rene fractions A, G and L in ethylcyclohexane–cyclohexanol mixtures were determined, therefore, at this temperature. Extrapolation of these data to obtain the infinite molecular weight binodials revealed that no critical consolute mixtures exist at 320°K. for this system. The curve of Fig. 4 lies somewhat below the curve for $M = \infty$ at zero concentration. Apparently this latter curve lies entirely above 320°K.

A desire to obtain a pair of critical consolute mixtures for this system prompted the determination of binodials for the same three polystyrene fractions at 330°K. Segments of the two regions of partial miscibility at this temperature are shown in Figs. 5a and 5b. Extrapolation of the data to $M = \infty$ was possible and the curves corresponding to those of Fig. 2 were essentially linear. The infinite molecular weight binodials thus obtained are represented by broken lines in Figs. 5a and 5b. Their extrapolation to infinite polymer dilution is complicated somewhat by their convexity to the ethylcyclohexane–cyclohexanol base-line. Admitting this possible source of error, we conclude that the two indicated critical consolute mixtures occur at $v_1(\text{CCM}) = 0.488$ and $v_1(\text{CCM}) = 0.945$ at 330°K.

Solving equation 3 for χ_{12} and substituting the values[4] $l = 1.357$, $\chi_{13} = 0.535$ and $\chi_{23} = 0.633$ yields $\chi_{12} = 0.44$ (or 2.35) for $v_1(\text{CCM}) = 0.488$. However, the same procedure yields conjugate complex solutions for χ_{12} at $v_1(\text{CCM}) = 0.945$. The value 0.44 is reasonable for the ethylcyclohexane–cyclohexanol interaction at 330°K. The reason for the failure of equation 3 to give a physically significant χ_{12} value at $v_1(\text{CCM}) = 0.945$ is to be found in the inadequacy of the chemical potential formulations used to describe mixtures containing an alcohol at low concentrations. Equations 1 and 2 do not even formally represent the desired chemical potentials in this concentration region. However, for the critical consolute mixture occurring at $v_1(\text{CCM}) = 0.488$ equations 1 and 2 should apply with sufficient accuracy to yield a satisfactory description of the plait point.

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Studies of the Diffusion of Mixed Solutes with the Gouy Diffusiometer

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In analyzing Gouy fringe patterns from two or more independently diffusing solutes it is convenient to separate certain time-dependent data, which provide a value of the height–area average diffusion coefficient, D_A, from the time-independent relative fringe positions, which contain all of the information about deviations of the boundary from Gaussian form. The latter data are conveniently recorded in the form of a graph of Ω_j, the relative fringe deviations from Gaussian positions, *versus* the reduced fringe numbers, $f(\zeta_j)$. Equations are developed which, when Ω_j is small, relate this graph to the fraction of the total gradient contributed by each solute and to ratios of the diffusion coefficients. A procedure for obtaining other average diffusion coefficients from D_A and this graph is also presented. Experiments with known mixtures of purified solutes confirm the validity of these methods of analysis. Since in favorable cases a few tenths of a per cent. of one solute as an impurity in another are detectable from the graph of Ω_j *versus* $f(\zeta_j)$, the Gouy diffusiometer can serve as a useful analytical tool. The application to protein diffusions of these methods for analyzing Gouy fringe patterns is illustrated by an experiment with bovine plasma albumin.

The purpose of this paper is to present new procedures for interpreting Gouy diffusiometer[1–6] data for mixed solutes and to test these procedures by experiments with known mixtures of purified compounds. It is expected that this work will be of particular utility in the study of proteins because most protein samples possess some heterogeneity,[7–13] and it is desirable that each protein diffusion experiment be analyzed to indicate the purity of the sample in addition to yielding an average diffusion coefficient. To illustrate the application of these methods to the study of proteins an experiment with crystallized bovine plasma albumin is included.

Equations which are derived for the analysis of mixtures also provide an estimate of the minimum amount of any impurity that can be detected with a given experimental error in the Gouy fringes. In support of these relations, experimental results show that a few tenths of a per cent. of potassium chloride in a sucrose sample produce measurable displacements of the Gouy fringes.

Theory

Three assumptions commonly made in studying the diffusion of mixed solutes in liquids[14–18] are used in the following development, the resulting

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[6] L. J. Gosting and L. Onsager, *ibid.*, **74**, 6066 (1952).

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[13] J. W. Williams, R. L. Baldwin, W. M. Saunders and P. G. Squire, THIS JOURNAL, **74**, 1542 (1952).

[14] O. Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, Series IV, **10**, No. 6 (1937).

[15] N. Gralén, Dissertation, Uppsala, 1944.

[16] A. G. Ogston, *Proc. Roy. Soc.* (*London*), **A196**, 272 (1949).

[17] A. G. Ogston, *Biochem. J.*, **45**, 189 (1949).

[18] P. A. Charlwood, *J. Phys. Chem.*, **57**, 125 (1953).

equations being in close agreement with Gouy fringe data reported below for dilute solutions. First, the flow of each solute is considered independent of the flow of every other solute; types of experiments for which this is not true and which require more complicated theoretical descriptions[19,20] are left for future investigation. Second, the diffusion coefficient, D_k, of each solute, k, is assumed to be constant throughout the boundary. Finally, when using optical methods which depend on the refractive index, n, of solution at a given level, x, in the cell, it is necessary to assume that across the diffusion boundary the variation of n with solute concentrations, C_k, expressed per unit volume of solution, is adequately represented by the first $(q + 1)$ terms of a Taylor expansion

$$n = n_{\bar{C}} + \sum_{k=1}^{q} R_k (C_k - \bar{C}_k) + \ldots \tag{1}$$

Here $n_{\bar{C}} = n(\bar{C}_1, \ldots, \bar{C}_q)$ is the value of n at the mean concentrations, $\bar{C}_k = [(C_k)_A + (C_k)_B]/2$ between solutions A and B forming the initially sharp boundary at level $x = 0$ and time $t = 0$. In general each differential refractive increment, $R_k = |[\partial n(C_1, \ldots, C_q)/\partial C_k]_{T,,P_{C l \neq k}}C_1 = |\bar{C}_1, \ldots C_q = \bar{C}_q$ will vary with temperature, T, pressure, P, and the nature and concentrations of each of the q solutes present. After free diffusion in such a boundary has proceeded for a time, t, the solution[21] of Fick's second law[22] may be written in the form

$$\mathrm{d}n/\mathrm{d}x = \sum_{k=1}^{q} [\Delta n_k/(2\sqrt{\pi D_k t})] e^{-x^2/(4D_k t)} \tag{2}$$

where x is considered positive below $x = 0$ and

$$\Delta n_k = R_k [(C_k)_B - (C_k)_A] \tag{3}$$

When equation 2 represents the experimental diffusion boundary we define reduced cell coördinates

$$z_{jk} = x_j/(2\sqrt{D_k t}) \tag{4}$$

and solute fractions on the basis of refractive index

$$\alpha_k = \Delta n_k / \sum_{l=1}^{q} \Delta n_l \tag{5}$$

so that the relative downward displacement of a Gouy fringe may be written[23-25]

$$\frac{Y_j}{C_t} = \frac{\sum_{k=1}^{q} (\alpha_k/\sqrt{D_k})\, e^{-z^2_{jk}}}{\sum_{k=1}^{q} (\alpha_k/\sqrt{D_k})} \tag{6}$$

where Y_j is the measured displacement of an intensity zero, j, from the undeviated slit image at time t ($j = 0, 1, 2, \ldots$, counting from the bottom upward on the fringe system). As in a single solute diffusion C_t is the maximum displacement of light at time t predicted by ray optics and is proportional to the maximum refractive index gradient in the cell. An extrapolation procedure described below makes possible its experimental determination from the lower Gouy fringes for mixtures. Since the total number of fringes is

$$j_m = \frac{a \sum_{l=1}^{q} \Delta n_l}{\lambda} = a(n_B - n_A)/\lambda \tag{7}$$

for a wave length, λ, and distance, a, between cell windows, the interference condition for intensity zeros becomes

$$f(\zeta_j) = \sum_{k=1}^{q} \alpha_k f(z_{jk}) = \frac{(j + 3/4 + \ldots)}{j_m} \simeq \frac{Z_j}{j_m} \tag{8}$$

where the first equality is not the derived interference condition but defines an easily measurable quantity, $f(\zeta_j)$, analogous to $f(z_j)$ for a single solute diffusion.[23,24] *When j_m is large* the Airy integral approximation,[5] Z_j, is nearly equal to the series expansion $(j + 3/4 + \ldots)$ and may be used to compute $f(\zeta_j)$.[25]

From equations 6 and 8 it is seen that for superimposed diffusion gradients values of $e^{-\zeta_j^2}$ corresponding to $f(\zeta_j)$ are not equal to Y_j/C_t but represent relative fringe displacements for a single-solute diffusion with the same j_m. Therefore, the relative fringe deviation

$$\Omega_j = (e^{-\zeta_j^2} - Y_j/C_t) \tag{9}$$

is chosen as an easily measurable quantity upon which to base the following procedures for studying diffusions of mixtures. A graph of Ω_j *versus* the reduced fringe number, $f(\zeta_j)$, is somewhat analogous to Lamm's plot in normal coördinates,[14] being independent of time, and contains all the information provided by the Gouy fringes about deviations of the experimental boundary from Gaussian form. The height-area average diffusion coefficient[26] on the basis of refractive index

$$D_A = \left[\sum_{k=1}^{q} \alpha_k/\sqrt{D_k}\right]^{-2} \tag{10}$$

obtained from[27]

$$D_A = \frac{(j_m \lambda b)^2}{4\pi C_t^2 t} \tag{11}$$

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

(20) O. Lamm, *J. Phys. Colloid Chem.*, **51**, 1063 (1947).

(21) J. Stefan, *Sitzber. Akad. Wiss. Wien, Abt. II*, **79**, 161 (1879).

(22) A. Fick, *Pogg. Ann.*, **94**, 59 (1855).

(23) In a single solute diffusion where $\alpha_1 = 1$, equations 4, 6 and 8 reduce to the relations obtained previously, ref. 2, 5 and 6

$$z_j = x_j/(2\sqrt{Dt})$$
$$Y_j/C_t = e^{-z^2 j}$$

and

$$f(z_j) = (j + \frac{3}{4} + \ldots)/j_m$$

(24) In both single and mixed solute diffusions the function, f, is defined by

$$f(\gamma) = (2/\sqrt{\pi})\left[\int_0^\gamma e^{-\beta^2} \mathrm{d}\beta - \gamma e^{-\gamma^2}\right]$$

(25) Equations 6 and 8 were presented without derivation by Ogston, ref. 16. They may be obtained by specializing the equations of ref. 6 to the case where $\mathrm{d}n/\mathrm{d}x$ is given by equation 2. It should be noted that only the first two terms of the series $(j + 3/4 + \ldots)$ for mixed solutes are identical with those in the corresponding series for a single solute, equation 62 of ref. 6. By arranging experiments so j_m is large, however, both series are adequately represented by the Airy integral approximation, Z_j. It can be seen from ref. 6 that this approximation may introduce serious theoretical errors if $j_m < 10$, but it should be entirely satisfactory for $j_m > 50$.

