Bird, R. B., W. E. Stewart, and E. N. Lightfoot, *Transport Phenomena,* Wiley, New York (1960).
Chapman, S., and T. G. Cowling, *The Mathematical Theory of Non-Uniform Gases,* 3 ed., prepared in co-operation with D. Burnett, Cambridge University Press, England (1970).
Gilliland, E. R., in *Absorption and Extraction,* T. K. Sherwood, 1 ed., McGraw Hill, New York (1937).
Hsu, H-W., and R. B. Bird, "Multicomponent Diffusion Problems," *AIChE J.,* **6,** 516 (1960).
Johns, L. E., and A. E. DeGance, "Diffusion in Ternary Ideal Gas Mixtures. I. On the Solution of the Stefan-Maxwell Equation for Steady Diffusion in Thin Films," *Ind. Eng. Chem. Fundamentals,* **14,** 237 (1975).
Krishna, R., "Interphase Transport of Mass and Energy in Multicomponent Systems," Ph.D. thesis, Department of Chemical Engineering, University of Manchester Institute of Science and Technology, Manchester, England (1975).
Standart, G. L., R. Krishna, and H. T. Cullinan, "Multicomponent Isothermal Mass Transfer," Paper presented at the AIChE Conference, Tulsa, Okla. (Mar., 1974).
Stewart, W. E., and R. Prober, "Matrix Calculation of Multicomponent Mass Transfer in Isothermal Systems," *Ind. Eng. Chem. Fundamentals,* **3,** 224 (1964).
Stewart, W. E., "Multicomponent Mass Transfer in Turbulent Flow," *AIChE J.,* **19,** 398 (1973).
Toor, H. L., "Diffusion in Three-component Gas Mixtures," *ibid.,* **3,** 198 (1957).
———, "Solutions of the Linearized Equations of Multicomponent Mass Transfer. II. Matrix Methods," *ibid.,* **10,** 460 (1964).

Manuscript received October 21, 1975; revision received December 9, and accepted December 11, 1975.

Evaluation of a Rapid Technique for Measuring the Diffusion Coefficients of Small Molecules

MARC LEMAGUER
F. H. WOLFE
and
T. G. SMYRL

Department of Food Science
University of Alberta
Edmonton, Alberta, Canada

The diffusion coefficients of sucrose, glycol, and glycine in water have been measured at 25.0°C by using the schlieren optical system of a preparative ultracentrifuge. The schlieren optical system was modified by the incorporation of a 35 mm single lens reflex camera. The values obtained were well within 2% of values found in existing literature and had an intrinsic precision of better than 2%.

SCOPE

The measurement of diffusion coefficients of small molecules has traditionally been restricted to well-known classical techniques such as those employing the Tiselius cell in conjunction with interference optics or those using the Stokes type of diaphragm cell. Although the reliability of these techniques is well established, they tend to require substantial amounts of time to obtain a diffusion measurement (Holmes, 1960; English and Dole, 1950).

For many years the ultracentrifuge with schlieren, absorption, or interference optical systems has been used in diffusion and sedimentation studies of macromolecular species in aqueous solution. Recently, however, Chandrasekhar and Hoelscher (1975) have extended the use of an analytical ultracentrifuge to the measurement of the diffusion coefficients of glycerol, glycol, and *n*-butanol in aqueous solution. Independently of Chandrasekhar and Hoelscher (1975), we have been studying the applicability of the ultracentrifugal technique to the measurement of diffusion coefficients of low molecular weight compounds using a preparative ultracentrifuge of which the schlieren optical attachment has been adapted to 35 mm photography. By using a double sector capillary synthetic boundary cell as the diffusion cell, the diffusion coefficients of sucrose, glycol, and glycine in water have been determined, and the results are compared to those obtained from interferometric techniques. In view of the fact that such preparative ultracentrifuges are commonly used, the rapid, simple, and accurate determination of diffusion coefficients of low molecular weight compounds is thus made available to a greater number of investigators.

