# Diffusion of Sucrose in Protein Solutions with Pore Restriction Effects Present

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**The effects of pore size on the diffusivity of sucrose in bovine serum albumin protein solutions were measured by using a diffusion cell with Millipore filter diaphragms. As expected, sucrose diffusivity decreased as pore size became smaller. This pore restriction effect compares closely with predictions using several equations from the literature. Sucrose diffusivity also decreased markedly as protein concentration increased because of physical blockage of diffusion by the large protein molecules. The presence of proteins did not interfere with the pore restriction effect in the range of protein concentrations studied. The smallest pores used, 0.05-$\mu$m diameter, were 45 times as large as sucrose and 8 times as large as the protein. The decrease of sucrose diffusivity with increasing protein concentration was larger than expected, and possible reasons for this are given.**

## Introduction

Molecular diffusion in biological solutions is frequently affected by interactions of the biological compounds present. The proteins which are present in many biological solutions can decrease the diffusion rate of smaller solutes. The large protein molecules can physically block the diffusion path (*1-6*). Proteins may also adsorb or bind some solute molecules, leaving fewer of these molecules free to diffuse. Serum albumin is an important protein molecule, partly because of its ability to bind a wide variety of organic and inorganic ligands. Bovine serum albumin (BSA) is frequently used for studies of diffusion in biological solutions because of its availability and close similarity with human serum albumin. Sucrose is another compound frequently occurring in biological fluids. It has been reported (*7*) that BSA apparently does not bind sucrose. This makes the BSA-sucrose pair a suitable choice for protein blockage experiments without the possible added complication of binding.

In some cases diffusion in biological solutions may be occurring in small pores of solids, such as in membrane separation processes, and the rate of diffusion is reduced by the small size of the pore. Several investigators (*1*, *8-12*) have proposed theories for the effect of this pore hindrance. Proteins may often be present in the solutions inside small pores. There does not seem to be any experimental data available where both pore restriction and protein blockage effects are present together and are affecting solute diffusion rates simultaneously. The purpose of this work was to obtain experimental data which are needed to determine whether these two effects are simply additive or whether there are some interactions between them. In the present work (*13*) a diaphragm cell was used to determine the diffusion of sucrose in BSA solutions within small pores. The resulting data were compared with existing theories for pore size effects and for protein blockage effects.

## Literature Review and Theory

***Diaphragm Diffusion Cell.*** Many investigators (*2*, *3*, *10*, *14*, *15*) have successfully used the diaphragm cell with Millipore-type filter diaphragms to measure diffusion rates. An important concern with the use of Millipore diaphragms is the possible existence of a boundary layer at the diaphragm surface. Some investigators used bulk stirring of the fluid in the cell to reduce the thickness of the boundary layers. Recently, it was found that direct contact of the stirring bars with the Millipore diaphragm (*10*, *12-14*, *16*) did not cause any damage and was the method which would best eliminate most of the boundary layer. The speed of these stirring bars is another important factor. Warren (*16*) studied the effect of stirring speed and found that, for a stirring speed range of 100-175 rpm, if the cell was calibrated and also used at the same stirring speed, then the measured diffusivities were the same.

The equation for the cell which is used to experimentally obtain the molecular diffusivity $D_{AB}$ of dilute solute A in solvent B is eq 1. The cell constant $\beta$ depends upon the geometry of

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

the diaphragm and the cell volumes. It is usually obtained (*2*) by calibrating the cell with the solute KCl of known diffusivity of $1.87 \times 10^{-9}$ m$^2$/s at 25 °C (*2*).

***Theories for Diffusion of Solutes in Protein Solutions.*** The diffusion rate of small solutes is decreased by the presence of very large molecules, such as proteins, in the solution. This effect, called protein blockage, has been studied by several early investigators, notably Prager (*5*), Stroeve (*6*), Colton et al. (*1*), and Jalan et al. (*4*). Geankoplis et al. (*2*, *3*) modified Colton's (*1*) equation as follows:

$$D_{AP} = [D_{AB}(1 - 1.2\alpha\phi_P) + D_{PB}k_P/(1 - \phi_P)]/[1 + k_P/(1 - \phi_P)] \quad (2)$$

This equation predicted diffusivity values within ca. ±5% of the experimental data for diffusion of solutes in protein solutions with binding. For nonbinding systems the predicted values and experimental values differed from each other by less than ±5%.