(26) O. Quensel, Dissertation, Uppsala, 1942.

(27) The optical lever arm, b, in a convergent light Gouy diffusiometer is measured from the center of the cell to the emulsion of the photographic plate and defined by $b = \Sigma L_i/n_i$ where L_i is the distance along the optic axis in each medium, i, of refractive index n_i, relative to air as unity.

contains the remaining information required for analysis of a mixture.

In principle equations 6, 8 and 10 are solvable for values of α_k and D_k by measuring a sufficient number of fringes. A general solution of this type does not appear practical, so we will solve equations 6 and 8 by means of series expansions to yield an expression for Ω_j which converges rapidly for experiments having relatively small values of Ω_j,[28] as should be the case for any reasonably well "purified" protein. To obtain values of α_k and D_k this expression for Ω_j can be solved simultaneously with equation 10.

In solving for Ω_j, α_1 is first eliminated by the relation

$$\alpha_1 = 1 - \sum_{k=2}^{q} \alpha_k \qquad (12)$$

so except where specifically indicated k may not hereafter be unity. Diffusion coefficient ratios are defined by

$$r_k = D_1/D_k \qquad (13)$$

so equations 8 and 6 become

$$[f(z_1) - f(\zeta)] = \sum_{k=2}^{q} \alpha_k[f(z_1) - f(\sqrt{r_k}z_1)] \qquad (14)$$

and

$$\frac{Y}{C_t} = e^{-z_1^2}\,\frac{\left\{1 - \sum\limits_{k=2}^{q} \alpha_k[1 - \sqrt{r_k}\,e^{-(r_k-1)z_1^2}]\right\}}{\left\{1 - \sum\limits_{k=2}^{q} \alpha_k(1 - \sqrt{r_k})\right\}} \qquad (15)$$

where the subscripts j have been dropped for simplicity. Since $f(z_1) \rightarrow f(\zeta)$ and $Y/C_t \rightarrow e^{-z_1^2} \rightarrow e^{-\zeta^2}$ when (1) all values of α_k are small or (2) all values of r_k are near unity, useful series expansions for Ω_j may be made either in α_k or in $(\sqrt{r_k} - 1)$.

Slightly Impure Solutes.—A series expansion for the relative fringe deviation, Ω, in terms of ζ, α_k and r_k will now be obtained from equation 15 by simultaneous solution with equation 14 to eliminate z_1. Defining

$$\sigma = [f(z_1) - f(\zeta)] \qquad (16)$$

the Taylor expansion of equation 14

$$\sigma = \sum_{l=0}^{\infty} \frac{\sigma^l}{l!}\,\frac{\partial^l}{\partial[f(\zeta)]^l}\left\{\sum_{k=2}^{q} \alpha_k[f(\zeta) - f(\sqrt{r_k}\zeta)]\right\} \qquad (17)$$

is solved for σ by successive approximations or by Lagrange's theorem[29]

$$\sigma = \sum_{k=2}^{q} \alpha_k[f(\zeta) - f(\sqrt{r_k}\zeta)] + \sum_{k=2}^{q}\sum_{l=2}^{q} \alpha_k\alpha_l[f(\zeta) - f(\sqrt{r_k}\zeta)][1 - r_l^{3/2}e^{-(r_l-1)\zeta^2}] + \ldots \qquad (18)$$

Equation 15 for Y/C_t may likewise be expanded in a Taylor series in σ

$$\frac{Y}{C_t} = \sum_{l=0}^{\infty} \frac{\sigma^l}{l!\left[1 + \sum\limits_{k=2}^{q}(\sqrt{r_k} - 1)\alpha_k\right]}\,\frac{\partial^l}{\partial[f(\zeta)]^l}\left[\left(1 - \sum_{k=2}^{q}\alpha_k\right)e^{-\zeta^2} + \sum_{k=2}^{q}\alpha_k\sqrt{r_k}e^{-a_k\zeta^2}\right] \qquad (19)$$

into which the substitution of σ from equation 18 yields the desired expression for Ω

$$(e^{-\zeta^2} - Y/C_t) = \Omega = \sum_{k=2}^{q} \alpha_k F(\zeta,r_k) - \sum_{k=2}^{q}\sum_{l=2}^{q} \alpha_k\alpha_l G(\zeta,r_k,r_l) + \ldots \qquad (20)$$

where

$$F(\zeta,r_k) = \sqrt{r_k}(e^{-\zeta^2} - e^{-r_k\zeta^2}) + \frac{[f(\zeta) - f(\sqrt{r_k}\zeta)]}{(2/\sqrt{\pi})\zeta} \qquad (21)$$

and

$$G(\zeta,r_k,r_l) = (\sqrt{r_l} - 1)F(\zeta,r_k) + \frac{[f(\zeta) - f(\sqrt{r_k}\zeta)][f(\zeta) - f(\sqrt{r_l}\zeta)]}{4(2/\sqrt{\pi})^2\zeta^4e^{-\zeta^2}} \qquad (22)$$

The rapid convergence of equation 20 for small values of α_k establishes its utility for diffusions of slightly impure compounds; but in cases where all values of $(\sqrt{r_k} - 1)$ are small, convergence occurs for any values of α_k as shown in the following section.

For a diffusion experiment with only two solutes, equation 20 reduces to

$$\Omega = \alpha_2F(\zeta,r_2) - \alpha_2^2G(\zeta,r_2) + \ldots \qquad (23)$$

where $F(\zeta, r_2)$ and $G(\zeta, r_2)$ are tabulated in Tables I and II. When α_2 is so small that the second term of equation 23 is negligible compared to the first, Ω is proportional to $F(\zeta, r_2)$ so that, from the position of the maximum of the experimental Ω *versus* $f(\zeta)$ curve, the ratio D_1/D_2 may be approximated by inspection from Table I.

Solutes with Similar Diffusion Coefficients.—When $r_k \simeq 1$, substitution of the Taylor expansions for equations 21 and 22

$$F(\zeta,r_k) = \zeta^2e^{-\zeta^2}(\sqrt{r_k} - 1)^2 + \cdots \qquad (24)$$

and

$$G(\zeta,r_k, r_l) = \zeta^2e^{-\zeta^2}(\sqrt{r_k} - 1)(\sqrt{r_l} - 1) + \cdots \qquad (25)$$

into equation 20 yields the expression

$$\Omega = \zeta^2e^{-\zeta^2}\sum_{k=2}^{q}\sum_{l=2}^{q} \alpha_k(\sqrt{r_k} - 1)[(\sqrt{r_k} - 1) - \alpha_l(\sqrt{r_l} - 1)]\cdots \qquad (26)$$

The dependence of this equation for Ω upon $(\sqrt{r_k} - 1)$ makes it converge rapidly, irrespective of the values of α_k, for experiments in which all solutes have nearly the same diffusion coefficient. Evaluation of the second term in this expansion requires terms of order α_k^3 in equation 20. These may be obtained without prohibitive effort for two-solute diffusions and the above procedure leads to the expansion

$$\Omega = \alpha_2(1 - \alpha_2)\,\zeta^2e^{-\zeta^2}(\sqrt{r_2} - 1)^2 + \frac{1}{3}\,\alpha_2(1 - \alpha_2)\,[(1 - 8\alpha_2) - 2\,\zeta^2(1 - 2\alpha_2)]\zeta^2e^{-\zeta^2}(\sqrt{r_2} - 1)^3 + \ldots \qquad (27)$$

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(28) Ogston's tables, ref. 16, using $\log(C_t/Y_j)$ for interpreting two-solute diffusion experiments, are not restricted in this way, but they allow only two of the Gouy fringes to be used.

(29) E. T. Whittaker and G. N. Watson, "A Course of Modern Analysis," Cambridge University Press, Cambridge, 1927, p. 133.

TABLE I

VALUES OF $F(\zeta,r_k)$ FOR EQUATIONS 20 AND 23

$f(\zeta)$	$r_k = 100$	$r_k = 49$	$r_k = 25$	$r_k = 9$	$r_k = 4$	$r_k = 2$	$r_k = 1.5$	$r_k = \frac{1}{1.5}$	$r_k = \frac{1}{2}$	$r_k = \frac{1}{4}$	$r_k = \frac{1}{9}$	$r_k = \frac{1}{25}$	$r_k = \frac{1}{49}$	$r_k = \frac{1}{100}$
0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.01	5.783	3.013	1.393	0.3151	0.0685	0.0104	0.0029	0.0017	0.0043	0.0115	0.0191	0.0260	0.0291	0.0314
.025	6.347	3.656	1.912	.5035	.1169	.0183	.0052	.0031	.0077	.0208	.0347	.0473	.0530	.0573
.05	6.380	3.864	2.192	.6675	.1677	.0272	.0078	.0048	.0118	.0322	.0540	.0738	.0828	.0896
.075	6.210	3.841	2.263	.7535	.2012	.0338	.0097	.0060	.0151	.0414	.0697	.0954	.1070	.1159
.10	5.991	3.751	2.258	.8001	.2247	.0388	.0113	.0071	.0178	.0492	.0831	.1141	.1280	.1387
.15	5.518	3.505	2.163	.8308	.2533	.0461	.0136	.0088	.0222	.0622	.1057	.1458	.1639	.1777
.20	5.050	3.235	2.025	.8181	.2660	.0508	.0152	.0102	.0257	.0727	.1244	.1723	.1941	.2107
.25	4.600	2.964	1.873	.7836	.2686	.0536	.0163	.0112	.0285	.0814	.1404	.1952	.2202	.2393
.30	4.172	2.700	1.718	.7373	.2641	.0549	.0170	.0120	.0307	.0886	.1540	.2152	.2431	.2644
.35	3.765	2.445	1.565	.6846	.2545	.0550	.0172	.0126	.0324	.0946	.1656	.2326	.2632	.2866
.40	3.378	2.199	1.414	.6283	.2412	.0542	.0172	.0129	.0336	.0994	.1755	.2477	.2808	.3063
.45	3.008	1.963	1.267	.5702	.2251	.0524	.0169	.0131	.0343	.1031	.1836	.2608	.2962	.3235
.50	2.656	1.737	1.124	.5117	.2070	.0500	.0164	.0132	.0347	.1058	.1902	.2718	.3094	.3384
.55	2.320	1.520	0.987	.4533	.1874	.0469	.0156	.0130	.0347	.1074	.1952	.2808	.3205	.3511
.60	2.000	1.312	.854	.3956	.1668	.0432	.0146	.0127	.0342	.1079	.1985	.2878	.3294	.3615
.65	1.695	1.114	.726	.3390	.1455	.0390	.0134	.0122	.0333	.1072	.2000	.2926	.3360	.3695
.70	1.404	0.924	.604	.2837	.1237	.0344	.0121	.0115	.0319	.1052	.1996	.2951	.3400	.3749
.75	1.128	.743	.486	.2300	.1019	.0293	.0105	.0106	.0299	.1017	.1969	.2948	.3412	.3773
.80	0.866	.571	.375	.1783	.0801	.0239	.0088	.0095	.0274	.0964	.1914	.2910	.3388	.3760
.85	.619	.409	.269	.1286	.0585	.0181	.0068	.0081	.0240	.0888	.1821	.2829	.3317	.3699
.90	.389	.257	.169	.0816	.0377	.0122	.0048	.0064	.0196	.0777	.1673	.2679	.3175	.3566
.925	.281	.186	.123	.0592	.0276	.0091	.0036	.0053	.0168	.0702	.1565	.2564	.3061	.3455
.95	.178	.118	.078	.0379	.0178	.0060	.0025	.0041	.0133	.0604	.1419	.2399	.2897	.3292
.975	.083	.055	.036	.0178	.0084	.0030	.0013	.0025	.0089	.0462	.1192	.2133	.2626	.3021
.99	.031	.020	.014	.0066	.0032	.0011	.0005	.0013	.0051	.0323	.0948	.1832	.2314	.2706
1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE II