CONCLUSIONS AND SIGNIFICANCE

A preparative ultracentrifuge equipped with a schlieren optical system has been used to obtain diffusion coefficients of sucrose, glycol, and glycine in water. The schlieren optical column has been modified so that a 35 mm single lens reflex camera can be used to record the schlieren patterns. With this apparatus, the diffusion coefficients were found to vary by less than 2% from values obtained by interference techniques. Replicate

Correspondence concerning this paper should be addressed to Marc LeMaguer.

measurements show that the diffusion coefficients obtained are precise to approximately 1 to 2%. Typically, a diffusion experiment consists of a run of less than 10 min. duration. This adaptation of the schlieren optical system to accommodate a 35 mm single lens reflex camera thus offers a quick method to accurately and precisely determine the diffusion coefficients of low molecular weight compounds.

The advantages of the modified photographic analysis lie in the ability of the single lens reflex camera to provide a visual display of the schlieren pattern while at the same time having the capability to record the schlieren pattern on a photograph. Neither the analytical nor the unmodified preparative ultracentrifuge is able to simultaneously display and record schlieren patterns. In addition, the time interval between successive photographs of the schlieren image may be reduced to a few seconds with the 35 mm single lens reflex camera. Another advantage of the system resides in the clear and sharp photographic images which are obtained when a high-speed film is used. Magnification of up to 40 × yields high clarity enlargements, thus affording a precise measurement of peak heights.

The double sector capillary synthetic boundary cell has been found to produce a sharp undistorted interface with very little mixing. Stabilization of the interface in the centrifugal field allows for almost ideal free diffusion.

As a result of the above advantages, an improved method of data treatment has been developed. This method of analysis will also permit the calculation of reduced moments in addition to classical D values.

EXPERIMENT

Materials

Sucrose, glycol, and glycine were all reagent grade and were used as received from the J. T. Baker Chemical Company. All solutions were prepared with distilled-deionized water.

Equipment

A Beckman Model L2-65B Preparative Ultracentrifuge equipped with a schlieren optical system was used in the diffusion experiments. A Miranda Sensorex 35 mm single lens reflex camera was mounted on the optical column to monitor and photograph the schlieren patterns. The diffusion cell was a double sector capillary synthetic boundary cell with a polished Epon centerpiece and was obtained from Beckman. An Analytical-D rotor was used to house the diffusion cell during the diffusion runs. The rotor, cell, and solutions were equilibrated at the required temperature in a Labline Environmental Chamber. Photographs were recorded on Tri-X film (400 ASA) at a shutter speed of ⅛ s.

PROCEDURE

Sucrose and glycine solutions were prepared on a weight-by-volume basis, whereas the glycol solutions were prepared on a weight-by-weight basis. All solutions were used on the same day of preparation to avoid effects of microbial growth. After cell assembly, the left-hand sector of the cell was completely filled with the less dense solution. The right-hand sector was flushed twice with the more dense solution and was then filled with the more dense solution so that the top of the meniscus just reached the lower capillary. For all aqueous solutions used in this study, the density was found to increase as the concentration of the solute increased. To avoid possible leakage from one sector to another, the cell was kept in a horizontal position until the time of the diffusion run. For runs at 25°C, the chamber was prewarmed, whereas the rotor, cell, and solutions were equilibrated for at least 1 hr. at 25° ± 0.2°C in the Labline chamber. For runs below room temperature, the chamber was precooled by using the refrigeration system of the instrument, and the rotor, cell, and solutions were precooled to the desired temperature in the Labline apparatus. After the chamber had been sufficiently evacuated, the rotor was accelerated to the desired final revolutions per minute. Runs were conducted at several final revolutions per minute values to determine if this parameter had any effect on the diffusion measurement. As the rotor begins to accelerate, the less dense solution flows through the lower capillary of the cell and layers smoothly onto the more dense solution in the right sector. The less dense solution was layered onto the more dense solution to maintain gravitational stability (Gosting and Fujita, 1957).

Photographing of the schlieren patterns in a diffusion run was initiated after the position of the air-liquid interface in the right sector had stabilized. The time intervals between successive photographs depended on the diffusion rate of the particular compound being studied. For example, in sucrose diffusion runs, 1 min. time intervals between successive photographs were found to be suitable, whereas in the glycol studies, a time interval of 30 s was used. In all cases, photographs were taken until the peak height had decreased to approximately 50% of the height of the first photograph. The angle of inclination of the schlieren diaphragm θ was selected so that peak height and peak sharpness were obtained.

