proceeds mainly by the unsymmetrical activated complex. Experiments we have done confirm this conclusion and extend it also to other alcohols. Two experiments were done for each alcohol, one without alcoholate ion, and one with alcoholate ion added. The reaction mixtures were made up to the same over-all composition by adding sodium alcoholate after reaction, also to the system which had none present during reaction. The NO_2 was introduced in CCl_4 solution, so that the reaction medium contained CCl_4 in volume equal to the alcohol. The average concentration of alcoholate ion was 0.6 *M*. The salt which separated after reaction was collected, washed, and the nitrite content determined.[6] The fraction (percentage) of the NO_2 converted to NO_2^- (corresponding to the alkyl nitrate as the co-product) for each alcohol, without and with Na alcoholate present was found as: EtOH, 0.025, 0.12; *n*-BuOH, 0.051, 0.095; *t*-BuOH, 0.051, 0.11. In each case the unsymmetrical cleavage is the predominant[1] process (to form RONO and $NaNO_3$), but in each case also, there is a distinct increase in the fraction forming the nitrate ester when the alcoholate ion is present. The difference between the mode of action of NO_2 with the alcohols, and that assumed for water under some conditions may be caused in part by the difference in rate of reaction. The rate difference was demonstrated by an experiment in which the oxide dissolved in CCl_4 was shaken with a solution containing 1 mole of water for every 100 of C_2H_5OH. After reaction, the solution was diluted with water and extracted with CCl_4. It was found that only $^1/_5$ of the NO_2 disappeared by the reaction which forms organic esters, so that the specific rate of reaction of the oxide with water exceeds that with alcohol by a factor of at least 400. In view of the rapid rate at which NO_2 reacts in the liquid, this ratio is probably much below the true value, because diffusion of water through the alcohol will have limited the extent of the reaction with water. The much lower rate of reaction of NO_2 with alcohol makes it seem possible that alcohol, but not water, allows time for rearrangement of N_2O_4 from a symmetrical (presumably the equilibrium structure) to an unsymmetrical structure. The increase in extent of the reaction to form NO_2^- and organic nitrate when alcoholate is present is consistent with this interpretation, because the ion may be expected to react more rapidly than the alcohol.

Experiments 7, 14 and 16 were performed to learn whether the exchange of NO_2^- with $NO_2(N_2O_4)$ is rapid enough to compete with the reaction of the oxide with water. All experiments agree in showing effects of the exchange of NO_2^- with NO_2, since the nitrate formed is found to be much closer to the isotopic composition of the NO_2^- (and of the solvent) than it is in experiments without added $NO_2{}^{*-}$. It is remarkable that the exchange of NO_2 with NO_2^- can compete in rate with the reaction of NO_2 with water, even though the NO_2^- is much less abundant than is water. The effects of this exchange appear to be little felt in the other experiments of Table I, although NO_2^- is delivered as product in isotopic composition approaching that of the solvent. No appreciable effect of the nitrite as a uniformly distributed product would be expected (the concentration of the nitrite product did not exceed 0.01 *M*) but there would seem to be the possibility of extensive exchange in a local reaction zone. However, the nitrite would be present as HNO_2 rather than NO_2^-, and this may account for the failure to observe any exchange effects caused by product nitrite.

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[6] H. A. Liebhafsky and E. H. Winslow, *Anal Chem.*, **11**, 189 (1939).

Further Studies of the Diffusion of Mixed Solutes with the Gouy Diffusiometer

BY PETER J. DUNLOP

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This note supplements previous diffusion data for dilute aqueous solutions of mixed solutes[1] by presenting experiments in which there was simultaneous diffusion of mixtures of (a) glycolamide and sucrose and (b) glycine and glycolamide. The latter experiment illustrates clearly that, when the ratio of the diffusion coefficients of the two simultaneously diffusing solutes is between 0.92 and 1.08, deviations of the Gouy fringes from their ideal positions are barely measurable. This problem of resolution is of major importance in interpreting diffusion experiments on proteins.

To aid in interpreting the second experiment, single-solute diffusion and refractive index data were obtained for the recrystallized sample of glycine; these data are also compared with previous measurements.[2,3] The notation of Akeley and Gosting[1] will be adopted and only the most important definitions repeated; hence constant reference should be made to this former work.

