Diffusion of Urea and Potassium Chloride in Albumin Solution

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The diffusivities of urea and KCl in aqueous albumin solutions were measured using a modified Teflon diffusion cell with millipore filters as diaphragms for biological solutions and were found to decrease markedly with increasing albumin concentration. The decreases are due primarily to binding of the solutes to the protein and partially to physical blockage of diffusion by the large protein molecules. Other data on similar systems also substantiate these trends. When the pH of the solution is decreased from 7.60 to 7.00, the diffusivity decreases markedly by 16% indicating a significant increase in binding and a decrease in unbound solute. Using data from the literature for nonbinding solutes, a modified empirical physical-blockage factor to correct the diffusivity was obtained. When combining this blockage effect with the binding effect given by others, diffusion data from this work and others agree reasonably well within 1–7% with predictions using this overall relationship.

The Wilke–Chang equation (*2, 5*) and other semiempirical equations are available to predict diffusion coefficients of ordinary dilute solutes such as urea and acetic acid in aqueous solution. The presence of other nonreacting dilute solutes has very little effect on the diffusivity of the solute. Fick's law can then be used to predict the diffusion flux.

In diffusion in biological solutions, however, interactions often occur between large protein molecules usually present in solution and ordinary solutes. Use of ordinary Fickian type equations in these solutions can be subject to errors since the small solute or ligand may bind to the surface of a large protein molecule. As a result, less solute is free to diffuse and the rate of diffusion can be substantially less than for nonbinding solutes. Few data on the combined effects of binding and of the physical blockage by large macromolecules on the rates of diffusion of solutes are available.

Albumin which is present in many biological fluids binds a wide variety of organic and inorganic ligands. For example, the solubility of fatty acids is increased markedly because of binding in albumin solutes compared to salt solutions.

The equations for diffusion in inorganic or organic solutions do not predict diffusion accurately in nonbinding polymer systems. The number of molecular species and the size of the macromolecules contribute to the complexity of such systems. The theories for predicting diffusion in polymer solutions do not consider the effects of binding, which can be considerable, on the diffusion flux.

In the present work, a diaphragm cell developed for measuring diffusion in protein solutions was used to determine the diffusion of urea in bovine serum albumin (BSA) solution and of KCl in the solution at different concentrations and pH. The data were compared with results obtained using a modified diffusivity equation when binding and blockage are present in such protein solutions.

Literature Review and Theory

Diaphragm Diffusion Cell. An important and accurate apparatus for measuring diffusion coefficients of solutes in liquid solutions is the diaphragm cell. In this cell molecular diffusion occurs in the small pores of a porous glass diaphragm separating two stirred compartments containing different concentrations of the diffusing solute. A review of the theory and cell construction is given elsewhere (*2, 5, 8*). The final equation used to obtain the molecular diffusivity D_{AB} of dilute solute A in water B is as follows

$$D_{AB} = (1/\beta t) \ln [(c_0 - c_0')/(c - c')] \quad (1)$$

where c is the concentration of A in the lower compartment at time t, c' the concentration in the uppper compartment at t, and c_0 the concentration at $t = 0$. The cell constant β depends upon the geometry of the diaphragm and the volumes of the compartments and is obtained by calibrating the cell with the solute KCl of known diffusivity of 1.87×10^{-9} m^2/s at 25 °C (*2*).

Binding of Solutes to Proteins. A macromolecule such as a protein may have a number of sites for interaction or reversible binding with small solute molecules (ligands) in solution. For an idealized case of reversible binding where the protein macromolecule P contains n independent and identical sites capable of binding the ligand A, the following result can be derived at equilibrium (*19*).

$$\bar{V} = nk[A]/(1 + k[A]) \quad (2)$$

Often there is interaction between the sites and also the sites are not identical. Then the equations become complex. Equilibrium measurements of binding can be done by equilibrium dialysis, ultrafiltration, gel filtration, etc. Experimental data are difficult to obtain and often data from different investigators do not check. The data are often represented as a protein binding coefficient k_P which can be related to the protein concentration c_P and a partition coefficient k' by

$$k_P = c_P k' \quad (3)$$

Human serum albumin has been shown to bind a large variety of solutes (*6*). Bovine serum albumin (BSA) has been demonstrated to be reasonably similar to human serum albumin in its behavior in solutions of ligands. Boyer et al. (*3*) found that up to 30–40 mol of sodium caprylate could bind per mole of BSA in solution. Hence, the diffusion rates of solutes diffusing in solutions of albumin could be greatly affected by binding to macromolecules which diffuse much more slowly.