The negatives were mounted in 35 mm glass slide mounts. A slide projector was then used to project the images onto a vertical gradient such that the peak height was magnified by a factor of 40 ×. The projector was adjusted so that the base line of the vertical gradient bisected the base line of the schlieren image, and the peak maximum fell at $X = 0$. From each photograph, values of the peak height at 5 mm intervals ($X = 0, \pm 5, \ldots, \pm 35$ mm) along the curve were obtained. Peak heights were measured from the middle of the base line of the image to a point corresponding to one half the vertical thickness of the peak image. The reference lines arising from the counterbalance in the rotor were used to calculate the magnification factor β. β was calculated from each photograph, and an average value was used in calculating the diffusion coefficient for that particular run.

EVALUATION OF DATA

The equation required to calculate the diffusion coefficient for a two-component system from measurements of the concentration gradient in a free diffusion experiment is (Longsworth, 1945)

$$\frac{\partial c}{\partial x} = \frac{\Delta c}{2\sqrt{\pi D t}} e^{-x^2/4Dt} \qquad (1)$$

where Δc is the concentration difference, D is the diffusion coefficient, and c is the concentration at a distance x from the interface at time t. With respect to the magnified

Table 1. Variation of Diffusion Coefficients ($m^2/s \times 10^{10}$) with Temperature $\bar{c} = 1.000$, $\Delta c = 2.000$ g/100 ml

Temperature, °C	$\bar{D}$exp	Dlit	% deviation
25.0	5.19 ± 0.05	5.148	0.8%
14.8	3.80 ± 0.06	3.866	+1.7%
2.6	2.54 ± 0.02	2.53	+0.4%

schlieren image, the concentration gradient is related to the vertical displacement y' (as measured on the vertical gradient) by

$$\frac{\partial c}{\partial x} = \frac{y' \tan \theta}{\beta_1 L a m_1 m_2 \dfrac{\partial n}{\partial c}} \tag{2}$$

where $\tan \theta$, L, m_1, and m_2 are constants associated with the schlieren optical system (Beckman Model E Manual, 1964), a is the cell thickness, $\partial n/\partial c$ is the specific refractive index increment, and β_1 is the magnification factor from the 35 mm slide to the enlargement. Similarly, the measured horizontal displacement x' is related to the horizontal displacement in the cell x by

$$x' = \beta_1 m_1 x \tag{3}$$

Substitution of Equations (2) and (3) into (1) and rearrangement yields

$$y' = \frac{\alpha \Delta c}{2\sqrt{\pi D t}} e^{-(x')^2/\beta^2 4Dt} \tag{4}$$

where

$$\alpha = \frac{\beta_1 L a m_1 m_2}{\tan \theta} \frac{\partial n}{\partial c} \tag{5}$$

and

$$\beta = \beta_1 m_1$$

Rearranging Equation (4) and taking the natural logarithm, we get

$$\ln(y' t^{1/2}) = \ln\left(\frac{\alpha \Delta c}{2\sqrt{\pi D}}\right) - \frac{x'^2}{\beta^2 4Dt} \tag{6}$$

Since α, Δc, π, D, and β are all constants for a particular diffusion experiment, a plot of ln $(y't^{1/2})$ vs. $(x')^2/t$ yields a straight line of slope $-1/\beta^2 4D$ and an intercept of $\ln(\alpha \Delta c/2\sqrt{\pi D})$, permitting a calculation of D. Since fifteen values of y' and x' are derived from each photograph, and since at least five photographs are taken during the diffusion run, a value of D is obtained from a linear regression involving at least 75 points. Zero time corrections (Longsworth, 1947) have been calculated by using the method outlined by Chervenka (1969). All reported diffusion coefficients have been calculated incorporating the zero time correction.

RESULTS

For an average concentration of 1.000 g/100 ml and a difference in concentration between solutions of 2.000 g/100 ml, the diffusion coefficient of sucrose in water was found to be $5.19 \times 10^{-10} m^2/s$ at 25.0°C. On the basis of repetitive runs, the precision of this value, assessed as the standard deviation, was calculated to be ±0.05. Using the Gouy interferometer, Gosting and Morris (1949) obtained a value of $5.148 \times 10^{-10} m^2/s$ at 24.95 ±0.005°C and with an estimated precision of between 0.05 and 0.10%. Thus, this technique appears to have a potential accuracy of 1%.