***Theories for Diffusion with Pore Hindrance Present.*** Beck and Schultz (*9*) used the Renkin equation to successfully correlate experimental data for diffusion of solutes inside small pores. The theory combines two theoretical phenomena, the exclusion effect and the wall drag effect. The exclusion effect is based on the solute molecule being excluded from the region near the pore wall. The wall drag effect can be compared to the increased drag on a sphere falling in a narrow cylinder of fluid. The Renkin equation as used by Beck and Schultz is

$$D_P/D_0 = (1 - \lambda)^2(1 - 2.104\lambda + 2.09\lambda^3 - 0.95\lambda^5) \quad (3)$$

Beck and Schultz also proposed a simplified equation which is

$$D_P/D_0 = (1 - \lambda)^4 \qquad 0 < \lambda < 0.2 \quad (4)$$

The results of the work of Anderson and Quinn (*8*) show fair agreement with eq 4. Satterfield (11) proposed a semiempirical equation which fitted his data slightly better than eq 4. The Satterfield equation is

$$\log (D_{PT}/D_0) = -2.0\lambda \quad (5)$$

Conlon and Craven (*10*) obtained a semiempirical equation which is similar in form to eq 5 but predicts a larger effect of pore restriction.

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## Experimental Methods

The diaphragm cell used was the same as the one used by Geankoplis et al. (*2*, *3*) except that the stirring bars rotate while in direct contact with the Millipore diaphragm. The stirring bars were rotated at 175 rpm for both calibrations and diffusivity measurements. The two types of diaphragms used were Millipore filters, made from mixed esters of cellulose or from poly(vinyl chloride), with pore diameters ranging from 0.05 to 0.65 $\mu$m.

Calibration of the cell to determine the cell constant $\beta$ was done as discussed elsewhere (*3*) with 0.1 M KCl in the lower chamber and water in the upper. An important difference in this work is that, in pores with diameters of 0.10 and 0.05 $\mu$m, even a small solute like KCl experiences a hindrance effect due to the pore restriction. Other investigators (*10*, *14*) have used molecules of different sizes to calibrate and measure diffusivities and also have used restricting pores in the diffusion measurements. An effective diffusivity obtained from eq 3 or 5 should have been used instead of the free solution diffusivity when using eq 1 to calculate $\beta$. This would have corrected the error, which can amount to ca. 3 or 4%.

In this work diffusion runs were made by using sucrose in various concentratiions of BSA. Sucrose diffused from the lower chamber where the initial concentration was 3 g/100 mL into the upper chamber which was initially sucrose-free aqueous BSA solution. The solvent was water with 0.1 M Tris buffer and 0.1 M KCl present to eliminate electrostatic effects. The albumin concentration was the same in the upper and lower chambers and remained constant during the experiment since there was no net diffusion of BSA. Both upper and lower solutions remained at pH 7.5 and 25.0 °C during the diffusion runs. The initial and final solutions were analyzed for sucrose concentration by using a half-shade polariscope and corrections made for the presence of BSA.

## Experimental Results and Discussion

***Diffusivity of Sucrose and Cell Accuracy.*** The aqueous diffusivity of sucrose at 25.0 °C was determined in the large, 0.65-$\mu$m pores where pore restriction should only affect diffusivity by a factor of 0.9928 as calculated from eq 4. The experimental sucrose diffusivity was found to be $0.577 \times 10^{-9}$ m$^2$/s with an average deviation of ±5%. This value would be equivalent to $0.581 \times 10^{-9}$ m$^2$/s in an unrestricted fluid. This equivalent value was obtained by dividing the experimental diffusivity by 0.9928. This value is somewhat higher than the Guoy diffusimeter (*17–19*) results of $0.511 \times 10^{-9}$ m$^2$/s and the commonly accepted value of $0.526 \times 10^{-9}$ m$^2$/s.

The reasons for the slightly high values of sucrose diffusivity in this work are not apparent. However, the consistent results of these experimental data are such that the results can be used to show relative effects. Evidence of experimental consistency includes ±2% reproducibility on cell-constant determinations and no more than ±2% differences in the material balances performed on the cell. Mixed esters of cellulose and also poly(vinyl chloride) were used as diaphragm materials and gave similar results. This should rule out any possible surface effects. Runs made without any buffer showed no effect on the results, eliminating the possibility of buffer interference. Also, experimental measurements of the diffusivity of urea and sodium caprylate in the cell gave values within ±2% of the accepted literature values. Finally, there was no evidence of protein denaturation or degradation caused by the stirring.

