

The higher the concentration of glucose, the more the slopes of the lines of Fig. 1 deviate from zero. Apparently, the magnitude of the slopes is in no way connected with the value of  $\beta$ . For any one solution, the slopes of the lines of Fig. 1 are independent of the  $C_t$  values; in other words independent of the time.

Referring to eq. 4, it can be seen that if  $\Delta c_2$  is made zero, as is possible by adding an extra amount of glucose to the solution to which the sucrose has been added, then  $\beta$  would equal zero and  $\bar{D}$  would equal  $D_1$  and again the system would behave like a two-component system. It would be interesting to carry out such an experiment.

If  $\Delta c_2 R_2$  is made equal to  $-\Delta c_1 R_1$ ,  $\beta$  equal to  $-1$ , then eq. 4 tells us that  $D_1$  would equal  $\bar{D}_2$ . This is an absurdity, but if  $\Delta c_2 R_2$  equalled  $-\Delta c_1 R_1$  there would be no boundary. This case is, therefore, meaningless.

As Gralén<sup>10</sup> has pointed out, it is possible to calculate a weight average diffusion coefficient defined by the equation (for two solutes)

$$D_{2,0} = \frac{D_1 + \beta D_2}{1 + \beta} \quad (6)$$

$D_{2,0}$  can be measured experimentally in terms of the second moment of the Gaussian curve. It is interesting to compute values of  $D_{2,0}$  from eq. 6 using the  $D_1$  and  $D_2$  data of Table I. Table II contains the results of this calculation.

TABLE II  
WEIGHT AVERAGE DIFFUSION COEFFICIENTS

Glucose, %	$-\beta$	$D_0$ , cm. <sup>2</sup> sec./ $\times 10^7$	$D_{2,0}$
5	0.0519	47.2	47.1
30	.299	25.71	24.6
40	.688	11.00	0.14
50	.495	11.37	9.1
60	.591	5.15	1.9
70	.700	1.32	-3.7

Vanishingly small or even negative values of  $D_{2,0}$  were obtained. Especially surprising was the extraordinarily low value of  $D_{2,0}$  in the case of the equilibrated solution experiment at 40% glucose. Apparently the greater the value of  $\beta$  the more unreasonable the calculated values of  $D_{2,0}$ .

If the diffusion of the two solutes is completely independent, as assumed in the derivation of eq. 4 and 6, then the concentration gradient established by glucose alone, by sucrose alone and for the