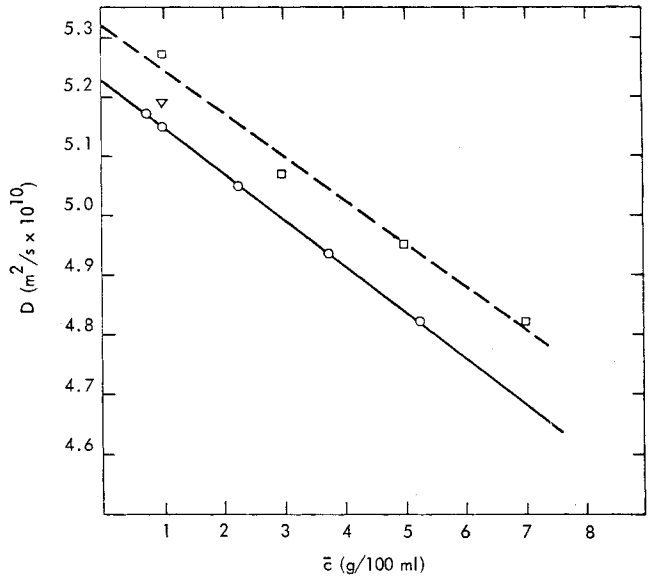

Fig. 1. D as a function of the average sucrose concentration. □, this work at 25.3°C; ○, Gosting and Morris (1949) at 24.95°C; ▽, this work 25.0°C; - - - best fit of data at 25.3°C.

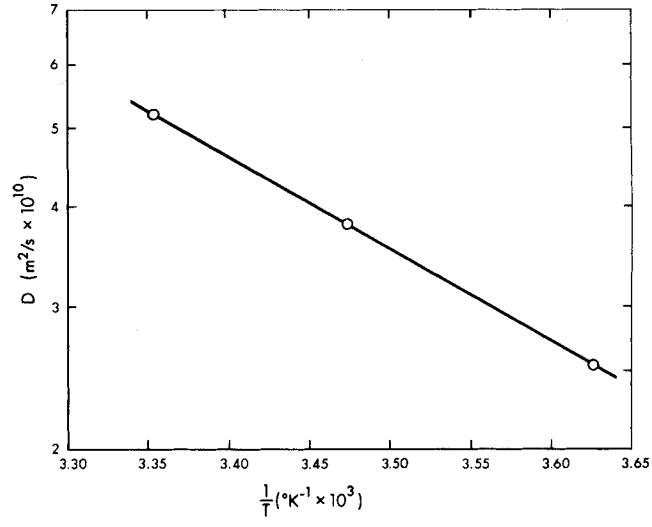

Fig. 2. Arrhenius type of plot of D vs. T.

The dependence of the diffusion coefficient on the average sucrose concentration is depicted graphically in Figure 1. In these experiments conducted at 25.3°C, the average concentration was systematically varied, while the difference in concentration was kept constant. The results of Gosting and Morris (1949) obtained at a slightly lower temperature of 24.95°C are also included in Figure 1. Extrapolation of the $\bar{D}$exp values to $\bar{c} = 0$ yields a $D°$exp of $5.32 \times 10^{-10} m^2/s$. Thus, this ultracentrifugal technique appears to have sufficient sensitivity to monitor the variation of the diffusion coefficient with small changes in the average concentration of the diffusing molecule.

Table 1 shows the variation of sucrose diffusion coefficients with temperature, where $\bar{c}$ and Δc have been kept constant at 1.000 g/100 ml and 2.000 g/100 ml, respectively. Again, the results of Gosting and Morris (1949) are included for comparative purposes. Dlit values for $T = 2.6°$ and 14.8°C have been obtained from the data of Gosting and Morris (1949) at 1° and 24.95°C, respectively, by employing the Stokes-Einstein relation; hence they should be considered as approximate. A plot

TABLE 2. DIFFUSION COEFFICIENTS ($m^2/s \times 10^9$) OF GLYCOL AND GLYCINE AT 25.0°C

Compound	$\bar{c}$	Δc	$\bar{D}$exp	D^0 exp	Dlit.	% deviation
Glycol	0.7507*	1.5003*	1.13 ± 0.04	1.14	1.16, 1.17	1.7
	10.2068*	2.0331*	1.02 ± 0.01			
	25.0109*	1.9625*	0.83 ± 0.01			
Glycine	0.5995†	1.1990†	1.02 ± 0.01		1.04	1.9

* = wt. %.
† = g/100 ml solution.

