Buffalo. We also express our gratitude to Dr. G. Patel for providing us with calf thymus tissue and to Dr. M. S. Wang for his assistance with the emission spectrograph. The generous financial aid and the use of the facilities of the Monsanto Company are gratefully acknowledged.

[CONTRIBUTION FROM THE BIOPHYSICS DIVISION, SAHA INSTITUTE OF NUCLEAR PHYSICS, CALCUTTA-9, INDIA]

Diffusion Studies of Bovine Plasma Albumin at 25° with the Help of Jamin Interference Optics

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Diffusion coefficients of bovine plasma albumin in acetate buffer (pH 4.55, ionic strength 0.2) were measured at 25° for different values of mean protein concentration $\bar{C}$ with the help of Jamin interference optics and using a microdiffusion cell. $D(\bar{C})$ was found to be linearly dependent on $\bar{C}$ with a small negative slope of 0.0837 × 10^{-7}, $\bar{C}$ being in g./100 ml. The extrapolated value of the diffusion coefficient was obtained as $D_{25}^{0,w}$ = 6.728 × 10^{-7} cm.^{2}/sec. The nature of the concentration dependence of the diffusion coefficient was in conformity with the theoretical deduction made by Creeth from a single diffusion experiment carried out at 25°. The probable theoretical basis of the present observations has been discussed.

The diffusion coefficient of bovine plasma albumin has been measured at a finite concentration by a number of workers[1-3] using either Gouy or Rayleigh interference optics. Unfortunately, experimental data on the nature of the concentration dependence of bovine plasma albumin have been lacking. Wagner and Scheraga[4] happen to be the only authors who carried out such studies at 1° and obtained a linear dependence with a small positive slope. On the other hand, Creeth[5] developed a theory with the help of which the nature of the concentration dependence could be deduced from a single diffusion experiment. From such an experiment at 25°, Creeth[6] observed that the straight line describing the concentration dependence of the diffusion coefficient of bovine plasma albumin (BPA) should have a negative slope, *i.e.*, just opposite in the sense to what was obtained by Wagner and Scheraga at 1°

The need for having experimental data on this aspect in the temperature region where many of the physicochemical studies on BPA are usually carried out is thus naturally felt. Accordingly, in the present investigation the diffusion coefficients of bovine plasma albumin have been measured at 25° over a range of concentrations.[7] The measurements have been carried out with the help of the Jamin interference optics and using a microdiffusion cell.

Experimental

Materials and Methods.—Crystalline bovine plasma albumin, fraction V, was obtained from L. Light & Co., Colnbrook, England. The sample was dissolved in acetate buffer (0.18 *M* NaCl + 0.02 *M* sodium acetate + 0.02 *M* acetic acid) of pH 4.55 and ionic strength 0.2. The solution was dialyzed against the said buffer for a total period of about 28 hr. In the course of dialysis, the solution outside the bag was constantly stirred with the help of a magnetic stirrer, changed twice (after 4 and 16 hr.) and then allowed to equilibrate for about 12 hr. The outer solution from the final equilibration was used, whenever necessary, for the dilution of the dialyzed protein solution. In all cases, the concentration of the final protein solution was measured by noting the absorbancy at 278 mμ ($E_{1\,cm.}^{1\%}$ = 6.67)[8] with the help of a Zeiss PMQ II spectrophotometer. In all the experiments, the difference in concentration between the diffusing and the diffusate protein solution was kept small and of the order of 2 × 10^{-3} g./ml.

Diffusion experiments were carried out at 25 ± 0.01° with the help of Jamin interference optics and a microdiffusion cell provided by the Antweiler microelectrophoresis diffusion equipment.[9] The instrument was standardized with the help of Merck reagent grade sucrose and the details of the theory and methods used in the present technique have already been described.[10] In short, for an average protein concentration $\bar{C}$, a number of symmetrical curves were obtained, describing the solute concentration distribution within the cell at different times after the layering had taken place. From each of such curves, an average value D', corresponding to the particular time t, was obtained. The zero time correction was performed as usual. This gave the value of the diffusion coefficient $D(\bar{C})$ corresponding to the mean protein concentration $\bar{C}$. The heterogeneity of the sample was checked from a number of ultracentrifugal runs carried out with the help of Spinco Model E ultracentrifuge.

Results and Discussion

Ultracentrifugal analysis of the sample revealed the presence of 4–6% of material of higher molecular weight. Fig. 1 gives an illustration of the nature of the solute distribution in the cell occurring in the course of the diffusion process. As stated beforehand, each of the curves gave an average value, D', corresponding to the particular time t at which the curve was obtained. In these averaging procedures the maximum standard deviation in any value of D' was found as ±0.5%. The zero time correction plot corresponding to the curves of Fig. 1 is shown in Fig. 2. The values of the diffusion coefficients, $D(\bar{C})$, thus obtained corresponding to different values of the average solute concentration

(1) R. L. Baldwin, L. J. Gosting, J. W. Williams, and R. A. Alberty, *Discussions Faraday Soc.*, **20**, 13 (1955).