Materials, Solutions and Experimental Procedure

The same sucrose and glycolamide samples were used in these experiments as in work reported earlier.[1,4] A commercially available sample of C.P. glycine[5] was once recrystallized from conductance water, drained centrifugally and dried at 60° for 48 hours *in vacuo*. The procedure for making up solutions and calculating their molarities, *C*, has been described elsewhere.[1] In these calculations the following molecular weights were used: sucrose 342.296, glycine 75.068 and glycolamide 75.068. For the mixtures, solution densities, *d*, were measured in 30-ml. Pyrex pycnometers; also, using the apparent molal volumes, ϕ, in ml. per mole, given below[6,7] for aqueous solutions of single solutes, corresponding densities for these three component solutions were

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

[2] M. S. Lyons and J. V. Thomas, *ibid.*, **72**, 4506 (1950).

[3] L. G. Longsworth, *ibid.*, **75**, 5705 (1953).

[4] P. J. Dunlop and L. J. Gosting, *ibid.*, **75**, 5073 (1953).

[5] Obtained from Pfanstiehl Chemical Company, Waukegan, Illinois.

[6] F. T. Gucker, Jr., W. L. Ford and C. E. Moser, *J. Phys. Chem.*, **43**, 153 (1939).

[7] G. Jones and S. K. Talley, THIS JOURNAL, **55**, 624 (1933).

TABLE I[a]

Exp. no.	Solute$_1$	Solute$_2$	$d_{\text{exp.}}$	C_1, moles/l.	C_2, moles/l.	α_2	j_m Calcd.	j_m Obsd.	$\frac{\Delta n}{\Delta C} \times 10^3$	$D_A \times 10^5$, cm.^{2}/sec. Calcd.[d]	$D_A \times 10^5$, cm.^{2}/sec. Obsd.
I	Glycolamide	Sucrose	1.00218[b]	0.2499_3	0.000259	0.0517	111.65	111.57	....	1.077_2	1.075_5
II	Glycine	....	......	$.0999_9$	......	....	....	61.77	13.568	....	1.050_4
III	Glycine	....	......	$.2000_1$	......	....	....	123.29	13.539	....	1.041_1
IV	Glycine	Glycolamide	1.00340[c]	$.1250_4$	0.1249_4	0.4068	130.15	130.06	....	1.082_8	1.079_0

[a] The concentration and density data are for the lower solutions used to form the initial boundary: in all experiments the upper phase was the solvent, doubly-distilled water, saturated with air. [b,c] The corresponding calculated values are 1.00217 and 1.00344. All starting time corrections Δt, were less than 11 seconds. [d] Calculated from equation 10, ref. 1.

calculated.[1] Good agreement was obtained between calculated and experimental values (see Table I).

$$\phi_{\text{sucrose}} = 212 \quad (1)$$

$$\phi_{\text{glycine}} = 43.199 + 0.8614C \quad (2)$$

$$\phi_{\text{glycolamide}} = 56.156 + 0.1595C \quad (3)$$

The Gouy diffusiometer used to measure the "height-area average" diffusion coefficients, D_A, and the refractive index increments, $\Delta n/\Delta C$, has been adequately described,[4,8] as have also the methods employed to obtain D_A, $\Delta n/\Delta C$, and the graph of the relative fringe deviations, Ω_j (equation 9[1]), *versus* reduced fringe number $f(\zeta_j)^1$. An average δ correction of -17 microns was applied in all experiments and in all cases the same Tiselius cell, with a 2.486_2 cm. "a" dimension, was used. All experiments were performed within ±0.005° of 25° and the diffusion coefficients corrected to 25.00_0° by means of the Stokes–Einstein relation.

Results

To test the applicability of equation 23[1] for predicting the relative fringe deviation graphs for mixtures of sucrose and glycolamide, an experiment was performed in which α_{sucrose}, the refractive index fraction of the boundary due to that solute, was approximately 0.05. The relative fringe deviation graph, given in Fig. 1, indicates that the experimental plot is in reasonable agreement with that calculated from equation 23[1] by using the previously reported[1,4] diffusion coefficients, D, and refractive index increments, $\Delta n/\Delta C$, from single-solute experiments at the same mean concentrations, $\bar{C}$. Reference to Table I, experiment I, shows that the experimental and calculated values of the total number of fringes, j_m, are in good agreement, while the calculated value of D_A is 0.2% high.