Experimental Data for Diffusion in Protein Solutions. Experimental data for the diffusivity of solutes in protein solutions are often reported as a diffusivity ratio, D_{AP}/D_{AB}, ratio of the diffusion coefficient of solute A in protein solution to the diffusion coefficient of A in the solution with no protein present. Goldstick and Fatt (*7*) measured the diffusivity of oxygen in isotonic BSA solution at 25 °C. At a protein concentration of 4 g/100 mL, $D_{AP}/D_{AB} = 0.91$ and at 11 g/100 mL, $D_{AP}/D_{AB} = 0.80$. Oxygen is not considered to bind to BSA. A value of $D_{AP}/D_{AB} = 0.616$ for one concentration for uric acid diffusing in human plasma solution containing about 4 g of albumin/100 mL and a total of 8.2 g of protein/100 mL was obtained by others (*4*). Uric acid has been shown to bind markedly to albumin and the other proteins in plasma (*1*) with about 33% of the total uric acid bound to the proteins giving a $k_P = 0.50$. The diffusivity ratio and binding coefficient have also been obtained (*4, 15*) for one concentration for urea.

A few other experimental data have been obtained for diffusion of oxygen and several other nonbinding solutes in albumin and plasma. Systematic studies of the effects of concentration

0021-9568/78/1723-0040$01.00/0

of the albumin and of pH on the diffusivity ratio of solutes which bind significantly do not appear to have been done.

Theories for Diffusion of Binding Solutes in Protein Solutions. The theories and correlations for predicting diffusion of dilute solute molecules in solutions generally do not apply directly to diffusion in polymer and protein solutions. Large macromolecules interfere with diffusion by blockage and interactions between solvent and polymer. Navari et al. (*14*) have developed a theory for diffusion of solutes in polymer and protein solutions. Their equation for the diffusivity ratio is

$$\frac{D_{AP}}{D_{AB}} = \left(\frac{V_P}{V_B}\right)^{1/3}\left(\frac{M_P}{M_B}\right)^{1/2} \exp\left(\frac{\Delta E}{RT}\right) \qquad (4)$$

where ΔE is an activation energy difference which is a function of the concentration and type of the polymer in solution and is independent of the solute. It is a measure of the strength of the polymer–solvent bonds. The description implies that the solute diffusing in the polymer solution moves through the solvent only and not along the polymer. The diffusivity is completely independent of the diffusing solute. This theory was tested by Navari et al. and they predicted diffusion of various solutes reasonably well in organic polymer solutions and of O_2 and CO_2 in albumin and plasma solutions. The theory does not apply to solutes that bind to proteins.

The usefulness of this equation, however, is somewhat limited in that it requires the prediction of the activation energy difference. Experimental viscosity measurements and determination of the intrinsic viscosity are needed for this. These are often not available. Hence, a simple expression for only protein polymers in solution which does not require any experimental measurements is desirable.

A more useful and specific equation for proteins was given by Wang (*20*) who derived an expression for the self-diffusion of water in protein solutions which includes an obstruction or blockage factor of the protein molecules and also the concentrations of water bound to the protein and unbound water. This equation was verified experimentally (*20, 21*) for protein solutions. It was found experimentally that 0.2 g of water was bound to 1.0 g of protein.

Colton et al. (*4*) developed an equation for diffusion of solutes in plasma solutions where the plasma was considered as a suspension of impermeable proteins in solution. The model accounts for the obstruction of the large protein molecules as given by Wang (*20*) and for reversible binding of solute by the proteins. The rate of reversible binding is assumed to be instantaneous. The solute binding isotherm is sometimes nonlinear but it is assumed to be linear which has been shown experimentally to be usually true in dilute solutions and also follows from eq 2 and 3.