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

(3) H. Hoch, *Arch. Biochem. Biophys.*, **53**, 387 (1954).

(4) M. L. Wagner and H. A. Scheraga, *J. Phys. Chem.*, **60**, 1066 (1956).

(5) J. M. Creeth, *J. Am. Chem. Soc.*, **77**, 6428 (1955).

(6) J. M. Creeth, *J. Phys. Chem.*, **62**, 66 (1958).

(7) In a previous publication [*Nature*, **194**, 1053 (1962)], preliminary data on the measurement of the diffusion coefficient of BPA at different protein concentrations were reported by the author. Unfortunately, at that time no serious attention could be given to some important experimental steps, *e.g.*, (i) the sample was not dialyzed, (ii) the diffusion runs were carried out for insufficient periods of time (maximum 5 hr.), etc. The data reported were thus very inaccurate and hence should no longer be relied upon, although the concentration dependence found in the present article has the same sign as that reported previously.

(8) M. D. Sterman and J. F. Foster, *J. Am. Chem. Soc.*, **78**, 50 (1956).

(9) H. J. Antweiler, *Chem. Ing. Tech.*, 284 (1952).

(10) A. Chatterjee, *J. Am. Chem. Soc.*, **86**, 793 (1964).

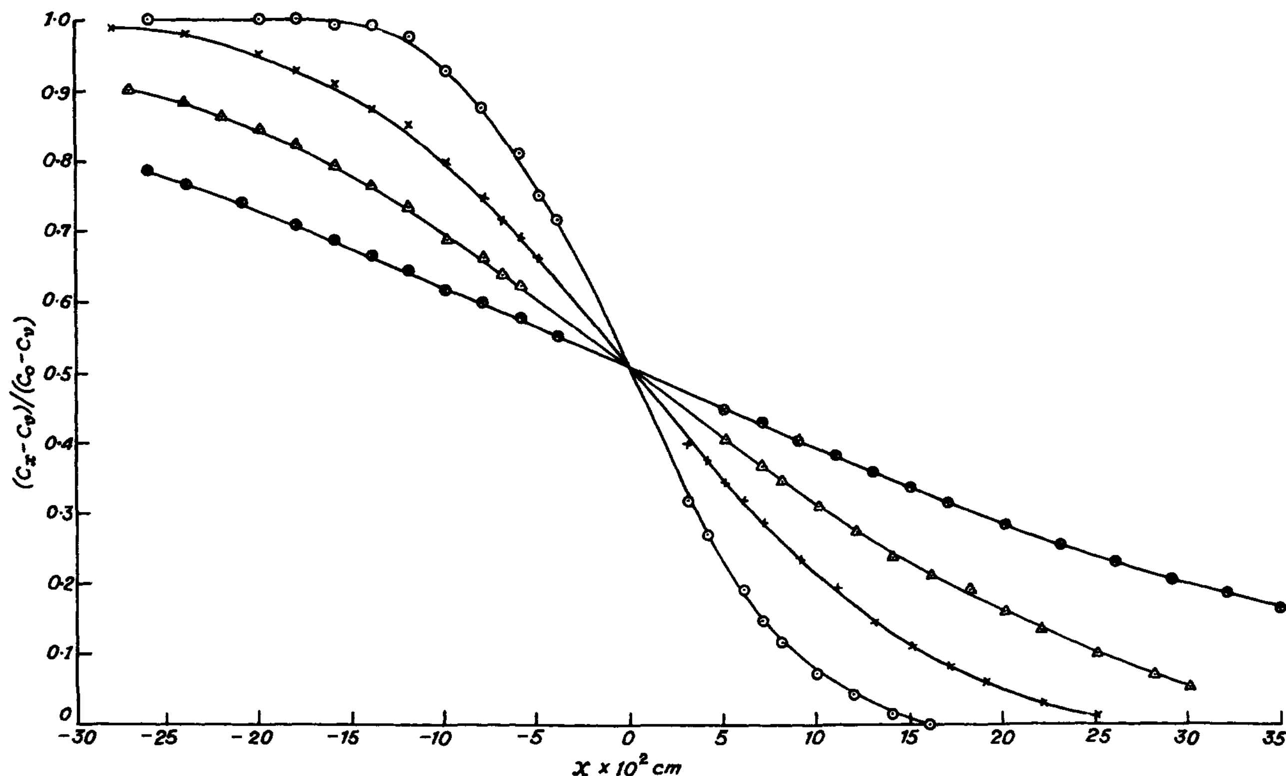

Fig. 1.—Solute concentration distribution in the cell in the course of diffusion with $\bar{C} = 3.3 \times 10^{-3}$ g./ml. and $\Delta C = 3.50 \times 10^{-3}$ g./ml.: ⊙, 0.3456×10^4 sec.; X, 1.1130×10^4 sec.; ◬, 2.9350×10^4 sec.; and ⊗, 8.5500×10^4 sec.