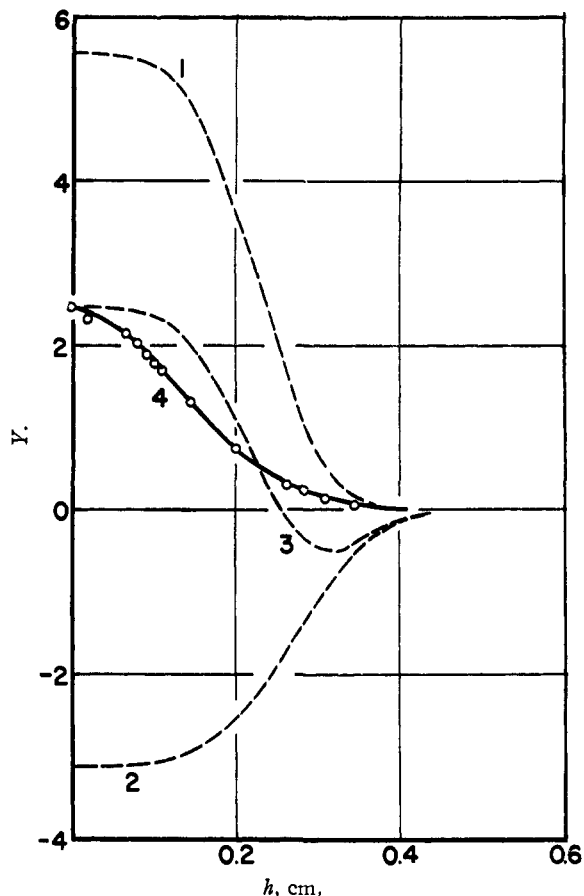


Fig. 4.—Theoretical Gaussian curves for the displacement of light due to refractive index gradients at the distance  $h$  from the center of the boundary; 40% glucose equilibrated experiment. Curve 1 represents the theoretical values calculated for single-solute sucrose diffusion; curve 2 for the glucose single-solute diffusion; curve 3 the resultant of curves 1 and 2. The solid line represents data calculated from the observed values of  $Y$  (curve 4).

mixture would give rise to the  $Y_j$  values illustrated in Fig. 4. The  $Y_j$  values of curve 3 change sign and pass through a minimum as the distance from the initial position of the boundary,  $h$ , increases.<sup>20</sup> Such a behavior would mean that an inverted density gradient in the solution and density unbalance would occur.<sup>21</sup> It is now clear why the calculated weight average diffusion coefficient may be very low, zero or negative because the calculated second moment may be zero or negative. Figure 5, dotted line, illustrates values of  $Y_j h^2$  for the resultant curve 3 of Fig. 4. Note that the area included by the dotted line of Fig. 5 sums to practically zero. Figures 4 and 5 represent calculations for the case of the equilibrated solution experiment at 40% glucose, and are typical of all of the experiments of three component systems.

In Figs. 4 and 5 the solid lines represent the experimental observations which agree with the theoretical Gaussian expectations (calculated for a single

(20) D. M. Clarke, Doctoral Dissertation, Northwestern University, 1953.

(21) This was first kindly pointed out to us by L. J. Gosting and independently by L. G. Longworth.

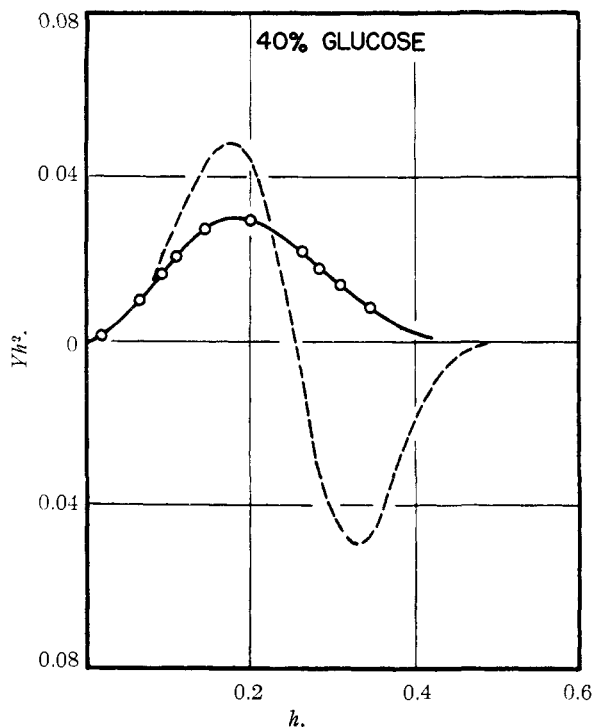


Fig. 5.—The dotted line represents values of  $h^2Y$  calculated from curve 3 of Fig. 4 while the solid line has the values calculated from the observed data. The area of these curves divided by the area of the corresponding curves of Fig. 4 is the second moment.

solute) so closely that the deviations cannot be detected on the scale of Figs. 4 and 5. Thus, we have the definite result that diffusion in the equilibrated solution experiment did not follow the expectations for independent glucose and sucrose diffusion, but occurred so as to produce results satisfying the expectations for a single solute system. Only in the case of the equilibrated solution experiment was this latter fact true.