VALUES OF $G(\zeta,r_2)$ FOR EQUATION 23

$f(\zeta)$	$r_2 = 100$	$r_2 = 49$	$r_2 = 25$	$r_2 = 9$	$r_2 = 4$	$r_2 = 2$	$r_2 = 1.5$	$r_2 = \frac{1}{1.5}$	$r_2 = \frac{1}{2}$	$r_2 = \frac{1}{4}$	$r_2 = \frac{1}{9}$	$r_2 = \frac{1}{25}$	$r_2 = \frac{1}{49}$	$r_2 = \frac{1}{100}$
0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
.01	112.7	64.60	26.65	3.075	0.3131	0.0233	0.0048	0.0010	0.0013	−0.0009	−0.0069	−0.015	−0.019	−0.025
.025	75.0	39.29	20.59	3.872	.4887	.0397	.0083	.0017	.0025	.0015	.0123	.026	.034	.045
.05	64.2	30.00	15.14	3.909	.6261	.0569	.0122	.0027	.0040	.0020	.0186	.040	.052	.070
.075	59.7	26.87	12.80	3.627	.6844	.0682	.0150	.0035	.0053	.0021	.0234	.052	.067	.090
.10	56.4	25.00	11.51	3.316	.7044	.0760	.0172	.0042	.0064	.0019	.0272	.061	.079	.107
.15	51.0	22.36	9.98	2.791	.6893	.0852	.0201	.0054	.0085	.0011	.0328	.076	.099	.135
.20	46.3	20.23	8.92	2.400	.6418	.0889	.0218	.0064	.0105	+0.0003	.0364	.088	.115	.157
.25	42.0	18.34	8.04	2.100	.5834	.0889	.0226	.0072	.0122	.0023	.0384	.096	.128	.176
.30	37.9	16.59	7.26	1.856	.5229	.0865	.0228	.0080	.0139	.0047	.0389	.103	.138	.191
.35	34.2	14.95	6.54	1.647	.4644	.0824	.0225	.0086	.0156	.0077	.0381	.107	.145	.203
.40	30.6	13.40	5.86	1.462	.4093	.0770	.0218	.0092	.0171	.0111	.0359	.109	.150	.212
.45	27.2	11.93	5.22	1.294	.3581	.0708	.0208	.0096	.0186	.0152	.0322	.109	.152	.218
.50	24.0	10.54	4.61	1.138	.3109	.0640	.0194	.0100	.0201	.0198	.0270	.106	.152	.221
.55	21.0	9.21	4.03	0.992	.2675	.0568	.0179	.0103	.0215	.0251	.0200	.101	.149	.221
.60	18.1	7.94	3.48	.854	.2274	.0495	.0161	.0104	.0228	.0313	.0108	.093	.142	.216
.65	15.3	6.73	2.95	.724	.1904	.0422	.0142	.0105	.0241	.0384	+0.0009	.081	.131	.208
.70	12.7	5.58	2.45	.600	.1562	.0349	.0122	.0105	.0253	.0468	.0160	.064	.115	.193
.75	10.2	4.48	1.97	.483	.1244	.0279	.0101	.0103	.0264	.0568	.0358	.041	.092	.170
.80	7.8	3.44	1.51	.371	.0948	.0212	.0079	.0099	.0273	.0692	.0624	.007	.057	.136
.85	5.6	2.46	1.08	.266	.0674	.0149	.0058	.0093	.0280	.0854	.1008	+0.044	.004	.082
.90	3.5	1.55	0.68	.168	.0423	.0091	.0037	.0083	.0283	.1087	.1630	.131	+0.089	+0.014
.925	2.5	1.12	.49	.121	.0305	.0064	.0026	.0076	.0281	.1258	.2140	.206	.170	.098
.95	1.6	0.71	.31	.077	.0194	.0040	.0017	.0066	.0276	.1511	.2981	.336	.310	.245
.975	0.7	.33	.15	.036	.0090	.0018	.0008	.0051	.0259	.1990	.4865	.645	.651	.602
.99	.3	.12	.05	.013	.0033	.0006	.0003	.0035	.0235	.2784	.8780	1.351	1.444	1.436
1.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Extrapolation Procedure for Obtaining C_t.—A limiting law for evaluating C_t from measurements of the lower fringes is readily derived by observing that as $\zeta \rightarrow 0$ both equation 20 and equation 26 reduce to the form

$$\Omega = (e^{-\zeta^2} - Y/C_t) = K\zeta^2 + \ldots \qquad (28)$$

where K need not be further determined. Since[30]

$$\zeta = w[1 + (1/5)w^2 + \ldots] \qquad (29)$$

and

$$w = [(3\sqrt{\pi}/4)f(\zeta)]^{1/3} \qquad (30)$$

equation 28 may be rewritten using these relations and equation 8 to give

$$\frac{Y_j}{e^{-\zeta_j^2}} = C_t - \{KC_t[3\sqrt{\pi}/(4j_m)]^{2/3}\}Z_j^{2/3} + \ldots \qquad (31)$$

The limiting slope, in braces, need not be determined since C_t is obtained as the intercept of a plot of $(Y_j/e^{-\zeta_j^2})$ *versus* $Z_j^{2/3}$. For convenience values of $Z_j^{2/3}$ are collected in Table III.

Average Diffusion Coefficients.—Average diffusion coefficients are well-defined quantities for any mixed solute diffusion and are of special im-

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(30) Equation 72 of ref. 6.

TABLE III

VALUES OF THE TWO-THIRDS POWER OF THE AIRY INTEGRAL APPROXIMATION

j	$z_j^{2/3}$	j	$z_j^{2/3}$
0	0.832	5	3.210
1	1.454	6	3.572
2	1.964	10	4.871
3	2.415	15	6.283
4	2.826	20	7.551

portance for polydisperse systems, which cannot be analyzed by the previous methods. In any diffusion experiment a graph of Ω *versus* $\mathrm{f}(\zeta)$ may be made, and from it and the experimental value of D_A, various refractive index–average diffusion coefficients can be calculated. The following development is based upon Gralén's[31] analysis of moments of the $\mathrm{d}n/\mathrm{d}x$ curve, which may be expressed in the form

$$\overline{D^s} = \frac{s!}{(2s)!}\left(\frac{2}{t^s\Delta n}\right)\int_0^\infty \frac{1}{(2s+1)}\left(\frac{\mathrm{d}n}{\mathrm{d}x}\right)\mathrm{d}(x^{2s+1}) \qquad s = 0,1,2,\ldots \quad (32)$$

where

$$\overline{D^s} = \sum_{k=1}^{q} \alpha_k D_k^s \qquad (33)$$

and $\Delta n = n_B - n_A$. If all solutes have the same refractive increment per gram per unit volume of solution, $\overline{D^1}$ is the weight average diffusion coefficient. Required values of the cell coördinate, x, are provided by Ogston's relation[17] which is in our notation

$$x = -\sqrt{\pi D_A t}\left[\frac{\partial \mathrm{f}(\zeta)}{\partial(Y/C_t)}\right] \qquad (34)$$

Substituting this relation and the definition of D_A in the form

$$\frac{\mathrm{d}n}{\mathrm{d}x} = \frac{\Delta n}{2\sqrt{\pi D_A t}}\left(\frac{Y}{C_t}\right) \qquad (35)$$

into equation 32 and replacing Y/C_t with $(e^{-\zeta^2} - \Omega)$ results in

$$\overline{D^s} = \frac{s!\pi^s D_A^s}{(2s)!(2s+1)}\int_{\zeta=0}^{\infty}(e^{-\zeta^2} - \Omega)\mathrm{d}\left[-\frac{\partial \mathrm{f}(\zeta)}{\partial(e^{-\zeta^2} - \Omega)}\right]^{2s+1} \qquad (36)$$

Since, for a given experiment, all the α's and D's are constants, Ω is a function only of $\mathrm{f}(\zeta)$ and[32]

$$\left[-\frac{\partial\, \mathrm{f}(\zeta)}{\partial(e^{-\zeta^2} - \Omega)}\right]_{\alpha_k, D_k} = \frac{\left(\frac{2}{\sqrt{\pi}}\zeta\right)}{\left[1 + \left(\frac{2}{\sqrt{\pi}}\zeta\right)\frac{\mathrm{d}\Omega}{\mathrm{d}\,\mathrm{f}(\zeta)}\right]} \qquad (37)$$

Defining, for convenience

$$\mathrm{H}(\zeta,s) = \frac{\left(1 - \frac{\Omega}{e^{-\zeta^2}}\right)}{\left[1 + \left(\frac{2}{\sqrt{\pi}}\zeta\right)\frac{\mathrm{d}\Omega}{\mathrm{d}\,\mathrm{f}(\zeta)}\right]^{2s+2}} \qquad (38)$$

equation 36 is rewritten in a form convenient for calculation

$$\overline{D^s} = D_A^s\left\{1 + \frac{2\pi^{s-1}(s!)}{(2s)!}\int_0^1\left(\frac{2}{\sqrt{\pi}}\zeta\right)^{2s-2}[\mathrm{H}(\zeta,s) - 1]\,\mathrm{df}(\zeta) + \frac{\pi^s(s!)}{(2s)!}\int_{\zeta=\infty}^{0}\left(\frac{2}{\sqrt{\pi}}\zeta\right)^{2s+2}e^{-\zeta^2}\,\mathrm{H}(\zeta,s)\mathrm{d}\left[\frac{\mathrm{d}\Omega}{\mathrm{d}\,\mathrm{f}(\zeta)}\right]\right\} \qquad (39)$$

The integrals, which are small compared to unity, are evaluated by graphical integration using data from the Ω *versus* $\mathrm{f}(\zeta)$ curve and requisite functions of s and ζ, values of which are tabulated in Table IV for $s = 1$ and $s = 2$.