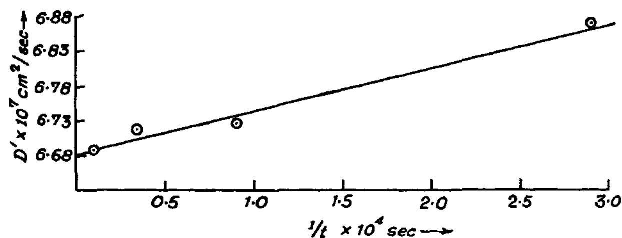

Fig. 2.—Zero time correction plot for the curves shown in Fig. 1.

$\bar{C}$ are given in Table I. In Table I, column 1 gives the values of ΔC, the concentration difference between the diffusing and the diffusate protein solutions, column 2 gives the values of C_1 the concentration of the diffusing solution, column 4 gives the values of Δt, the reciprocal of the intercept made by the straight line in the zero time correction plot on the abscissa, and the last column gives the values of the average per cent deviations of the individual points in any zero time correction plot. It can be seen from Fig. 3 that $D(\bar{C})$ is linearly related with $\bar{C}$ and the resulting straight line, drawn by the least-square method, has a small negative slope given by $\delta D/\delta C = -0.0837 \times 10^{-7}$, C being in g./100 ml. Some of the experimental runs were carried out for slightly different values of ΔC; the corresponding experimental points, as can be seen from Fig. 3, were found to scatter around about the resultant line within the limits of the experimental error. The value of $D(\bar{C})$ extrapolated to $\bar{C} = 0$ has been obtained as $D_{25}{}^0 = 6.695 \times 10^{-7}$ cm.²/sec. After conversion to water at 25°, the value of the diffusion coefficient has been obtained as $D_{25}{}^{0,w} = 6.728 \times 10^{-7}$ cm.²/sec. This is in good agreement with the value $D_{25}{}^{0,w} = 6.751 \times 10^{-7}$

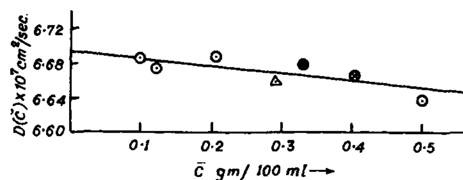

Fig. 3.—Concentration dependence of the diffusion coefficient of bovine plasma albumin at 25° in acetate buffer of pH 4.55 and ionic strength 0.2: ⊙, $\Delta C \simeq 2 \times 10^{-3}$ g./ml.; ◬, $\Delta C = 1.7 \times 10^{-3}$ g./ml.; ⊗, $\Delta C = 2.5 \times 10^{-3}$ g./ml.; ●, $\Delta C = 3.5 \times 10^{-3}$ g./ml.

cm.²/sec. calculated[11] from Creeth's experimental data at a finite concentration $\bar{C} = 0.518$ g./100 ml. following the theory developed by him. It is to be admitted, however, that a critical comparison with the value obtained from Creeth's experimental data is not justified, as apart from other factors, the samples were of different commercial sources. In this respect, further, reference to the work of Wagner and Scheraga carried out at 1° is thought quite relevant. When compared with the present work, it is striking to note that the nature of the concentration dependence of D at 1° is of opposite sense to what is presently obtained at 25°. Although it has to be admitted again that the material used in the former work was of different commercial source and the pH of the protein solution was not also the same as in the present work, this simple comparison is thought significant at least in pointing out that the temperature may have an important role in deciding the nature of the concentration dependence of the diffusion coefficient of BPA.

(11) L. J. Gosting, *Advan. Protein Chem.*, **11**, 520 (1956)

TABLE I

DIFFUSION DATA OF BOVINE PLASMA ALBUMIN AT 25°

ΔC, g./100 ml.	C_1, g./100 ml.	$\bar{C}$, g./100 ml.	Δt, sec.	$D(\bar{C})$, 10^{-7} cm.^{2}/sec.	Average deviation, %
0.198	0.198	0.099	2	6.686	0.096
0.204	0.222	0.102	130	6.675	0.16
0.200	0.305	0.205	2	6.688	0.08
0.170	0.375	0.290	55	6.660	0.064
0.350	0.505	0.330	90	6.680	0.13
0.250	0.530	0.405	64	6.669	0.13
0.202	0.602	0.501	42	6.638	0.08