A sensitive test of the deviation of the results from strictly Gaussian values is to plot  $\Omega$  as a function of  $f(\zeta)$  as recommended by Akeley and Gosting.<sup>15</sup> The relative fringe deviation,  $\Omega$ , is defined by the equation

$$\Omega_i = e^{-\zeta_i^2} - Y_i/C_i \quad (7)$$

In Fig. 6 such a plot is shown for the equilibrated solution experiment at 40% glucose and for an experiment in 60% glucose where  $\beta$ , sucrose concentrations and time after formation of the boundary were nearly the same. Again the close approach of the data to the ideal Gaussian values can be seen in the case of the equilibrated solution experiment. However, in comparing the curves of Fig. 6 with those of Akeley and Gosting<sup>15</sup> it should be noted that the ordinate scale of Fig. 6 is one hundred times greater than that used by Akeley and Gosting. Akeley and Gosting give theoretical expressions for  $\Omega$  in terms of a power series in  $\alpha_2$  where

$$\alpha_2 = \frac{\beta}{1 + \beta} \quad (8)$$

In many of our experiments the absolute value of  $\alpha_2$  was greater than unity; hence for this reason, in

addition to the fact that they are based on the assumption of independent diffusion of the solute species, the equations of Akeley and Gosting are inapplicable. This study also demonstrates that the use of the relative fringe deviations as an analytical tool as suggested by Akeley and Gosting<sup>15</sup> is impossible in these concentrated solutions. The two curves of Fig. 6 represent solutions containing the same "impurity" percentage of sucrose, yet the values of  $\Omega$  differ by an order of magnitude.

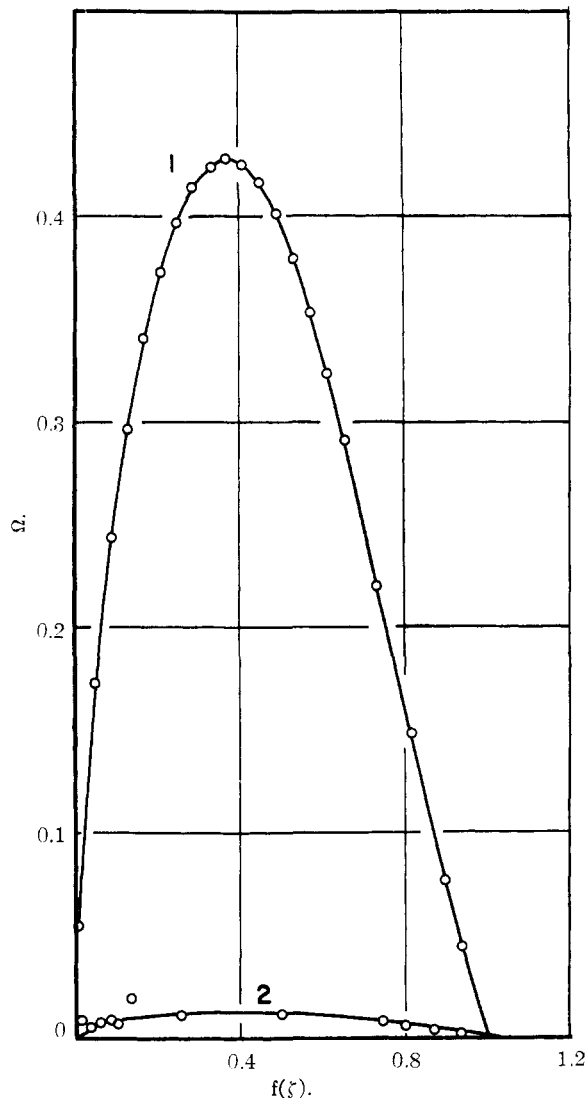


Fig. 6.—Akeley-Gosting function  $\Omega$  (the relative fringe deviations) plotted as a function of  $f(\zeta)$ . The upper curve represents data calculated from an experiment carried out in accordance with the standard procedures described in this paper (60% glucose,  $C_1$  5.2547, 1.520% sucrose,  $\bar{D}' = 4.796 \times 10^{-7}$ ,  $\Delta C_2/\Delta C_1 = 0.6068$ ,  $t = 8955$  sec.); the lower curve, from an experiment in which the vapor pressure of water was brought to equality in the two interdiffusing solutions (40% glucose,  $t = 7395$  sec.,  $C_1 = 2.4756$ ,  $\bar{D}' = 11.39 \times 10^{-7}$ , 1.50% sucrose,  $\Delta C_2/\Delta C_1 = -0.6942$ ).