The total diffusion flux J_A of the solute is assumed to be the sum of diffusion as unbound or free solute and of the solute–protein complex.

$$J_A = \left[-D_{AB}\frac{\psi_P}{1-\phi_P}\frac{dc_{Af}}{dx}\right] + \left[-D_P\frac{dc_{Ab}}{dx}\right] \qquad (5)$$

where D_{AB} is the diffusivity of unbound solute A in protein-free solution, the term $\psi_P/(1-\phi_P)$ is the Wang blockage term caused by the proteins, and D_P is the diffusivity of the protein–solute complex in the protein solution (assumed the same as the protein alone). Assuming the binding coefficient k_P is constant for dilute solutions and the total solution concentration c_A is the sum of bound plus unbound solute, the final equation is

$$J_A = -\left[\frac{D_{AB}\psi_P + D_P k_P}{(1-\phi_P) + k_P}\right]\frac{dc_A}{dx} \qquad (6)$$

where the term in the brackets is D_{AP}, the diffusivity of A in the protein solution.

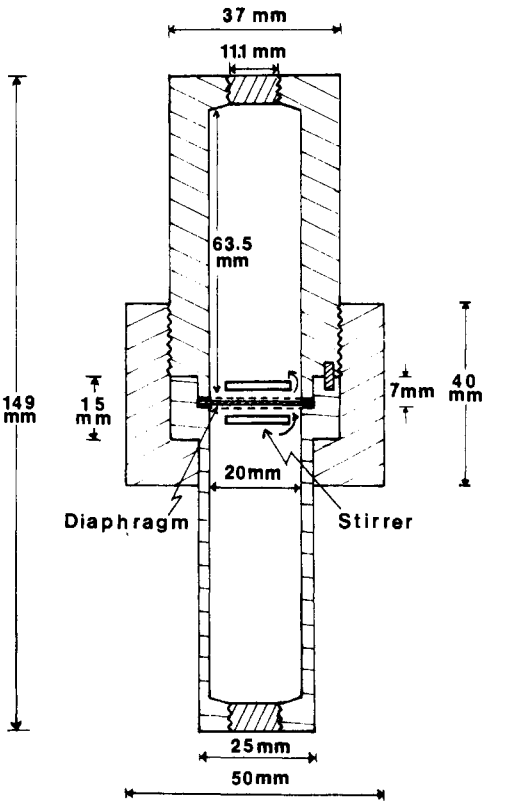

Figure 1. Teflon diaphragm cell for diffusion studies.

$$D_{AP} = \left[\frac{D_{AB}\psi_P + D_P k_P}{(1-\phi_P) + k_P}\right] = \left[\frac{D_{AB}\psi_P/(1-\phi_P) + D_P k_P/(1-\phi_P)}{1 + k_P/(1-\phi_P)}\right] \qquad (7)$$

It should be noted that in this derivation dilute solutions of the solutes are assumed which allows the flow of the other components to be neglected.

The Wang factors in the blockage term are given by the equations

$$\phi_P = c_P(\bar{v}_P + H_P/d_0) \qquad (8)$$

$$\psi_P = (1 - \alpha_P\phi_P) \qquad (9)$$

where H_P is protein water of hydration and is 0.2 g of bound water/g of protein (*20*), α_P varies from 1.5 for spheres to 1.67 for prolate ellipsoids with infinite axial ratios (α_P = 1.615 for albumin), $\bar{v}_P$ = 0.733 mL/g for the partial specific volume of albumin, and d_0 = 1.0 for the density of water.

Experimental Methods

The diaphragm cell used is a modification of that used by Perkins and Geankoplis (*16*) and of Keller and Friedlander (*12*) but constructed of Teflon as shown in Figure 1. The diaphragm was a cellulose acetate Millipore filter with a pore size of 1.2 μm and was held in the apparatus by Teflon gaskets. A new filter was used for each run. It is assumed that the cell constant is essentially independent of the concentration of protein since the ratio of pore diameter to the diameter of the protein molecule (*11, 12*) is of the order of several hundred. Hence, wall effects, if present, should be small compared to the large effects of blockage and binding.