TABLE IV

FUNCTIONS OF ζ FOR EQUATION 39

$\mathrm{f}(\zeta)$	$\frac{2}{\sqrt{\pi}}\zeta$	$e^{-\zeta^2}$	$\left(\frac{2}{\sqrt{\pi}}\zeta\right)^4 e^{-\zeta^2}$	$\left(\frac{2}{\sqrt{\pi}}\zeta\right)^2$	$\left(\frac{2}{\sqrt{\pi}}\zeta\right)^6 e^{-\zeta^2}$
0.01	0.2704	0.9442	0.0050	0.0731	0.0004
.025	.3706	.8977	.0169	.1374	.002
.05	.4733	.8387	.0421	.2240	.009
.075	.5482	.7898	.0713	.3005	.021
.10	.6099	.7466	.1033	.3720	.038
.15	.7126	.6711	.1731	.5079	.088
.20	.8000	.6050	.2477	.6399	.159
.25	.8786	.5454	.3250	.7719	.251
.30	.9520	.4908	.4031	.9063	.365
.35	1.0223	.4401	.4806	1.045	.502
.40	1.0908	.3928	.5561	1.190	.662
.45	1.1588	.3483	.6281	1.343	.844
.50	1.2273	.3064	.6950	1.506	1.047
.55	1.2972	.2667	.7551	1.683	1.271
.60	1.3695	.2292	.8064	1.876	1.512
.65	1.4457	.1937	.8461	2.090	1.768
.70	1.5275	.1600	.8711	2.333	2.032
.75	1.6172	.1282	.8770	2.615	2.294
.80	1.7190	.09819	.8574	2.955	2.534
.85	1.8398	.07005	.8026	3.385	2.717
.90	1.9949	.04391	.6954	3.980	2.768
.925	2.0966	.03167	.6120	4.396	2.690
.95	2.2305	.02009	.4973	4.975	2.474
.975	2.4395	.009333	.3306	5.951	1.967
.99	2.6874	.003439	.1794	7.222	1.296
.995	2.8588	.001630	.1089	8.173	0.890
.999	3.2180	.0002936	.0315	10.355	.326

It should be noted that while the series expansions for Ω, equations 20, 23, 26 and 27, are useful only for experiments in which Ω is relatively small, equation 39 is applicable to any experiment.

Experimental

The Gouy diffusiometer described previously[4] was used for these experiments after installation of an improved camera, light source assembly and cell frame suspension.[33] All measurements were made with the 5460.7 Å. mercury line isolated from an A-H 4 lamp with a Wratten 77A filter. Since this filter does not remove all of the red light a Wratten 40 filter was also inserted in one experiment, but no effect on the values of C_t was observed. At least four sets of photographs were taken in each experiment to determine

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

(32) These equations help to clarify the meaning of ζ. Defining

$$(z_A)_j = x_j/(2\sqrt{D_A t}) = \sum_{k=1}^{q} \alpha_k z_{jk}$$

it might seem from analogy with a single solute diffusion that $z_A = \zeta$. That this is *not* true is readily seen by combining the definition of z_A with equations 34 and 37 to give

$$z_A = \frac{\zeta}{\left[1 + \left(\frac{2}{\sqrt{\pi}}\zeta\right)\frac{\mathrm{d}\Omega}{\mathrm{d}\,\mathrm{f}(\zeta)}\right]}$$

(33) P. J. Dunlop and L. J. Gosting, THIS JOURNAL, **75**, 5073 (1953).

the reference correction,[5,34] δ, which was considered negative since light from the central portion of the cell filled with homogeneous solution gave a slit image position above that for light through the twin double-slit reference mask. An average δ correction of -14 microns was applied to experiments I through IV (May through August, 1952) while for the subsequent experiments (September through December) an average value of -17 microns was used. The maximum deviation from these averages was 4 microns except in experiment XI for which the measured value of -10 microns was used. Measurement of the optical lever arm,[27] b, yielded values of 307.85 to 307.88 cm. with this small drift over the eight-month period being due to inelastic bending of the cell frame suspension. These values for b are relative to gage blocks[35] which had been calibrated at 68°F., as had the comparator[36] used in measuring the photographic plates. A long center section Tiselius cell with a 2.486_2-cm. a dimension served as a diffusion cell, and boundaries were sharpened with either a single- or double-pronged capillary at rates comparable to those used previously.

In most experiments about ten Gouy fringe photographs were taken over a period of time up to $D_{\rm A}t \cong 0.15$. Values of $D_{\rm A}$ were then extrapolated to infinite time,[1] giving starting time corrections between 3 and 16 seconds for all but the much slower diffusing bovine plasma albumin, experiment XIV, for which Δt was 52 sec. Mean deviations from these least-squared straight lines were always less than 0.08%. Using a series form

$$D_{25} = D_T[1 + 0.0264(25 - T) + \ldots] \quad (40)$$

of the Stokes–Einstein relation these diffusion coefficients were corrected to 25° from the temperatures of measurement,[37] which never differed from 25° by more than 0.006° and were constant to ±0.002° during each experiment.

Materials.—Sucrose, potassium chloride and urea were used in preparing mixtures to test the above theory. These compounds were chosen because their diffusion behavior is known, and by using only one salt the complication of independent motion of different kinds of ions is avoided. In all experiments the solvent was doubly distilled water which was air saturated and stored in glass at room temperature. The urea used by us previously[38] was redried at 60° in vacuum for five hours for these experiments, while National Bureau of Standards sucrose (sample no. 17, lot numbers 5005 and 5105) was used as received. Reagent grade potassium chloride was recrystallized once from conductance water, centrifugally drained, fused in air after drying in vacuum, and broken up in an agate mortar.

The crystallized bovine plasma albumin (BPA) was part of a sample (Armour and Co., control no. 284–8) which had been analyzed for heterogeneity by electrophoretic spreading.[12] Drying at 110° for 36 hours in air[39] indicated that it contained 8.4% moisture by weight. Reagent grade potassium acetate, acetic acid and potassium chloride were used without further purification to prepare the buffer for experiment XIV.

Solutions.—All solutions were made up by weight using calibrated weights. The weight fraction of each solute, corrected to vacuum,[40] was converted to molarity, C, using values of 342.296, 74.557 and 60.058 for the molecular weights of sucrose, potassium chloride and urea, respectively. For solutions of a single solute, the required solution densities at 25° were obtained using a value of 0.997075 g./ml. for the density of water[41] and the following data[42-44] for apparent molal volumes, ϕ, in ml. per mole

$$\phi_{\rm sucrose} = 212 \quad (41)$$

$$\phi_{\rm KCl} = 26.50 + 3.26\sqrt{m} - 1.12m \quad (42)$$

$$\phi_{\rm urea} = 44.218 + 0.13999C - 0.002601C^2 \quad (43)$$

where m is molality. Solution densities, d, in g./ml. for mixed-solute solutions were also calculated from these expressions by assuming the absence of volume interactions between the solutes; *i.e.*, using the molalities, m_k, of each solute in a solution, values of ϕ_k from equations 41 to 43 were substituted in the expression for solution volume

$$V = N_0\overline{V_0^0} + \sum_{k=1}^{q} N_k\phi_k \quad (44)$$

where N_k is the number of moles of each component in a solution and $\overline{V_0^0}$ is the volume of a mole of solvent at 25°. As will be seen in Table VI, densities computed in this way are in good agreement with densities determined experimentally for several of the mixed-solute solutions using 30-ml. Pyrex pycnometers filled to measured positions in their capillary necks. In computing $d_{\rm B}$ for experiment XII a value[45] of 0.734 ml./g. was used for the partial specific volume of BPA.

Results

Single Solutes.—Before studying the diffusion of mixtures, experiments were carried out to determine whether the relative fringe deviation, Ω, equation 9, is actually zero at all values of $f(\zeta)$, equation 8, for diffusions of each solute separately. That this is true within experimental error is seen from Fig. 1 in which, as in all subsequent relative fringe deviation diagrams, the dots at each value of the reduced fringe number, $f(\zeta)$, represent observed values of Ω at the different times. Since many of the dots are obscured by superposition, and since no definite drift of Ω with time was observed in any experiment, the arithmetic mean of Ω for all the times was computed for each fringe and indicated by a cross. In calculating Ω for these single-solute diffusions, C_t was taken as the mean value of $Y_j/e^{-\zeta_j^2}$, which showed no drift with fringe number, for fringes 1 through 6 inclusive. The fact that Ω is approximately zero for all values of $f(\zeta)$ at different times and for different solutes indicates that the Gouy fringes correspond to Gaussian dn/dx *versus* x curves and that the optical quality of the diffusiometer is adequate for studies of mixtures.

The effect on Ω of a small error in $j_{\rm m}$ or δ is proportional to that error and inversely proportional to $j_{\rm m}$ or C_t, respectively, for both single-solute and mixed-solute diffusions. When $j_{\rm m} = 100$ an error of $+0.10$ fringe would produce an error in Ω indicated by the dashed line in Fig. 1, while when $C_t = 2$ cm., an error of $+10$ microns in δ would result in the corresponding dotted line. To ensure that errors of this type do not produce a maximum combined uncertainty of greater than 2×10^{-4} in Ω, $j_{\rm m}$ must be known to 0.02% and δ to 1 micron per cm. of C_t.

As seen in Table V these experiments, II, VI and X, which were made during the past year after re-

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[34] L. J. Gosting, THIS JOURNAL, **72**, 4418 (1950).

[35] Micrometer Microgage set M21, The Van Keuren Co., Watertown 72, Mass.