***Pore Restriction Effect on Diffusion of Sucrose in BSA Solutions.*** In Figure 1, the experimental data for the restriction effect of pore size on the diffusivity of sucrose in various BSA solutions are plotted as the sucrose normalized diffusivity, $D_P/D_0$, vs. the pore size ratio. The free diffusivity $D_0$ is the diffusivity of sucrose in the various protein solutions with no pore restriction effects present. This is obtained by using the experimental value of sucrose diffusing in the largest pore of 0.65-$\mu$m size ($1/\lambda = 586$) and dividing by the factor of 0.9928 from eq 4 which corrects the diffusivity to a pore size of $1/\lambda = \infty$ (no pore restriction). The theoretical line shown in Figure 1 is calculated by using the Satterfield equation, eq 5. Using the theory of Beck and Schultz, eq 4 gives a line very close to that of eq 5. As can be seen, the experimental data for pore restriction effects follow the equations closely.

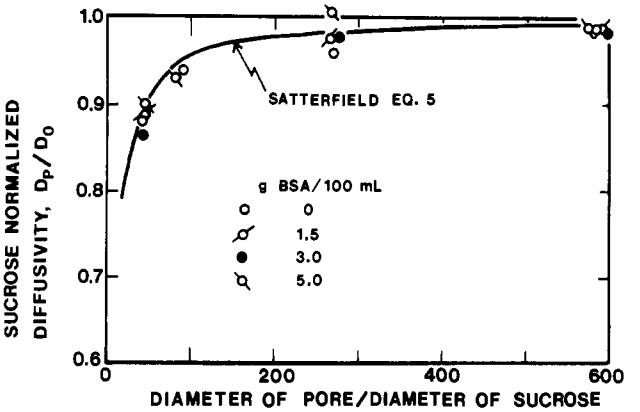


**Figure 1.** Pore restriction effect on sucrose normalized diffusivity in various concentrations of protein.

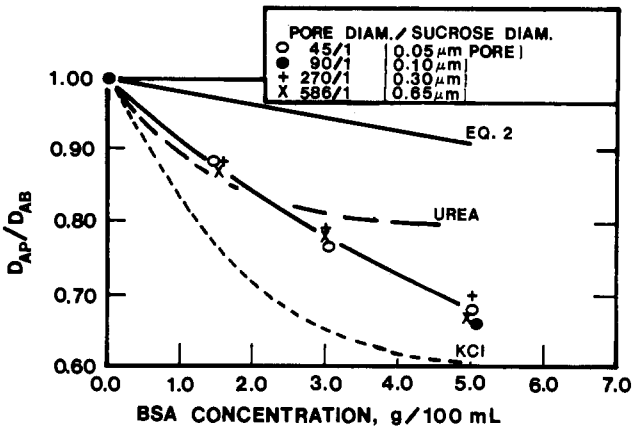


**Figure 2.** Protein blockage effect. Sucrose diffusivities of this work vs. protein concentration and comparison with the results of others for urea (*3*), KCl (*3*), and eq 2 (*2*).

It can also be seen in Figure 1 that the protein concentrations of 0–5 g of BSA/100 mL have no interaction effects on the behavior of the pore restriction phenomena in the range of these experiments. All of the data, regardless of protein concentration, follow the same trend. The smallest pore used in this work was 8 times as large as the BSA molecule. As pore size approches protein size, there should be an interaction effect as the limit is approached where the protein completely fills the pore.

***Protein Blockage Effect on Diffusion of Sucrose.*** To observe the protein blockage effect when in pores, we converted the data to $D_{AP}/D_{AB}$ where $D_{AB}$ is simply the experimental value of sucrose diffusivity at 0 g of BSA/100 mL for the appropriate pore size. These ratios were then plotted in Figure 2 vs. protein concentration. It can be seen in Figure 2 that the protein blockage data from this work for all pore sizes fall on a single line. For a given concentration of BSA, pore size has no effect on the protein blockage effect. However, the data are below the prediction in free solution, eq 2, for a nonbinding, blockage protein and solute. The line from eq 2 is the lowest of the theoretical or empirical equations proposed by others (*1–3*, *5*, *6*). The explanation for the low diffusivity ratios of sucrose in pores containing BSA solutions is not clear.