Two Teflon screens with large holes for liquid circulation past the surface of the diaphragm were placed immediately above and below the diaphragm to protect it from the two polyethylene stirring bars. The bars were rotated at 133 rpm by permanent

Table I. Experimental Diffusivity Data at 25 °C

g of BSA/ 100 mL	pH	D_{AB}, m^2/s	D_{AP}, m^2/s	D_{AP}/D_{AB}	k_P (calcd, eq 7)	k_P (calcd, eq 10)	D_{AP}/D_{AB} (predicted, eq 10)
			Diffusing Solute Urea (0.25 g/L)[a]				
0	7.35	1.374×10^{-9}					
1.49	7.35		1.146×10^{-9}	0.834	0.198	0.174	0.888
2.97	7.35		1.146×10^{-9}	0.834	0.185	0.139	0.862
1.49	7.60		1.237×10^{-9}	0.900	0.105	0.084	
1.49	7.00		1.035×10^{-9}	0.753	0.334	0.308	
3.72	7.35		1.089×10^{-9}	0.793	0.244	0.185	0.851
			Diffusing Solute KCl (0.097 M)[a]				
0	7.35	1.87×10^{-9}					
1.49	7.35		1.461×10^{-9}	0.781	0.278	0.254	
2.96	7.35		1.181×10^{-9}	0.632	0.574	0.515	
1.49	7.60		1.471×10^{-9}	0.787	0.270	0.245	
4.42	7.35		1.174×10^{-9}	0.628	0.562	0.473	
1.49	7.00		1.320×10^{-9}	0.706	0.422	0.394	

[a] Initial concentrations in the bottom compartment of the diffusion cell.

magnets rotating outside the cell. The cell and external rotating magnets were in a bath held at 25.0 °C. Teflon and cellulose acetate were used since proteins tend to bind to glass.

Calibration of the cell to determine the cell constant β was done as discussed elsewhere (*16*). A concentration of 0.10 M KCl with a diffusivity $D_{AB} = 1.87 \times 10^{-9}$ m^2/s at 25 °C was used in the lower chamber and water in the upper. For three different calibrations using a new diaphragm each time the maximum deviation between the cell constants was 2.8%. Hence, further calibrations with KCl were not needed for each new filter. Three diffusion runs were also made using urea having a concentration of 0.25 g of urea/L in a buffer solution having a pH of 7.35. The average value obtained for the diffusivity D_{AB} was 1.374×10^{-9} m^2/s.

Diffusion runs were made using 0.097 M KCl in various concentrations of albumin and at different pH levels. In all diffusion runs a small amount of Tris buffer was used to control the pH at the desired level during the run. The KCl concentration was determined by titrating with silver nitrate with appropriate blanks being used because of the presence of albumin. Diffusion runs were also made using 0.25 g of urea/L in various concentrations of albumin at a constant pH of 7.35 and also at varying pH values. The concentration of urea in solution was determined by using the enzyme urease with the procedure of Neilands (*13*). The urea analysis was found to not be affected by the presence of protein. All diffusion runs were made with the solutes KCl or urea diffusing from the lower to the upper chamber of the cell in Figure 1. The pH and buffer concentrations were kept equal in both chambers as were the albumin concentrations.

To calculate the experimental value of D_{AP} for KCl or urea in albumin solutions, the experimentally determined concentrations in the cell compartments, time t, and the known cell constant β were substituted into eq 1 and the diffusivity D_{AP} (D_{AB} when no albumin was present) was obtained. These experimental values of D_{AP} and the value of D_{AB} for KCl and urea were substituted into eq 7 to obtain an approximate protein binding coefficient k_P from diffusion data. A value of D_P of 7.0×10^{-11} m^2/s at 25 °C was used for BSA in water (*18*). Experimental and calculated data are given in Table I.

Experimental Results and Discussion

Diffusivity of Urea and Cell Accuracy. In order to determine the accuracy of determination of the diffusivities in this modified cell, the diffusivity of urea was obtained in aqueous solution. This value of 1.374×10^{-9} m^2/s given in Table I compares closely to the value of 1.382×10^{-9} m^2/s at 25 °C obtained by Gosting and Akeley (*9*) in dilute concentration using the Gouy interference method. Also, different cell calibrations using a new diaphragm each time gave results close to each other with a maximum deviation of 2.8%. This indicates that using Millipore cellulose acetate diaphragms and also Teflon screens to protect them from scraping by the stirrers gives reasonably accurate results.