[36] Most photographic plate measurements were made with a Gaertner M2001RS toolmakers' microscope using projection attachment M2001PA to reduce eye strain. This instrument measures in two dimensions with an accuracy of 0.002 mm. per inch and is designed to allow direct calibration of one screw against a 5-cm. gage block.

[37] The authors are indebted to Professor P. J. Bender and A. D. Kaiser, Jr., for calibration against a Bureau of Standards certified platinum resistance thermometer of the two mercury-in-glass thermometers used in the diffusion bath.

[38] L. J. Gosting and D. F. Akeley, THIS JOURNAL, **74**, 2058 (1952).

[39] G. E. Perlmann and L. G. Longsworth, *ibid.*, **70**, 2719 (1948).

[40] Solid state densities used in these corrections are; potassium chloride, 1.984; sucrose, 1.588; and urea, 1.330 g./cc.

[41] N. E. Dorsey, "Properties of Ordinary Water-Substance," Reinhold Publ. Corp., New York, N. Y., 1940, p. 201.

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

[43] D. A. MacInnes and M. O. Dayhoff, *ibid.*, **74**, 1017 (1952).

[44] F. T. Gucker, Jr., F. W. Gage and C. E. Moser, *ibid.*, **60**, 2582 (1938).

[45] M. O. Dayhoff, G. E. Perlmann and D. A. MacInnes, *ibid.*, **74**, 2515 (1952).

TABLE V

SINGLE-SOLUTE DIFFUSION DATA AT 25°

Exp. no.	Solute	ΔC, moles/l.	$\bar{C}$, moles/l.	This work $D \times 10^5$, cm.^{2}/sec.	This work $\frac{\Delta n}{\Delta C} \times 10^3$	Former work[a] $D \times 10^5$, cm.^{2}/sec.	Former work[a] $\frac{\Delta n}{\Delta C} \times 10^3$
I	Sucrose	0.04375_9	0.02187_9	0.5170	48.93_3	0.5175	48.97
II	Sucrose	$.04383_2$	$.02191_6$	0.5170	48.94_2	0.5175	48.97
VI	Urea	.24999	.12500	1.370_6	8.608	1.372_0	8.602
IX	KCl	.20002	.19999	1.833_9	9.944	1.838 (1.837)	9.946
X	KCl	.19337	.22042	1.835_8	9.921	1.838 (1.837)	9.922

[a] These values are from refs. 5, 34 and 38, except for those in parentheses which are from ref. 46.

building and improving parts of the diffusiometer, yielded values of D slightly lower than those reported previously. A similar effect is noted in experiments I and IX which were also made during the same period. While it is not yet certain that the true values of D are actually lower than reported earlier, it seems desirable in analyzing the following mixed-solute diffusions to use values of D consistent with the present single-solute data from this diffusiometer. No differential diffusion coefficients for one solute in the presence of others are available, so values of D_k at 25° needed in computation of predicted average diffusion coefficients in Table VII were obtained from the relations for single solutes

$$D_{\text{sucrose}} \times 10^5 = 0.5228 - 0.264_8\, \bar{C}_{\text{sucrose}} \quad (45)$$

$$D_{\text{urea}} \times 10^5 = 1.380_3 - 0.0782_2\, \bar{C}_{\text{urea}} + 0.00464_1(\bar{C}_{\text{urea}})^2 \quad (46)$$

which are 0.1% lower than the previous data. Similarly a value of $D_{\text{KCl}} \times 10^5 = 1.836_0$ was used in the range $0.20 \leqslant \bar{C}_{\text{KCl}} \leqslant 0.23$ while data of Harned and Nuttall[46] were used for very dilute potassium chloride solutions.

Previous Gouy diffusiometer data for $\Delta n/\Delta C$ are seen to be in substantial agreement with present results and are used in approximating values of R_k at 25° for the mixtures.[47]

$$R_{\text{sucrose}} \times 10^3 = 48.95 \quad (47)$$

$$R_{\text{KCl}} \times 10^3 = 10.370 - 0.8715(\bar{C}_{\text{KCl}})^{1/2} - 0.1951\bar{C}_{\text{KCl}} \quad (48)$$

$$R_{\text{urea}} \times 10^3 = 8.613 - 0.0886\, \bar{C}_{\text{urea}} + 0.00549(\bar{C}_{\text{urea}})^2 \quad (49)$$

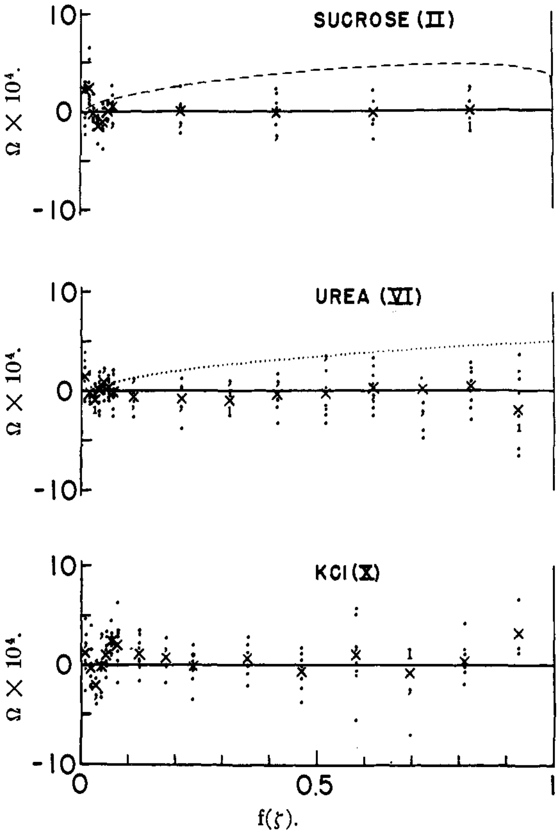

Fig. 1.—Relative fringe deviation diagrams for representative single solute experiments II, VI and X, Table V. — — —, the effect on Ω of an error of +0.10 fringe in j_m when $j_m = 100$; ……. the effect on Ω of an error of +10 microns in δ when $C_t = 2$ cm.

Slightly Impure Solutes.—To test the applicability of equation 20 to the diffusion of mixed solutes with each $\alpha_k << 1$, a series of experiments was performed using artificial mixtures of known composition. The description of each diffusion in terms of the solute molarities, C_k, the total number of fringes, j_m, and the fraction, α_k, of the total refractive increment due to each impurity is found in Table VI. For this case, there are two experiments, III and V, with sucrose as a slower diffusing impurity in potassium chloride, two, IV and XI, with potassium chloride as a faster diffusing impurity in sucrose and one, XII, with faster diffusing potassium chloride and slower diffusing bovine plasma albumin impurities in sucrose. When potassium chloride was the main solute, $\bar{C}_{\text{KCl}}$ was made approximately 0.2 M because D_{KCl} varies little from 0.1 to 0.3 M. Since BPA was itself an impurity in experiment XII, possible impurities in it were assumed to have no measurable effect on the Gouy fringes.

The extrapolation procedure, equation 31, for obtaining C_t for each Gouy photograph of mixed-

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[46] H. S. Harned and R. L. Nuttall, THIS JOURNAL, **71**, 1460 (1949).

[47] Equation 47 for R_{sucrose} represents an average between present data and those in ref. 5, while equation 49 for urea is from ref. 38. Equation 48 was obtained by letting $\Delta C \rightarrow 0$ in the semi-empirical relation

$$(\Delta n/\Delta C)_{\text{KCl}} \times 10^3 = 10.370 - 0.581[(C_{\text{B}}^{3/2} - C_{\text{A}}^{3/2})/\Delta C] - 0.0867[(C_{\text{B}}^{3/2} - C_{\text{A}}^{3/2})/\Delta C]^2 \quad (48a)$$

which fits the data in ref. 34 when

$$0.4 \leqslant [(C_{\text{B}}^{3/2} - C_{\text{A}}^{3/2})/\Delta C] \leqslant 1.5$$

Small errors may therefore be introduced by using equation 48 for very dilute KCl solutions. The fact that values of $(\Delta n/\Delta C)_{\text{KCl}}$ computed from equations 3 and 48 for $\bar{C} \simeq 0.2$ are slightly smaller than those obtained from equation 48a is due to the omission of terms in $(\Delta C)^2$, etc., in equation 3 and will be neglected.

TABLE VI

SOLUTIONS USED IN MIXED-SOLUTE DIFFUSIONS AT 25°

Exp. no.	Solutes 1	Solutes 2	Solutes 3	Solution A d	Solution A C_1	Solution A C_2	Solution B d	Solution B C_1	Solution B C_2	Solution B c_3[a]	α_2[b]	α_3[c]	j_m[c,d] calcd.	j_m obsd.
III	KCl	Sucrose	..	1.00294	0.12455	0	1.01229	0.32481	0.000451	.....	0.0110	....	91.39	91.39
IV	Sucrose	KCl	..	......	0	0	1.00287	.043818	.00099	.....	.0048	....	98.12	98.10
V	KCl	Sucrose	..	1.00191	0.10244	0	1.01130	.30224	.000833	.....	.0201	....	92.27	92.30
VII	KCl	Urea	..	1.00413	.15000	0	1.01059[e]	.24999	.11542	.....	.4999	....	90.49	90.20
VIII	KCl	Urea	..	1.00273	.12001	0	1.01069[f]	.28000	.03257	.....	.1499	....	85.18	85.09
XI	Sucrose	KCl	..	......	0	0	1.00305[g]	.043775	.00490	....	.0231	....	99.86	99.81
XII	Sucrose	KCl	BPA	......	0	0	1.00305[h]	.043831	.00322	0.00407	.0152	0.0035	99.55	99.62
XIII	KCl	Sucrose	..	1.00189[i]	0.10002	0.000573	1.01108	.30002	0	.....	− .0143	....	89.25	89.27

[a] This concentration is in g./100 ml. It has been corrected for water content of the BPA, which was subtracted from the weight of the BPA sample and added to the solvent weight. [b] Obtained from equations 3, 5, 47, 48 and 49. [c] A value of 1.881×10^{-3} (g./100 ml.)$^{-1}$, ref. 39, was used for the specific refractive increment of BPA. [d] Obtained using equations 3, 7, 47, 48 and 49. [e–i] While other densities in this table were calculated using equation 44, these values were measured pycnometrically. For comparison the calculated values are 1.01061, 1.01068, 1.00305, 1.00298 and 1.00187 g./ml., respectively.