A theoretical support to the possibility of such a reversal of slope with the change in temperature may probably be obtained from the work of Mandelkern and Flory.[12] According to these authors the concention dependence of the diffusion coefficient can be expressed by the equation

$$D/D_0 = [1 + 2\Gamma\bar{C} + (15/8)\Gamma^2\bar{C}^2]/(1 + k\bar{C}) \ldots \quad (1)$$

where both Γ and k are dependent, apart from other factors, on temperature. For a sufficiently dilute protein solution the slope of the D *vs.* $\bar{C}$ curve can be assumed equal to $(2\Gamma - k)$. Thus with the change in temperature, there arises a possibility that the expression $(2\Gamma - k)$ can change from a positive to a negative value or *vice versa*. In fact, an approximate calculation of the expression $(2\Gamma - k)$ at 25° and at a pH near about 4.6 has been made from the experimental data of different authors (Γ from the osmotic pressure data of Scatchard, *et al.*,[13] and k deduced from the S_0 value of Harrington, *et al.*,[14] after conversion to 25° and the value of the slope of the S *vs.* $\bar{C}$ curve taken as 0.2×10^{-13}, $\bar{C}$ being in g./100 ml.) and a negative value (−1.0) was obtained. No quantitative agreement with the present experimental value of the slope can, however, be expected as the calculations were not based upon the experimental data obtained under exactly the same conditions.

From the diffusion experiments performed thus far with the help of the Jamin interference optics and a microdiffusion cell it can be concluded that the straight line describing the concentration dependence of the diffusion coefficient of BPA at 25° has a small negative slope. This is, at least, in qualitative agreement with the theoretical deduction of Creeth referred to above.

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(12) L. Mandelkern and P. J. Flory, *J. Chem. Phys.*, **19**, 984 (1951).

(13) G. Scatchard, A. C. Batchelder, and A. Brown, *J. Am. Chem. Soc.*, **68**, 2320 (1946).

(14) W. H. Harrington, P. Johnson, and R. H. Ottewill, *Biochem. J.*, **62**, 569 (1956).

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Reactions of Coordinated Ligands. VIII. The Reactions of Alkyl Halides with Mercapto Groups in Transition Metal Complexes of Mercaptoamines

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The reactions of mercaptoamine complexes of nickel(II) and palladium(II) with typical alkylating agents have been investigated. The complexes utilized contain the ligands: 2-aminoethanethiol, N-methyl-2,2'-dimercaptoethyldiethylamine, 2-(2-mercaptoethyl)pyridine, and α-diketobismercaptoethylimines. Pure products have been isolated in all cases and characterized insofar as possible, by determination and interpretation of elemental analyses, infrared spectra, molar conductances, magnetic moments, molecular weights, and electronic spectra. Reaction occurs at the coordinated mercaptide group, producing a thioether *in situ*. The relatively lesser coordinating ability of the newly formed thioether group is evident in the nature of the products. The planar nickel(II) reactant $[Ni(NH_2CH_2CH_2S)_2]$ yields the octahedral products $[Ni(NH_2CH_2CH_2SR)_2X_2]$; α-diketobis(mercaptoimine)nickel(II) complexes give similar results. Under the conditions employed bis[2-(2-mercaptoethyl)pyridine]nickel(II) yields no isolable thioether products. The altered ligand is displaced by the coordinated mercaptide groups of unreacted starting material yielding bridged complexes. Thus $C_6H_5CH_2Br$ produces $[Ni_2(C_5H_4NCH_2CH_2S)_2Br_2]$ and $(CH_3)_2SO_4$ yields $[Ni\{Ni(C_5H_4NCH_2CH_2S)_2\}_2](CH_3SO_4)_2$; bis(N-methyl-2-2'-dimercaptodiethylamine)dinickel(II) reacts with alkyl halides only at the terminal sulfur atoms, not at the bridged sulfurs.

Introduction

In view of the ability of coordinated metal ions to influence the course of many complex reactions, particularly those which occur in living organisms,[1] an investigation of the reactivity toward nucleophiles of the coordinated sulfur atom might be expected to yield results of broad significance. Further, extensive investigation into the specific influence of metal ions on the reactions of organic species should reveal much new fundamental chemistry.[2,3] Among the principal limitations on the understanding of the functions of metal ions in influencing the course of complicated organic and biochemical reactions is the fact that the systems of interest are often labile. Although unusual effects may be observed. the actual nature of the sub-

(1) G. L. Eichhorn, Advances in Chemistry Series, No. 37, American Chemical Society, Washington, D. C., 1963, p. 37.

(2) D. H. Busch, ref. 1, p. 1.

(3) H. L. Bender, ref. 1, p. 19.