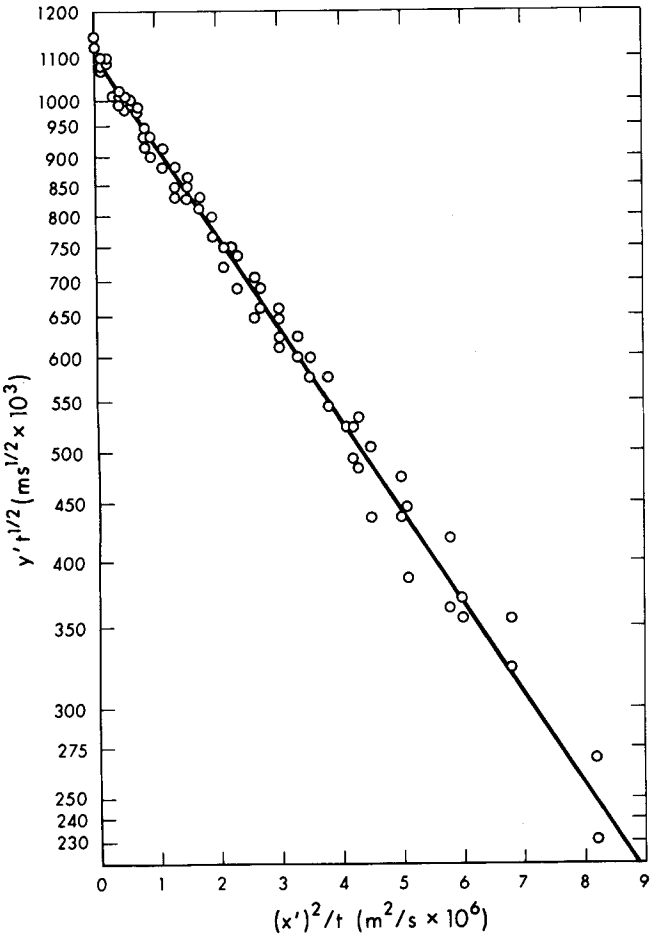

Fig. 3. Plot of Equation (6) for glycine at 25.0°C.

of ln (Dexp) vs. $1/T$(°K) (Glasstone et al., 1941) follows the expected linear relationship as evidenced in Figure 2.

Diffusion coefficients of glycol and glycine at 25°C are listed in Table 2. Extrapolation of $\bar{D}$exp for glycol to $\bar{c} = 0$ yields a D^0exp of $1.14 \times 10^{-9} m^2/s$ which compares well with $D^0 = 1.16 \times 10^{-9} m^2/s$ obtained by Garner and Marchant (1961) using the interference technique and $D^0 = 1.17 \times 10^{-9} m^2/s$ obtained by Byers and King (1966) using the diaphragm cell technique. Results for glycine agreed quite well with those from previous interferometric studies (Lyons and Thomas, 1950).

DISCUSSION

Neurath (1942) has described several standard methods for determining diffusion coefficients from schlieren photographs. The frequently used maximum height-area method was used by Chandrasekhar and Hoelscher (1975) and English and Dole (1950). Plots involving the maximum height-area technique appear to yield excellent linear relationships; however, as pointed out by English and Dole (1950), there is uncertainty on the absolute magnitude of the diffusion coefficient because of the fact that there is considerable individual latitude in the selection of both peak height and area. Since peak height determinations are limited to an accuracy of about 1% (Gosting, 1956), the diffusion coefficients calculated by using this method cannot be expected to give results with a precision better than 4% (the maximum height-area method involves the square of the peak height and the square of the peak area). The maximum ordinate method relies on a single measurement of the peak height, while the statistical method of analysis requires knowledge of the peak area.