TABLE VII

OBSERVED AND PREDICTED VALUES OF D_A, $\overline{D^1}/D_A$ AND $(\overline{D^2})^{1/2}/D_A$ FOR MIXED SOLUTES AT 25°

1 Exp. no.	2 $D_1 \times 10^5$, cm.²/sec.	3 $D_2 \times 10^5$, cm.²/sec.	4 $D_3 \times 10^5$, cm.²/sec.	5 Predicted $D_A \times 10^5$, cm.²/sec.	6 Predicted $\overline{D^1}/D_A$	7 Predicted $(\overline{D^2})^{1/2}/D_A$	8 Observed $D_A \times 10^5$, cm.²/sec.	9 Observed $\overline{D^1}/D_A$	10 Observed $(\overline{D^2})^{1/2}/D_A$
III	1.8360	0.5227	....	1.8012	1.0113	1.0142	1.7979	1.0094	1.0109
IV	0.5170	1.97	....	0.5194	1.0089	1.0271	0.5197	1.0062	1.0141
V	1.8360	0.5227	....	1.7731	1.0206	1.0259	1.7748	1.0223	1.0295
VII	1.8360	1.3758	....	1.5811	1.0157	1.0261	1.5804	1.0124	1.0186
VIII	1.8360	1.3790	....	1.7542	1.0076	1.0119	1.7518	1.0078	1.0114
XI	0.5170	1.95	....	0.5288	1.0403	1.1171	0.5280	1.0255	1.0749
XII	0.5170	1.95	0.067[a]	0.5182	1.0367	1.0918	0.5180	1.0293	1.0599
XIII	1.8360	0.5227	....	1.8828	0.9851	0.9815	1.8769[b]	0.9870	0.9823

[a] From Experiment XIV under "Bovine Plasma Albumin." [b] Since the observed time at which sharpening was stopped would have given a value of $\Delta t = -285$ sec., it is almost certain that this time was misread by 5 minutes and that the correct value of Δt is 15 sec. There remains a slight possibility, however, that some disturbance of the starting boundary caused the discrepancy between observed and predicted values of D_A for this experiment.

solute diffusions is illustrated in Fig. 2. Using these extrapolated values of C_t, the relative fringe deviations, Ω, were calculated, and the deviation graphs for the above experiments are shown in Figs. 3, 4 and 5 where dashed lines are theoretical curves from equations 20 or 23 using only terms to the first power in α_k, and solid lines include all the terms of order α_k^2. The required coefficients, $F(\zeta, r_k)$ and $G(\zeta, r_2)$, were interpolated from Tables I and II except in experiment XII for which the coefficients, $G(\zeta, r_k, r_l)$, had to be computed separately. Values of $r_k = D_1/D_k$ were calculated from the single-solute diffusion coefficients tabulated in columns 2, 3 and 4 of Table VII. Agreement between average experimental values of Ω, represented by crosses, and the solid theoretical lines validates within experimental error the use of equation 2 to describe these experiments.

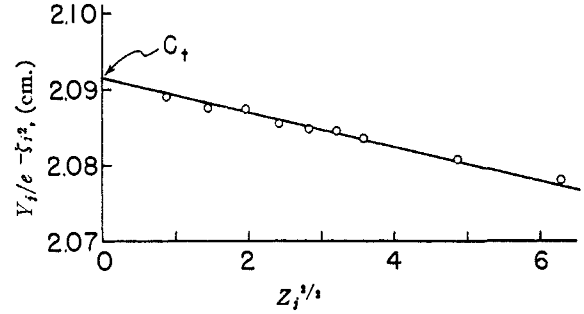

Fig. 2.—A typical extrapolation for obtaining C_t. Data are for t = 2460 sec., experiment V.

As predicted by the theory (equations 20 and 21 and Table I) the maxima in Figs. 3 and 4 lie to the left of $f(\zeta) = 0.428$ when $D_1/D_2 > 1$ and to the right when $D_1/D_2 < 1$. Furthermore, when α_2 is small enough that only the first term of equation 23 need be considered, the height of each point on these curves is proportional to the fraction of impurity, α_2. Assuming an experimental uncertainty in Ω of 2×10^{-4}, the smallest detectable amount of an impurity is

$$(\alpha_2)_{\min} = 2 \times 10^{-4}/[F(\zeta,r_2)]_{\max} \quad (50)$$

where $[F(\zeta, r_2)]_{\max}$ denotes the maximum value of $F(\zeta, r_2)$ for the value of r_2 considered. It is helpful to note that, when C_t = 1 cm., $\Omega_j \times 10^4$ is the difference in microns between an observed fringe displacement, Y_j, and the corresponding displacement, $e^{-\zeta_j^2}$, expected from a Gaussian boundary with the same j_m. Difficulty in extrapolating to the correct C_t may be encountered when $D_1/D_2 >> 1$, as would be true if a protein impurity were present in a salt, since the slope of Ω *versus* $f(\zeta)$ becomes very large as $f(\zeta) \rightarrow 0$.

Comparing Fig. 5 with Figs. 3 and 4, it is seen that at least two impurities, one with $D_2 > D_1$ and another with $D_3 < D_1$, must be present in Fig. 5 because the slope of this relative fringe deviation graph is steep at *both* $f(\zeta) = 0$ and $f(\zeta) = 1$.

To show what might be expected if incomplete dialysis for a protein diffusion resulted in an inverted buffer gradient, experiment XIII was performed with an inverted gradient of sucrose impurity in KCl, producing the inverted deviation graph of Fig. 6.

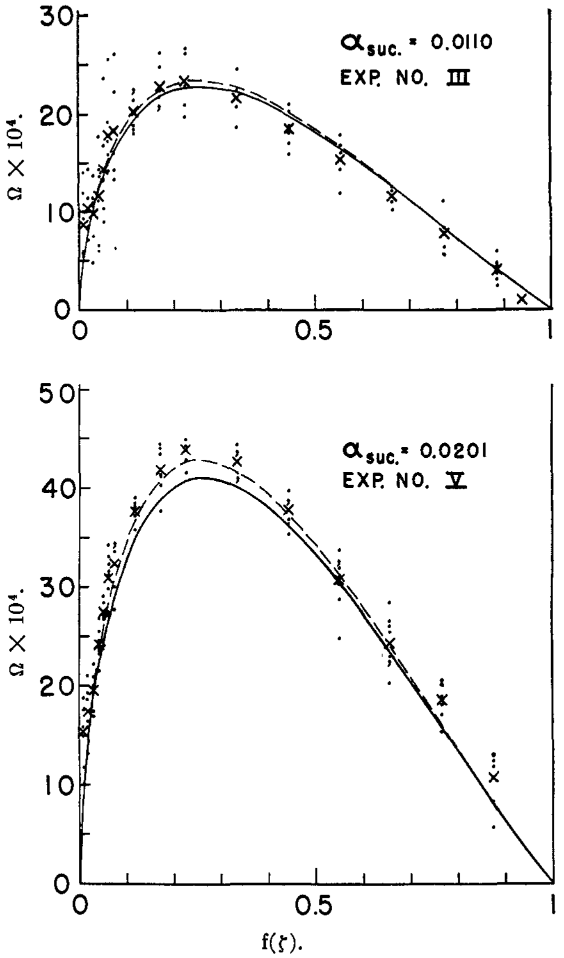

Fig. 3.—Relative fringe deviations for sucrose impurity in KCl.

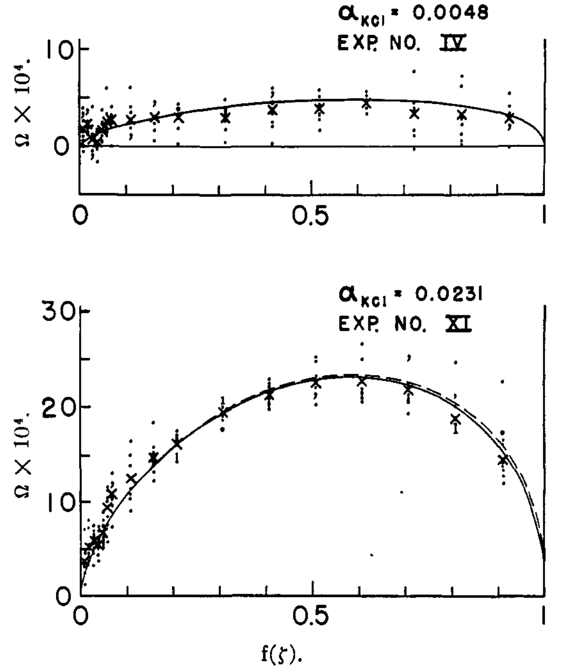

Fig. 4.—Relative fringe deviations for KCl impurity in sucrose.

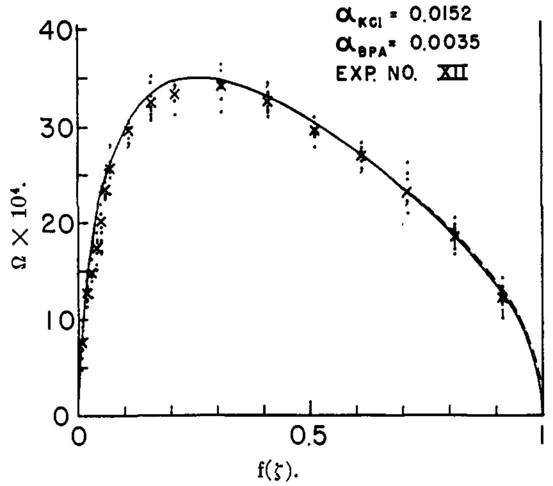

Fig. 5.—Relative fringe deviations for KCl and bovine plasma albumin impurities in sucrose.

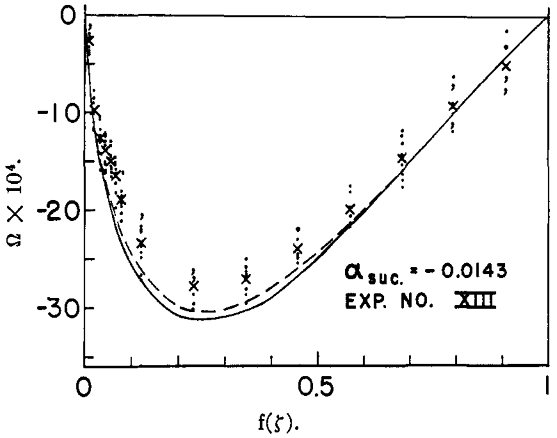

Fig. 6.—Relative fringe deviations for an inverted gradient of sucrose impurity in KCl.