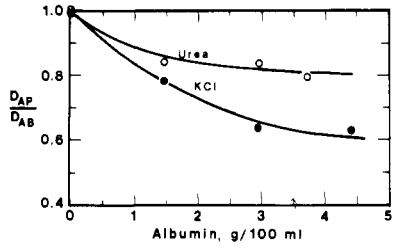

Figure 2. Diffusivity ratio vs. albumin concentration. Original concentration = 0.097 M KCl or 0.25 g of urea/L and pH 7.35.

Diffusion of Urea in Albumin Solutions. Diffusivity ratio D_{AP}/D_{AB} data from Table I are plotted in Figure 2 vs. the BSA concentration in g/100 mL. All of these data are at a constant pH of 7.35. The data for KCl are for a constant concentration of 0.097 M KCl and for urea for a constant concentration of 0.25 g of urea/L. The curve for urea shows that the diffusivity ratio of urea in albumin solution decreases from a value of 1.0 at zero albumin concentration to 0.793 at 3.72 g of albumin/100 mL of solution. This large decrease is much greater than the value predicted from eq 7 with no binding ($k_P = 0$).

These data indicate that an appreciable amount of binding is occurring. Colton et al. (*4*) in their studies with plasma and serum at 37 °C, which contain about 4 g of albumin/100 mL plus other proteins, also found a value for the diffusivity ratio of 0.807 which is comparable to this work as shown in Figure 3 for urea. Experimental binding data for urea (*15*) in plasma give a value for the binding coefficient k_P of about 0.1. Using eq 7 at 37 °C with this binding coefficient and the total protein concentration shown in Figure 3, a diffusivity ratio of 0.87 is predicted which is 8% greater than the experimental value of Colton et al. It should be noted that experimental binding data are difficult to obtain accurately.

Alternatively, eq 7 was used to calculate approximate binding coefficients for urea using experimental diffusivity ratio data in Table I. These calculated values of k_P given in Table I for a pH 7.35 range from 0.185 to 0.244 and are appreciably higher than the experimental binding of 0.1 or less. This could mean that the experimental value is low or that eq 7 does not predict D_{AP} correctly. The blockage term $\psi_P/(1 - \phi_P)$ in eq 5 and 7 could be underestimating the true effect.

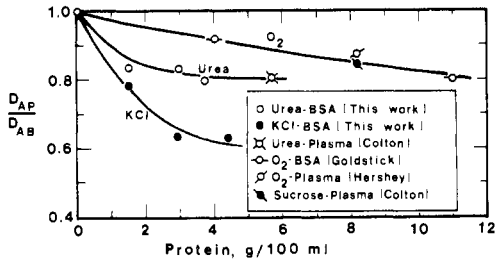

Figure 3. Diffusivity ratio vs. protein concentration. Comparison of data from different investigators.

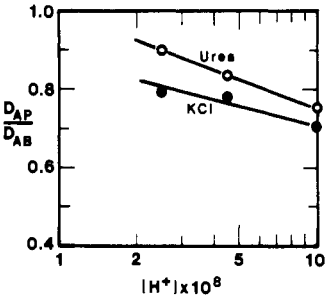

Figure 4. Diffusivity ratio vs. hydrogen ion concentration. Original concentration = 0.097 M KCl or 0.25 g of urea/L and 1.49 g of albumin/100 mL.

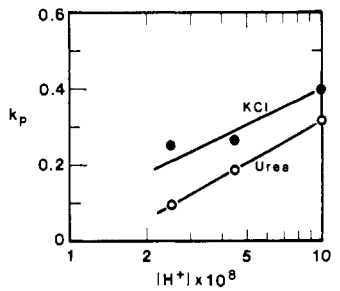

Figure 5. Approximate protein binding coefficient from diffusion data vs. hydrogen ion concentration. Original concentration = 0.097 M KCl or 0.25 g of urea/L and 1.49 g of albumin/100 mL.