Since a recrystallized sample of glycine was prepared for the glycine–glycolamide experiment, two single-solute diffusion measurements, experiments II and III of Table I, were performed with this preparation. Both experiments yielded data which were lower by 0.3% in D and 0.1% in $\Delta n/\Delta C$ than those of Lyons and Thomas. Hence the two equations given below were adopted for the present work.[9]

$$D_{\text{glycine}} \times 10^5 = 1.060_2 - 0.192_4\bar{C} \quad \bar{C} < 0.56 \quad (4)$$

$$\Delta n/\Delta C_{\text{glycine}} \times 10^3 = 13.60_1 - 0.64_2\bar{C} \quad \bar{C} < 0.56 \quad (5)$$

In each equation the slopes are those previously reported[2,4] but the limiting values of D and $\Delta n/\Delta C$ have been adjusted to fit the present experimental data. Relative fringe deviation graphs for the single-solute diffusion of both the glycine and glycolamide samples are shown in Fig. 2. That values of Ω_j are zero within experimental error at all values of $f(\zeta_j)$ indicates that the boundaries were Gaussian within the limits of experimental measurement.

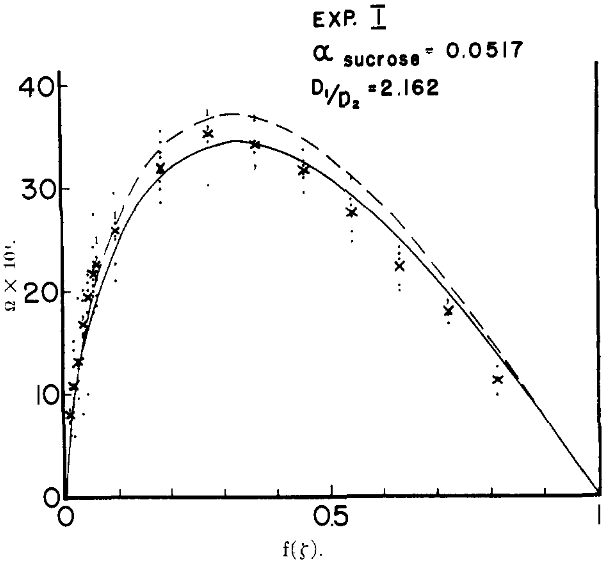

Fig. 1.—Relative fringe deviations for sucrose impurity in glycolamide, $\alpha_{\text{sucrose}} = 0.0517$. The dashed line gives the plot predicted from the first term of equation 23 and the solid line that from the first two terms. At a given value of $f(\zeta)$ crosses indicate the average of the experimental points obtained from ten different Gouy photographs of the same boundary. Individual points are represented with dots.

Equation 27[1] describes the relative fringe deviation graphs when r_2, the ratio of the diffusion coefficients of the two solute components, is approximately unity. This equation shows that the deviation graph tends to zero as r_2 approaches unity, regardless of the relative amounts of the two solutes, and that it becomes impossible from this graph to detect impurities with diffusion coefficients similar in value to the main component. Glycine and glycolamide, two isomers, have similar diffusion coefficients and provide an excellent system for testing and illustrating this point. Figure 3 shows the deviation graph for a mixture, experiment IV of Table I, in which the refractive index fractions of glycine and glycolamide are almost equal. The deviations from Gaussian form are barely detectable experimentally and are of the same order of magnitude as an error of

(8) L. J. Gosting, E. M. Hanson, G. Kegeles and M. S. Morris, *Rev. Sci. Instruments*, **20**, 209 (1949).

(9) Neither Lyons and Thomas nor Longsworth recrystallized their glycine samples. Presumably the difference between our values and those of the previous workers is due to the fact that the present sample was recrystallized. In any case, the values of D and $\Delta n/\Delta C$ in equations 4 and 5 which describe measurements on this sample in the present apparatus should be the best values to use for predicting D_A for the glycine–glycolamide experiment IV. For comparison, Longsworth (ref. 3) with $\bar{C} = 0.03988$ and $\Delta C = 0.07976$ obtained $D \times 10^5 = 1.055_4$ and $\Delta n/\Delta C = 13.567$. The corresponding values calculated from equations 4 and 5 are $D \times 10^5 = 1.052_5$ and $\Delta n/\Delta C = 13.575$.