The sucrose–BSA data in Figure 2 fall between the data of others (*2*, *3*) for urea and potassium chloride in BSA solutions. Since urea and KCl are both known definitely to bind to BSA, it could appear that sucrose binding to BSA might be the cause of the effect seen in Figure 2. However, it has been reported by Giles and McKay (*7*) that disaccharides could not bind with proteins. However, sucrose and BSA were studied (*7*) for binding along with several other sugars and proteins, but sucrose was never actually tested with BSA. Sucrose was tested with casein. Some other disaccharides were tested with BSA, but sucrose was not. In a diffusion experiment, Colton et al. (*1*) found a binding coefficient $k_P$ of 0.131 for sucrose in 4% BSA solution with 4.2% other proteins present. However, the diffusion equation that Colton et al. used to calculate $k_P$ is very sensitive to errors in diffusivity (*1*, *2*). Their reported binding coefficient could possibly occur because of errors in the diffusivity measurements, or the presence of 4.2% of other proteins could possibly cause the binding. In view of this limited and seemingly contradictory evidence of possible binding, a sucrose–BSA binding study should probably be performed in the future.

### Glossary

| | |
|---|---|
| A | diffusing solute |
| B | solvent |
| $c$ | concentration of A in lower chamber of diaphragm cell at time $t$, g-mol/m$^3$ of solution |
| $c'$ | concentration of A in upper chamber at time $t$, g-mol/m$^3$ of solution |
| $c_0$, $c_0'$ | concentration of A at time $t = 0$, g-mol/m$^3$ of solution |
| $D_{AB}$ | diffusivity of A in solution with no protein present, m$^2$/s |
| $D_{AP}$ | diffusivity of A in protein solution, m$^2$/s |
| $D_{PB}$ | diffusivity of protein–solute complex in solution (assumed that of the protein), m$^2$/s |
| $D_P$ | diffusivity of A in protein solution inside pore, m$^2$/s |
| $D_0$ | free diffusivity of A in protein solution outside pore, m$^2$/s |
| $k_P$ | protein binding coefficient (concentration dependent), [(g of bound solute)/(mL of solution)]/[(g of free solute)/(mL of protein-free solution)] |
| $t$ | time, s |
| $\alpha$ | diffusivity reduction shape factor for protein (1.5 for sphere, 1.615 for BSA) |
| $\beta$ | cell constant, m$^{-2}$ |
| $\lambda$ | inverse pore size ratio, solute size/pore size |
| $\tau$ | tortuosity, effective pore length/diaphragm thickness |
| $\phi_P$ | volume fraction of proteins in protein solution |

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# Vapor–Liquid Equilibria of the Formic Acid–Dimethylformamide System

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**Vapor–liquid equilibria have been measured for the system formic acid–dimethylformamide at 200, 300, 400, 600, and 760 mmHg. The system presents associations in the vapor phase which have to be taken into account for a thermodynamically consistent reduction of the data. The nonideal behavior is assumed for the vapor mixture of true chemical species.**

Deviations from ideal behavior in the vapor phase of systems containing components which can form intermolecular hydrogen bonds are frequently interpreted in terms of associations between like or unlike molecules (*1*–*12*). The chemical theory of vapor imperfections, in contrast to the physical theory, has been widely used in many recent works to fit vapor–liquid equilibrium data at low or moderate pressure. The calculation of activity coefficients from $x$–$y$ data, ignoring the presence of the true species in the vapor phase, can lead to values without thermodynamic meaning, i.e., inconsistent with the Gibbs–Duhem equation. In a simplified form, the chemical theory of vapor-phase nonideality assumes ideal behavior of the mixture of "true" species (monomer, dimer, etc.), whose concentrations can be evaluated by the chemical equilibrium constants of association reactions. More sophisticated formulations, however, take into account physical interactions of species present in the vapor phase (*5*). In the present paper the nonideal approach of Nothnagel et al. (*5*) is applied to the correlation of the vapor–liquid isobaric equilibria of the formic acid (FA)–dimethylformamide (DMF) system.

Very few data of vapor–liquid equilibrium are available in the literature for the system examined. Ruhoff and Reid (*13*) observed a homogeneous azeotrope at 153.2 °C and atmospheric pressure with 97.4 wt % DMF. Du Pont observations (*14*) indicate the azeotrope position at 67 wt % DMF and a

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