Modified Equation for Predicting Diffusion. In Figure 3 data are plotted for the diffusivity ratio of O_2 in primarily BSA, a system which is considered to have no binding. This could be considered as the line for urea for no binding. For the run with a concentration of 3.72 g of BSA/100 mL in Table I a diffusivity ratio of 0.92 is obtained from Figure 3 for the O_2 curve. This value of 0.92 for the blockage term was substituted into eq 7 and a value of $k_P = 0.165$ obtained. This is considerably lower than the value of 0.244 calculated using the blockage term in eq 7 and is closer to the experimental binding value. Similar results were obtained for the other data in Table I for urea.

Further results indicating that the blockage term in eq 7 is low are shown using data correlated for nonbinding solutes in protein solutions given by Goldstick and Fatt (*7*), Hershey and Karhan (*10*), and Navari et al. (*14*). The blockage term reduction effect predicted in eq 7 appears to be underestimated. Using the oxygen diffusion data in BSA of Goldstick and Fatt (*7*) and Navari et al. (*14*), an empirical blockage factor to be used in eq 7 in place of the term $[\psi_P/(1 - \phi_P)]$ in BSA and protein solutions is obtained as $(1 - 1.2\alpha\phi_P)$. The modified equation to predict D_{AP} becomes

$$D_{AP} = \frac{D_{AB}(1 - 1.2\alpha\phi_P) + D_P k_P/(1 - \phi_P)}{1 + k_P/(1 - \phi_P)} \qquad (10)$$

Using eq 10 experimental values of D_{AP} were used to again calculate approximate binding coefficients and these values are tabulated in Table I. These three values for pH 7.35 give an average k_P value of 0.16 which is close to the experimental of 0.1. Also, the k_P values do not appear to vary with concentration of albumin implying a linear isotherm.

Alternatively, using the experimental binding k_P value of 0.1 and eq 10 at a pH of 7.35, the diffusivity ratio for urea in albumin was predicted for the three concentrations used in this work (Table I). The three predicted values check the experimental within 6, 3, and 7% or an average of 5%.

Again predicting the diffusivity ratio for urea in plasma but using eq 10 instead of eq 7 and the experimental binding coefficient, a value of 0.816 is obtained. This predicted value checks the experimental of 0.807 within 1% as compared to 8% for eq 7.

The effect of pH on the diffusivity ratio was also investigated for urea and the results from Table I are plotted vs. hydrogen ion concentration in Figure 4. The plot shows a substantial reduction in the diffusivity ratio being 0.90 at a pH of 7.60 and decreasing to 0.753 at a pH of 7.00. The approximate binding coefficients calculated from the modified eq 10 are plotted in Figure 5 and show a substantial increase as pH is decreased. This is consistent with data for binding BSA with sodium caprylate by Boyer et al. (*3*) and binding human serum albumin with chloride ions by Skatchard and Yap (*17*). The data by Boyer et al. given large values of k_P for binding from about 0.1 to 0.3.

Diffusion of KCl in Albumin Solutions. The data from Table I for the diffusivity ratio D_{AP}/D_{AB} for KCl in BSA are plotted in Figure 2 vs. the concentration of BSA at a constant pH of 7.35. The curve shows a substantial decrease in diffusivity ratio with increasing BSA concentration. Since the theory of Navari et al. (*14*) and experimental data show that the diffusivity ratio for nonbinding solutes is independent of the diffusing solute, this decrease, which is considerably greater than for urea and for nonbinding solutes, indicates a large amount of binding of KCl.

Using the modified eq 10 and experimental values of D_{AP}, approximate binding coefficients were calculated and tabulated in Table I. At a pH of 7.35 the values range from 0.254 to 0.515 which are substantially higher than for urea.

Experimental binding data for KCl in BSA are not available but data for NaCl binding on human serum albumin (*17*) at very low salt concentrations and mainly low pH indicate less binding than found in this work for KCl on BSA and also that binding increases as pH decreases. Differences may occur since data indicate that binding depends also on the treatment, preparation, and source of the protein. They (*17*) deionized freeze-dried human serum albumin by electrodialysis and subsequently freeze dried it again before use. In the present work freeze-dried BSA crystallized from solution was used.