Solutes with Similar Diffusion Coefficients.—Mixtures of potassium chloride and urea were used in experiments VII and VIII, Table VI, to test equation 27 for the relative fringe deviations, Fig. 7. The convergence of equation 27 for these experiments is observed by comparing the dashed curve, representing only the first term, with the solid curve, which includes the term in $(\sqrt{r_2} - 1)^3$. As expected from the first term in equation 27 the maxima in both graphs are located near $\zeta = 1$, or $f(\zeta) = 0.42759$, while for a given pair of solutes their heights are approximately proportional to $\alpha_2(1 - \alpha_2)$. When the maximum uncertainty in Ω is 2×10^{-4}, the minimum fractional difference in diffusion coefficients which will produce detectable displacements of the fringes is seen from equation 27 to be

$$\frac{D_1 - D_2}{D_2} \simeq 2(\sqrt{r_2} - 1) \simeq \frac{0.05}{\sqrt{\alpha_2(1 - \alpha_2)}} \quad (51)$$

or 0.1 for the most favorable case of $\alpha_2 = 0.5$.

Though observed and calculated values of j_m, Table VI, are in good agreement for the solutions of slightly impure solutes, greater discrepancies result from the urea–KCl mixtures. These discrepancies are attributed to the use of values of R_k for

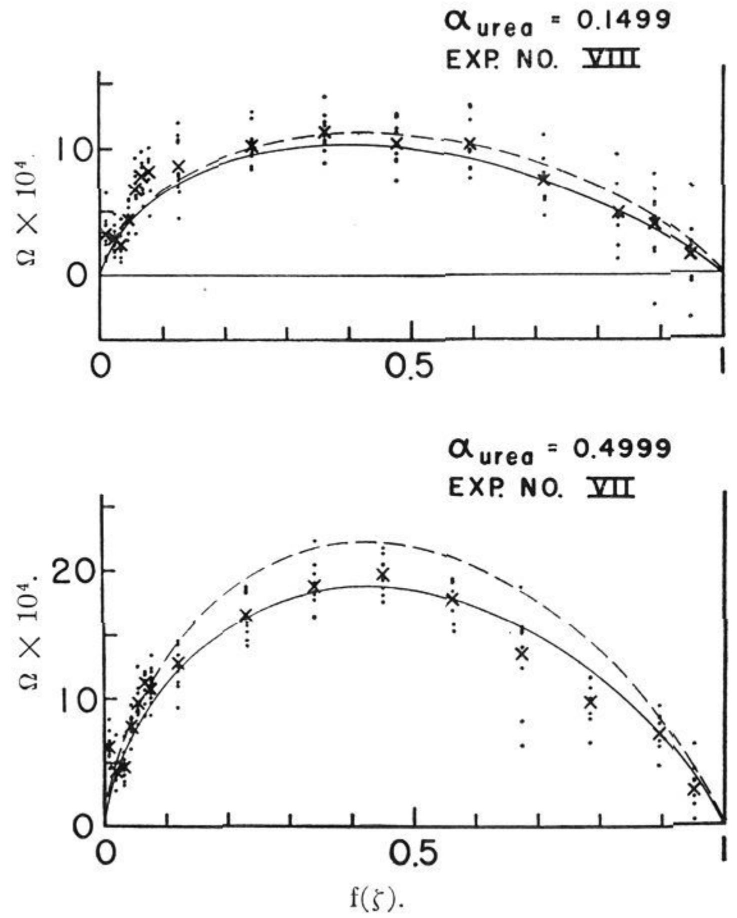

Fig. 7.—Relative fringe deviations for urea–KCl mixtures.

single solutes, equations 47–49, which should be nearly correct when only small amounts of impurity are present, but which may differ appreciably from the true values of R_k when larger amounts of a second component are present.

Average Diffusion Coefficients.—Observed values of D_A and the ratios $\bar{D}^1/D_A$ and $(\bar{D}^2)^{1/2}/D_A$, obtained from the relative fringe deviation graphs using equation 39, are listed in columns 8, 9 and 10 of Table VII for comparison with predicted values, columns 5, 6 and 7, from equations 10 and 33. For the latter calculations, values of α_k were taken from Table VI and, as described under "Single Solutes," each D_k was approximated, columns 2, 3 and 4 of Table VII, by single-solute diffusion coefficients at the appropriate mean concentrations for each experiment. The good agreement between predicted and observed values of D_A (except in experiment XIII which perhaps should not be compared because of the possibility of convection) indicates that neither the use of equation 2 nor the substitution of single-solute diffusion coefficients for values of D_k introduces serious errors in these experiments.

In every experiment graphs for the integration of equation 39 were prepared using, in conjunction with Table IV, measured heights and slopes of smooth relative fringe deviation curves drawn through the crosses representing average experimental data. Figures 8 and 9 for two experiments with widely different values of r_k illustrate the areas measured in obtaining $\bar{D}^1/D_A$. Similar graphs were obtained in the evaluation of $(\bar{D}^2)^{1/2}/D_A$, but experimental errors are magnified in obtaining these ratios.

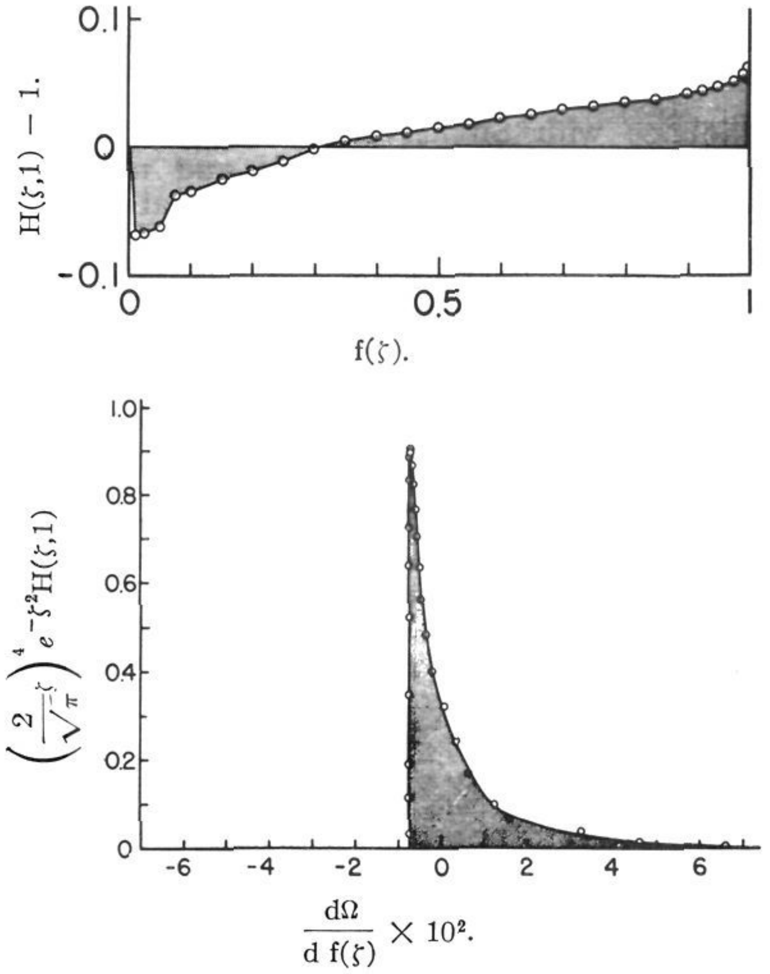

Fig. 8.—Graphical integrations in equation 39 for the case of a slower diffusing impurity, experiment V.

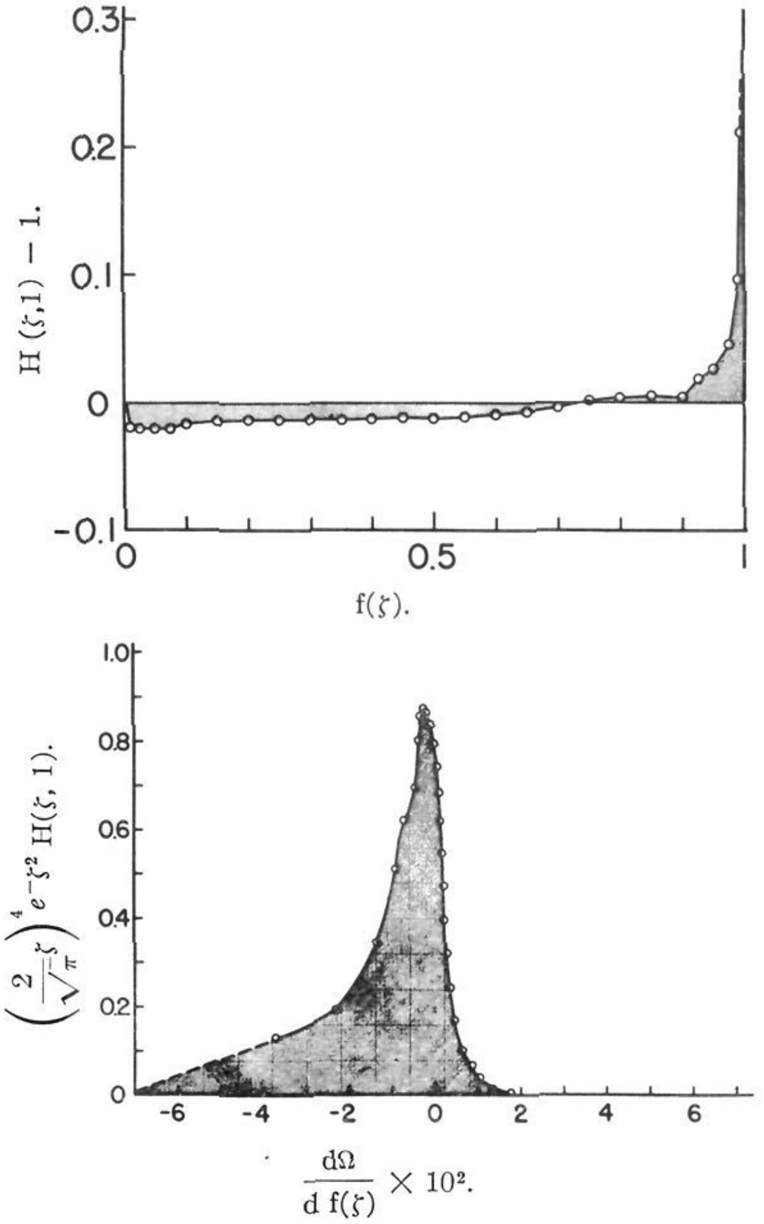

Fig. 9.—Graphical integrations in equation (39) for the case of a faster diffusing impurity, experiment XI.