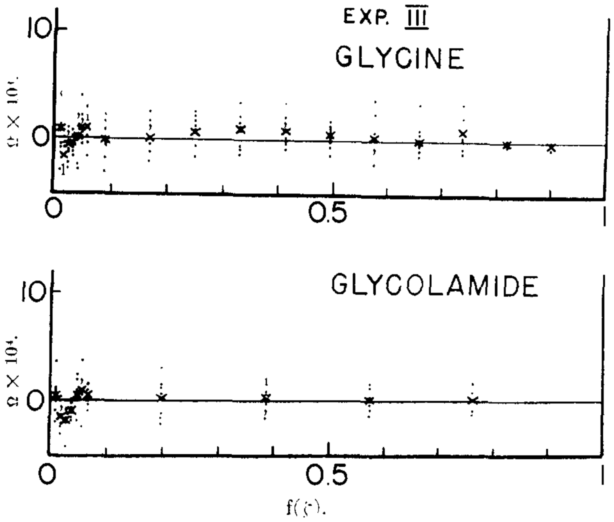

Fig. 2.—Relative fringe deviation graphs for glycine and glycolamide. The glycolamide deviation graph is for an experiment (ref. 4) in which the mean concentration was $\bar{C}$ = 0.1249$_9$ and the difference in concentration across the boundary was ΔC = 0.2499$_8$.

two or three microns in δ. Calculated and experimental values of j_m are in good agreement while those for D_A differ by 0.4%.

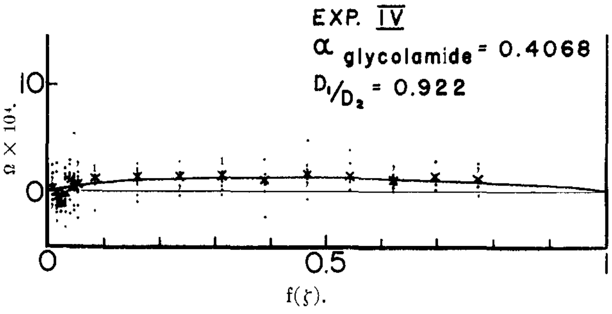

Fig. 3.—Relative fringe deviations for the glycine–glycolamide mixture, $\alpha_{\text{glycolamide}}$ = 0.4068.

It should be noted that, for three component systems in which r_2 is close to unity, as α_2 varies from one to zero the experimental D_A values vary from D_2 to D_1 even though the relative fringe deviation graph remains close to zero in all cases (see Fig. 3). For example, at the same mean concentration, $\bar{C}$, used in experiment IV, equation 10[1] indicates that D_A values will range from 1.048 (α_{glycine} = 1) to 1.136 ($\alpha_{\text{glycolamide}}$ = 1). It is important that this point be remembered when using relative fringe deviation graphs to study heterogeneity in proteins, since in the range $0.92 < r_2 < 1.08$ the presence of impurities cannot be detected without still greater experimental accuracy. For this case it is thus impossible to know whether the diffusion coefficient measured experimentally represents a value for a single-solute or is a "height–area average."

That the calculated values of D_A for both the above mixtures differ a little from the experimental values could be due to two factors which have been assumed negligible.[1] Firstly, the solute flows in three-component systems may interact slightly, and secondly, the differential diffusion coefficient of a solute, measured for a two-component system, could differ from the value obtained when that solute diffuses in a system of three components. In the absence of interaction between the solute flows the correct values of the differential diffusion coefficients, D_k, to use in equation 10 should be measured by experiments in which all solutes, except the diffusing component "k," are at the same concentration in both solutions forming the initial boundary. However the experiments described in this paper and those previously reported[1] show that, when Fick's first law[10] is sufficient to describe the flows of solutes in dilute solution, the differential diffusion coefficients, obtained from two component systems, are adequate to a first approximation for computing height–area average diffusion coefficients from equation 10. Further experiments with other three component systems are planned to investigate the case when the solute flows interact.

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[10] Adolf Fick, *Pogg. Ann.*, **94**, 59 (1855).

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Polarographic Study of Copper(II) Complexes with Ethanolamine and Some Derivatives

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The literature[1] records several studies of complexes of copper(II) with ethanolamine and its derivatives, usually involving analysis of precipitates. In some of these studies it has been suggested that both the hydroxyl and the amino groups are coördinated to the copper(II) ion. The present research was undertaken to determine the formulas of some of the complexes and their formation constants by a polarographic method.

Experimental Method

Chemicals.—Solutions for electrolysis were prepared from reagent chemicals, used without further purification, with the exception of the ethanolamines which were of the best commercial grades and were vacuum distilled just before use. The diethanolamine and the ethylethanolamine were supplied by Carbide and Carbon Chemicals Company and the monoethanolamine was provided by Sharples Chemicals, Incorporated.

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