In conclusion, it would seem that the average diffusion coefficient for the three-component system measured in terms of the height and area of the  $dn/dh$  versus  $h$  curve is the most logical average to use.

The  $D_1$  values calculated from this average by means of eq. 4 (after replacing  $\Delta c_2/\Delta c_1$  by  $\beta$ ) seem reasonable. The good agreement with the other data of the value of  $D_1$  obtained in the equilibrated solution experiment, where conditions were markedly different, lends credence to the results. However, the results demonstrate convincingly that diffusion does not occur independently for the two solutes; probably because the diffusion or displacement of the solvent molecules cannot simultaneously satisfy the requirements for independent diffusion of both the glucose and sucrose molecules. Unfortunately, there seems to be no way in which what might be called the "interaction" diffusion coefficients,  $D_{ik}$ , discussed by Onsager<sup>22</sup> can be evaluated from the data of this research.

For the remainder of this paper the diffusion coefficients,  $D_1$  and  $D_2$ , will be treated in the same way as diffusion coefficients calculated in two-component experiments.

**2. Discussion of the  $D_1$  Values.**—As can be seen from Fig. 3, the diffusion coefficients of sucrose in glucose solutions as solvent become relatively greater than corresponding values in the pure sucrose-water system as the concentration of glucose increases. This is contrary to the observation of Scheibling<sup>14</sup> who found that the solute with the higher diffusion coefficient diffuses more rapidly and the solute with the lower diffusion coefficient more slowly in the three-component mixture than in their pure solutions. However, he compared sucrose and galactose at the same weight percentage rather than at the same mole fraction. When our data are compared at the same weight percentage of glucose and sucrose, then the diffusion coefficient of sucrose is less in the glucose solution than in the pure sucrose solution. But it should be noted that the ratio  $D_2/D_1$  increases from 1.29 at 0% glucose to 1.88 at 70% glucose. Increasing the concentration of glucose decreases the diffusion coefficient of sucrose relatively more than it decreases the diffusion coefficient of glucose.

(22) L. Onsager, *Ann. N. Y. Acad. Sci.*, **46**, 241 (1945).

The activation energy for the diffusion of sucrose in 60% glucose is 8.2 kcal. as compared to a value of 9.7 kcal. in sucrose solutions of the same mole fraction (0.13) and as compared to 7.8 kcal. for glucose in its own solutions at this mole fraction. Thus the activation energy approaches that for the glucose-water system. At 60% glucose there are about 6 molecules of water per molecule of glucose; hence a large part of the water will be hydrated to the glucose and if the mechanism of diffusion of sucrose in such a solution is based on transitions of water molecules as previously suggested,<sup>2,3</sup> the activation energy for diffusion would be expected to be that of transitions of the water molecule in the neighborhood of a glucose molecule. This explains the higher diffusion coefficient of sucrose in the glucose solutions than in its own solutions at the same mole fraction.

It is true that the viscosity of sucrose solutions at a mole fraction of 0.130 is 34.3 times as great as that of glucose solutions at the same mole fraction, but the diffusion coefficient of sucrose in the glucose solution is only greater by a factor of 4.0 than in its own solutions at 0.130 mole fraction. This strikingly illustrates the previous observations and conclusions of English and Dole<sup>2</sup> and Gladden and Dole<sup>3</sup> that the viscosity of the solutions increases more rapidly than the diffusion coefficient decreases, and that there is little connection between the two in these highly concentrated solutions.

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