Inspection of Table VII shows the best agreement between predicted and observed ratios of average diffusion coefficients is obtained in experiments with slower diffusing impurities and the poorest agreement in those with faster diffusing impurities. This behavior is expected from the dependence of the graphical integrations of equation 39 upon the magnitudes and slopes of the fringe deviation curves between $f(\zeta) = 0.9$ and 1.0. When only slower diffusing impurities are present, Figs. 3 and 8, these integrals can be quite accurately evaluated since the deviation graphs approach zero almost linearly with small slopes as $f(\zeta) \rightarrow 1$. Faster diffusing impurities, Figs. 4 and 9, produce large slopes in this region, leaving uncertain the shapes of the graphical integration curves over a range which may contain as much as half the area.

The above method for obtaining higher-average diffusion coefficients is similar to procedures developed by Ogston[17] and Charlwood[18] except that approximation of an integral by a summation has been avoided through use of the relative fringe deviation graphs. Photographs from an integral fringe[48–52] diffusiometer when subjected to Svensson's analysis[51] also yield average diffusion coefficients of the type defined by equation 33, but this procedure likewise depends on approximation of an integral by a summation. The two methods complement each other because most of the integral fringes arise from the central part of the diffusion boundary whereas, due to the effect of the air path on the Gouy method, the "tails" of the boundary contribute significantly to the Gouy fringes. For example, in a 100 fringe pattern from a Gaussian boundary the 99th integral fringe, for which $(2/\sqrt{\pi})\int_0^z e^{-\beta^2}\,d\beta = 0.98$ and $z = 1.645$, corresponds to $f(z) \simeq 0.856$ or approximately the 85th Gouy fringe. A characteristic of the Gouy method which may give it an advantage over the integral fringe method in studying mixtures is that at any given time its fringe system possesses *two* reference points, C_t and the undeviated slit image position, which can be accurately and easily determined in addition to j_m. In both methods two such points are required to locate the fringe positions corresponding to a Gaussian boundary if relative fringe deviations are to be calculated.

The data in Table VII indicate that fairly accurate values of the average diffusion coefficients, equation 33, can usually be determined with the Gouy diffusiometer though this accuracy is always lower than for D_A. When only a qualitative indication of whether detectable impurities are present is desired, without quantitative evaluation, the relative fringe deviation graph provides a more direct test of homogeneity than does comparison of D_A with the higher averages.

Bovine Plasma Albumin.—To show how the above methods for analyzing Gouy fringes may be applied to protein diffusions, BPA was allowed to diffuse, experiment XIV, at 25.002° near its isoelectric point in pH 4.59 acetate buffer. This buffer, which was 0.0100, 0.0100 and 0.1500 M in acetic acid, potassium acetate and potassium chloride, respectively, had a density of 1.00470 g./ml. and a relative viscosity of 1.004, requiring only a small correction to convert observed diffusion coefficients to those in water. Before diffusion the BPA solution was twice dialyzed for several hours at 1° after which 10 hours were allowed for attaining final dialysis equilibrium at room temperature against the second buffer sample. Assuming that no buffer gradient exists after this dialysis and that the specific refractive increment,[39] 1.922×10^{-3} (g./100 ml.)$^{-1}$, for BPA at this temperature and wave length in sodium chloride also applies to this buffer, substitution of the total number of fringes, $j_m = 87.22$, in equations 3 and 7 yields the protein concentration after dialysis, $\Delta c_{BPA} = 0.997$ g./100 ml., or $c_{BPA} = 0.498$ g./100 ml.

The measured value of 6.670×10^{-7} cm.²/sec. for D_A in this buffer corrects to 6.697×10^{-7} cm.²/sec. in water at 25°, or 5.855×10^{-7} cm.²/sec. in water at 20°. Until additional experiments can be carried out it is not known whether the difference between this result and Creeth's value[53] of 6.112×10^{-7} cm.²/sec. for $D_{20,w}$ is due to the difference in pH, buffer or albumin sample.

Figure 10a shows the relative fringe deviation graph for this experiment. These positive deviations, Ω, correspond to a small decrease of $Y_j/e^{-\zeta_j^2}$ with increasing fringe number, similar to that observed by Charlwood[18,54] for both BPA and human plasma albumin. Application of equation 39 to Fig. 10a yields the ratios $\bar{D}^1/D_A = 1.0049$ and $(\bar{D}^2)^{1/2}/D_A = 1.0068$. The presence of one or more *slower* diffusing impurities is seen from the position of the maximum in Fig. 10a, and this result is supported by the velocity sedimentation diagram, Fig. 11, which indicates the presence of a small amount of faster sedimenting impurity. Since $\Omega \rightarrow 0$ quite linearly in the region $0.7 \leqslant f(\zeta) \leqslant$

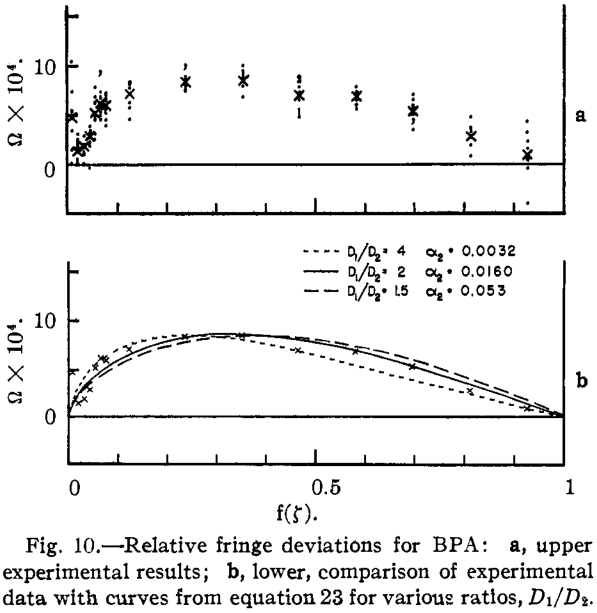

Fig. 10.—Relative fringe deviations for BPA: **a**, upper experimental results; **b**, lower, comparison of experimental data with curves from equation 23 for various ratios, D_1/D_2.

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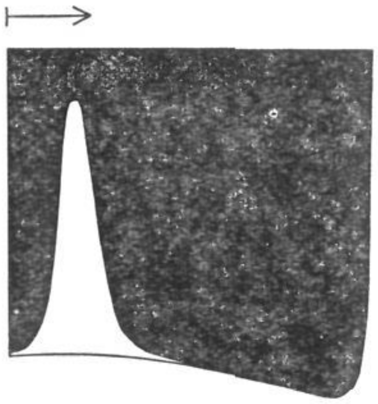

Fig. 11.—Sedimentation diagram for the BPA in the same buffer used for diffusion experiment XIV: 60,000 r.p.m., 2580 sec., 0.91 g./100 ml. and 24°.

1 any contribution, Ω_{buffer}, to this curve is probably less than 1.5×10^{-4} for which equation 23 and Table I indicate that any residual buffer gradient ($r_{\text{buffer}} \simeq 1/25$) is restricted to $\alpha_{\text{buffer}} < 0.0006$. According to equation 10 such a buffer gradient cannot influence D_A by more than 0.1%, so in this experiment the spacings of the Gouy fringes near the slit image have proved that any buffer gradient was too small to influence significantly the observed diffusion coefficient.

Assuming that only one impurity is present, we may attempt to determine D_1/D_2 and α_2 by selecting the two values which enable equation 23 to fit best the crosses of the relative fringe deviation graph. To illustrate how this may be done, curves are shown in Fig. 10b representing three pairs of values of D_1/D_2 and α_2 which yield the observed value of $\Omega_{\max} = 8.5 \times 10^{-4}$. If each of these ratios, 4, 2 and 1.5, for D_1/D_2 is assumed to be independent of buffer and temperature and is substituted with its corresponding value of α_2 into equation 10, together with $(D_A)_{20,w} = 5.855 \times 10^{-7}$ cm.²/sec., the three values 5.892, 5.932 and 5.995, respectively, are obtained for $(D_1)_{20,w} \times 10^7$, the diffusion coefficient of the albumin alone. While the best fit of the crosses, Fig. 10b, would be given by a ratio of D_1/D_2 between 2 and 4, it is seen that each of the three ratios considered corresponds to the experimental points within their probable errors.

It should be emphasized that interpretation of protein diffusions by assuming only one impurity to be present may often be a gross oversimplification. In such cases quantitative interpretations appear to be limited to evaluation of different average diffusion coefficients with equation 39. The interpretation of the BPA experiment in terms of a single impurity was included to illustrate how, when this assumption is justified, the above theory for mixed solute diffusions is used to obtain D_1, and how much these values are influenced by experimental error.

Further experiments with BPA are contemplated to corroborate and extend the results from the single experiment reported above. The above data, however, when considered with those of Ogston,[16,17] Creeth[53] and Charlwood[18,54] indicate that the Gouy diffusiometer is both a sensitive and a very useful tool for the study of proteins.

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MADISON, WISCONSIN

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Self-diffusion Coefficients of Iodide, Potassium and Rubidium Ions in Aqueous Solutions[1]

BY REGINALD MILLS AND JOSEPH W. KENNEDY

RECEIVED AUGUST 14, 1953

The self-diffusion coefficients of iodide, potassium and rubidium ions have been measured in a series of aqueous metal iodides of moderate to high concentration at 25°. The open-ended capillary method has been used throughout. In view of serious discrepancies that have arisen between self-diffusion coefficients measured by this capillary method and by the diaphragm cell method, an examination of possible sources of error has been made. The self-diffusion coefficients of large unhydrated ions as obtained by the improved method are found to exhibit a linear relationship up to comparatively high concentrations when their product with the specific viscosity is plotted against $\sqrt{c}$. A correlation of the slopes of the series of lines obtained in this way is attempted by taking into account the mean distance of approach of the ions and its effect on the potential energy of the ion in its ionic atmosphere. A simple picture of the mechanism of the self-diffusion process for unhydrated ions, based on this evidence, is suggested.

The increasing availability of artifically produced radioisotopes over the last decade has given impetus to the study of certain types of diffusion phenomena. In particular, the self-diffusion coefficients of ions in electrolytic solution have been measured by several methods, chief among which are the diaphragm cell method and various forms of the single capillary method. In the results first reported, the two kinds of method gave fairly good agreement with one another. Thus Adamson[2] using a diaphragm cell and Wang and Kennedy[3] using a capillary cell obtained comparable values for the self-diffusion coefficients of Na^+ in aqueous

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(1) Submitted as a part of the requirements for the Ph.D. degree at Washington University, St. Louis, by Reginald Mills. Mr. Mills' present address is in care of the Department of Chemistry, University of Southern California, Los Angeles 7, Calif.

(2) A. W. Adamson, *J. Chem. Phys.*, **15**, 760 (1947).

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