Variations in binding may also be due to the fact they (*17*) adjusted pH by adding NaOH to their isoionic solutions which greatly increased the ionic strength. In this work Tris buffer (tris(hydroxymethyl)aminomethane) was used which did not affect the very low ionic strength of the albumin solutions. Also, in the derivation of eq 10 it was assumed that the rate of reversible binding was instantaneous which may not be the case in some systems (*1*). Some solutes may be irreversibly bound.

Diffusivity ratio data for KCl in Figure 4 show that as pH decreases the diffusivity ratio decreases which is consistent with the fact that when binding increases, the diffusivity ratio decreases as shown by eq 10. The binding coefficients for KCl calculated from eq 10 and the diffusion data are plotted in Figure 5 and follow the same trends as urea. Since BSA is not at its isoelectric point at the pH of this study, binding should be expected to depend upon pH.

High values for binding have been reported by others in protein

solutions. For uric acid in plasma an experimental value of D_{AP}/D_{AB} of 0.616 was obtained (*4*). The experimental binding coefficient $k_P = 0.50$ at 37 °C (*1*). Using eq 10, $D_{AB} = 1.21 \times 10^{-9}$ m²/s, $D_P = 0.091 \times 10^{-9}$ m²/s, 37 °C, and 8.2 g of total protein/100 mL, a value of $k_P = 0.40$ is calculated which is reasonably close to the experimental of 0.50. Instead, using the experimental k_P of 0.50 a predicted value of $D_{AP}/D_{AB} = 0.58$ is obtained which is close to the experimental of 0.616 with an error of 6%.

Considerably more experimental binding and diffusivity data in BSA and other protein solutions are needed to confirm the modified diffusion equation. For highly binding solutes, the majority of the decrease in diffusivity ratio is due to the binding and not the blockage factor as seen in Figure 3. Hence, errors in this blockage factor will not greatly affect the overall diffusivity ratio predicted from the modified diffusion equation presented. The main use of the modified diffusion equation is in predicting diffusion of binding solutes in solution. Using experimental binding data in the literature, this equation has been shown to predict the experimental diffusivity ratio for urea in albumin within about 5%, urea in plasma within 1%, and uric acid in plasma within 6%. Predictions of binding from experimental diffusion data are not very accurate since small errors in diffusion measurements are magnified in the calculation of the binding coefficient.

Glossary

- $[A]$ concentration of free A in solution, g-mol of A/L
- c concentration of A, g-mol/m³ solution
- c_A concentration of total solute A, g/m³ of solution
- c_{Ab} concentration of protein-bound solute A, g/m³ of solution
- c_{Af} concentration of free solute A, g/m³ of solution
- c_P concentration of protein, g/mL of solution
- d_0 density of water, g/mL
- D_{AB} diffusivity of A in solution with no protein present, m²/s
- D_{AP} diffusivity of A in protein solution, m²/s
- D_P diffusivity of protein in solution, m²/s
- H_P protein water hydration, g of bound water/g of protein
- J_A total flux of A, g/(m² s)
- k empirical constant in eq 2
- k' protein partition coefficient, [(g of bound solute)/(g of protein)]/[(g of free solute)/(mL of protein-free solution)]
- k_P protein binding coefficient, [(g of bound solute)/(mL of solution)]/[(g of free solute)/(mL of protein-free solution)]
- M_B molecular weight of liquid solvent
- M_P molecular weight of polymer solution
- n number of binding sites
- R gas law constant
- t time, s
- T temperature, K
- $\bar{v}_P$ partial specific volume of protein, mL/g
- V_B molar volume of liquid solvent
- $\underline{V}_P$ molar volume of polymer solution
- $\overline{V}$ molar binding ratio, (g-mol of A bound)/(g-mol of protein)
- x distance, m
- α_P diffusivity reduction shape factor for protein
- β cell constant, m⁻²
- ΔE activation energy difference
- ϕ_P volume fraction proteins in protein solution
- ψ_P ratio permeability in protein solution/permeability in protein-free solution

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Received for review March 9, 1977. Accepted August 17, 1977.