When Equation (6) is used to determine diffusion coefficients from schlieren peaks, the only error involved is that corresponding to the height measurement y'. Since peak heights are measured at fifteen x' values on each peak, the resulting diffusion coefficient is based on many y' values, not just y' max. The subsequent regression analysis using $\ln y'$ leads to a maximum error of 2% for a single run as has been verified on numerous runs. In addition, the use of Equation (6) avoids the need to find the peak area, thus eliminating any uncertainty in the diffusion coefficient arising from this quantity. Diffusion coefficients calculated by the maximum height-area method are typically based on a plot consisting of five to ten points, whereas a linear regression involving at least 75 points is used following the method of Equation (6). Figure 3 illustrates a plot of Equation (6) for glycine at 25.0°C.

Two critical factors required to achieve a valid free diffusion experiment are the formation of a sharp initial boundary between the two solutions and the absence of restricted diffusion conditions in the diffusion cell. Several types of synthetic boundary cells are available to achieve the layering of one liquid onto another. The valve type of synthetic boundary cell was used by Chandrasekhar and Hoelscher (1975), whereas the double sector capillary synthetic boundary cell was used in this study. In the double sector capillary synthetic boundary cell, the less dense solution flows from the full sector to the partially full sector as the rotor begins to accelerate. The solution from the full sector enters the partially filled sector at the same level as the solution already present, hence minimizing the turbulence or mixing which could occur as the boundary is formed and at the same time sharpening the boundary.

In all experiments using the double sector capillary synthetic boundary cell, the zero time corrections Δt were invariably of the order of a few seconds, indicating a sharp initial boundary formation. Because the compounds employed in this study are of low molecular weight, the

effect of the centrifugal field is not expected to be significant. Diffusion runs carried out at angular velocities between 5 000 and 15 000 rev./min. showed the experimentally determined diffusion coefficient to be constant within experimental error. Earlier work in this laboratory with the valve type of synthetic boundary cell revealed that a considerable amount of turbulence occurred at the interface of the two liquids as boundary formation was taking place. This phenomenon was observed by taking a rapid succession of photographs (3 s intervals) as the cup emptied. This was made possible by the 35 mm modification. This turbulence probably arises from the momentum acquired by the solution as it falls from the cup to the lower layer of solution already present in the cell and results in the distortion of the schlieren peak leading to unreliable diffusion coefficient values.

Adaptation of the schlieren optical column of the preparative ultracentrifuge to accommodate a 35 mm single lens reflex camera is an important factor in increasing the precision of peak measurements over that of other techniques (Chervenka, 1969; Schachman, 1957). Since the 35 mm negative may be enlarged by a factor of about 40 without serious distortion of the schlieren image, the magnitude of the relative error on the peak height measurements can be minimized. As stated previously in the discussion of turbulence at the interface, the 35 mm adaptation to the schlieren optical column allows for the analysis of very rapidly diffusing substances because the time interval between successive photographs may be reduced to seconds. In addition, the use of the single lens reflex camera permits the constant viewing of the diffusion experiment under study to insure, for example, that peaks are not recorded while the diffusion run reaches a point of restricted diffusion.

Figure 4 illustrates the comparison between diffusion coefficients obtained by using interference techniques (horizontal axis) and diffusion coefficients arising from other methods (vertical axis). The closeness of our diffusion coefficients to the 45 deg. line asserts their validity and reflects the substantial improvement in the accuracy of the ultracentrifugal technique compared to previous work with an analytical ultracentrifuge (Chandrasekhar and Hoelscher, 1975). The dashed lines in Figure 4 represent the limits of the 5% deviation interval.

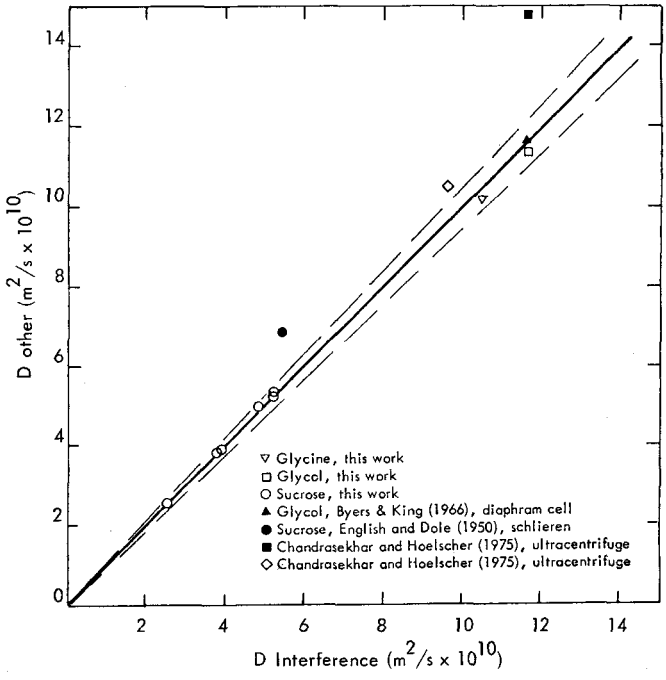

Fig. 4. *D* interference vs. *D* other.

NOTATION

c = solute concentration
D = diffusion coefficient, m^2/s
x = coordinate normal to interface
x' = measured distance normal to interface
y' = measured height of schlieren peak above base line
L = distance from the middle of the cell to the plane of the schlieren analyzer
a = thickness of the centerpiece
m_1 = horizontal magnification due to camera lens
m_2 = vertical magnification due to cylindrical lens
t = time
n = refractive index
θ = angle of inclination of schlieren diaphragm
β_1 = magnification due to slide projector
β = total magnification in x direction

ACKNOWLEDGMENTS

The authors are grateful to the National Research Council of Canada for their financial support of this work. Thanks are due to Mr. R. Currie for preliminary work on this project.

LITERATURE CITED

Beckman Model E Analytical Ultracentrifuge, Spinco Div., Beckman Instruments (1964).

Byers, C. H., and C. Judson King, "Liquid Diffusivities in the Glycol-Water System," *J. Phys. Chem.*, **70**, 2499 (1966).

Chandrasekhar, S., and H. E. Hoelscher, "Mass Transfer Studies Across Liquid/Liquid Interfaces (Use of an Analytical Ultracentrifuge)," *AIChE J.*, **21**, 103 (1975).

Chervenka, C. H., "A Manual of Methods for the Analytical Ultracentrifuge," Spinco Div., Beckman Instrument (1969).

English, A. C., and M. Dole, "Diffusion of Sucrose in Supersaturated Solutions," *J. Am. Chem. Soc.*, **72**, 3261 (1950).

Garner, F. H., and P. J. M. Marchant, "Diffusivities of Associated Compounds in Water," *Trans. Inst. Chem. Engrs.*, **39**, 393 (1961).

Glasstone, S., K. J. Laidler, and H. Eyring, *The Theory of Rate Processes*, McGraw-Hill, New York (1941).

Gosting, L. J., "Measurement and Interpretation of Diffusion Coefficients of Proteins," *Advan. Prot. Chem.*, **11**, 429 (1956).

———, and H. Fujita, "Interpretation of Data for Concentration-dependent Free Diffusion in Two-Component Systems," *J. Am. Chem. Soc.*, **79**, 1359 (1957).

Gosting, L. J., and M. S. Morris, "Diffusion Studies on Dilute Aqueous Sucrose Solutions at 1 and 25° with the Gouy Interference Method," *ibid.*, **71**, 1998 (1949).

Holmes, J. T., U.S. Atomic Energy Commission Report UCRL-*9145* (1960).

Longsworth, L. G., "The Diffusion of Electrolytes and Macromolecules in Solution: A Historical Survey," *Ann. N.Y. Acad. Sci.*, **46**, 211 (1945).

———, "Experimental Tests of an Interference Method for the Study of Diffusion," *J. Am. Chem. Soc.*, **69**, 2510 (1947).

Lyons, M. S., and J. V. Thomas, "Diffusion Studies on Dilute Aqueous Glycine Solutions at 1° and 25° with Gouy Interference," *ibid.*, **72**, 4506 (1950).

Neurath, H., "The Investigation of Proteins by Diffusion Measurements," *Chem. Rev.*, **30**, 357 (1942).

Schachman, H. K., "Ultracentrifugation, Diffusion and Viscometry," *Methods in Enzymology*, **4**, 32 (1957).

Manuscript received September 22, 1975; revision received December 29, and accepted December 30, 1975.