

Mutual Diffusion Studies of Bovine Serum Albumin in Water at Different Concentrations and pH with Laser Beam Deflection Technique

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Abstract : Eventhough the structural studies of protein with various methods is flourishing fast; the application of laser beam deflection sensor to study the diffusion mechanism in Albumin Bovine Serum-Water system is not yet reported. Diffusivity of the globular protein Albumin in water is relevant in drug delivery, pharmacology and many medicinal fields. Here we well enforced Laser Beam Deflection technique with a fanned out laser beam from a semiconductor diode laser to study the diffusion mechanism with varying pH, temperature and concentration. Laser beam deflection technique based on Fickian laws of diffusion is proved to be an efficient technique with less investment. Even if there is not a remarkable change in the diffusion in the temperature range from 293 K to 303K, the pH variations alter the diffusion process of protein considerably which signal the structural change of Bovine Serum Albumin from linear to globular.

Keywords - Laser Beam Deflection, Diffusion coefficient, Bovine Serum Albumin, concentration gradient, structural variation of protein

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I. INTRODUCTION

Diffusion is one of the major transport mechanisms in fluids causing molecular movement creating concentration gradient. The study of diffusion properties is important in various fields like chemical energy studies, drug delivery, biology, pollution control, metallurgy etc. Diffusivity measurement is necessary for the design of chemical and biological equipments and for mass transfer studies [1-3]. The diffusion coefficient of substance in a fluid can be predicted theoretically or found from empirical correlations [4]. Theoretical predictions can be verified by experimental techniques. There are many methods to study diffusivity with their own advantages and disadvantages. Diffusivity values can be accurately found by employing optical methods like multiple beam interferometry, fringe projection Michelson interferometry, holographic interferometry and electronic speckle pattern interferometry [5, 6]. Laser beam deflection (LBD) is a non interferometric method which is much sensitive and less expensive. LBD is already developed technique based on the concentration gradient and refractive index gradient (RIG)[7-9].

In this paper we discuss the development of LBD to study the diffusion properties of Albumin Bovine Serum (BSA) which is a globular protein. BSA is the most abundant protein in blood plasma [10]. Analysis of the protein in the crystal state by X-ray method showed that the protein is a 585 amino acid residue monomer that folds into three structurally homologous α -helical domains. These domains assemble to form a heart shaped molecule [11]. In a solution the confirmation of BSA depends on the pH of the solution and the protein undergoes reversible conformational transformations with a change in pH. The normal form is predominant from pH4.3 to 8.0 and the BSA molecule is globular. Below 4.3 pH BSA conformation changes to a highly charged fast migrating form [12-14]. Random Brownian motion of protein molecule is necessary to perform most of the physiological functions of the protein. In particular diffusion phenomenon plays an important role in passive intracellular transport which regulates cellular functions such as signal transduction, self assembly of supramolecular structures, the kinetics of reactions and embryogenesis and plays a crucial role in the transport of small molecules and ions. The diffusion coefficient (D) of proteins can be measured by using many sophisticated models which require high resolution X-ray crystallographic or nuclear magnetic resonance (NMR) data which are not always available [15-17]. So LBD serves as a fast and cheap solution.

II. THEORY AND EXPERIMENTAL METHODS

LBD is based on the Fickian laws of diffusion. Considering a step like variation of concentration in a plane we can write [10]

$$\frac{\partial c}{\partial y} = \frac{c}{2\sqrt{(\pi Dt)}} e^{\left(\frac{-y^2}{4Dt}\right)} \quad (1)$$

This is a Gaussian function and will be of the same shape traced out by the deflected beam at the boundary. The shape of the beam trajectory inside the liquid is given by the Gaussian function.

$$Z(y) = k e^{\left(\frac{-y^2}{4Dt}\right)} \quad (2)$$

Here $Z(y)$ is the deflection of the laser beam on the screen, y is the depth of the deflecting region, within the cell below the interface, k is the proportionality constant. As time evolves the boundary smears out until the concentration gradient disappears. This results in the broadening of the Gaussian function. We can evaluate the diffusion coefficient D from the half width $y_{1/2}$ of the Gaussian function as

$$D = \frac{y_{1/2}^2}{4t \ln 2} \quad (3)$$

A properly fanned out laser beam is passed through a glass cuvette containing the slowly diffusing solution. The laser beam after passing through the interdiffusing media follow a Gaussian profile which is projected to a screen placed at a distance. The image of the profile which in turn represents the RIG at various time intervals is studied and graph with $Y_{1/2}^2$ versus time is plotted. The slope of it is applied to the formula to calculate the diffusion coefficient (D)

2.1 MATERIALS USED

BSA was purchased from Sigma Aldrich and used without further purification. The BSA crystals were dissolved in distilled water to make the test solution at various concentrations and pH. The glass cell has dimensions 1cmx1cmx4cm. The laser beam used was from a semiconductor diode laser. A cylindrical lens was used to fan-out the laser beam. Two solutions were micropipetted to the glass cuvette. The experimental set-up is shown in figure 1

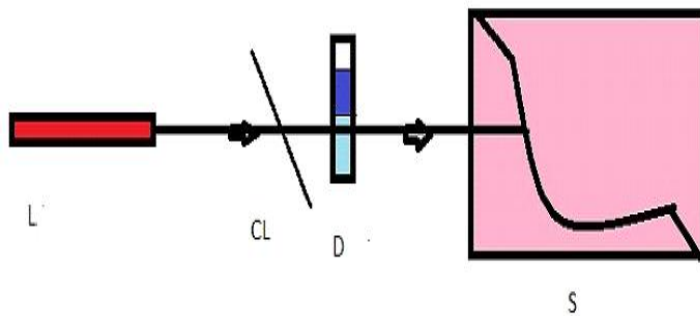


Fig 1: Experimental set up for determining the diffusion coefficient using laser beam deflection method. L: diode laser of wavelength 632nm, CL: cylindrical lens, D: cell containing diffusing liquids, S: screen

III. RESULTS AND DISCUSSIONS

The first paragraph under each heading or subheading should be flush left, and subsequent paragraphs should have a five-space indentation. A colon is inserted before an equation is presented, but there is no punctuation following the equation. All equations are numbered and referred to in the text solely by a number enclosed in a round bracket (i.e., (3) reads as "equation 3"). Ensure that any miscellaneous numbering system you use in your paper cannot be confused with a reference [4] or an equation (3) designation.

The diffusion coefficient of BSA in water was studied as a function of pH, concentration and temperature by varying one of these properties while the other two were held constant. Figure 2 depicts the variation of concentration gradient with time. Initially there was a sharp peak which smears as time goes on.

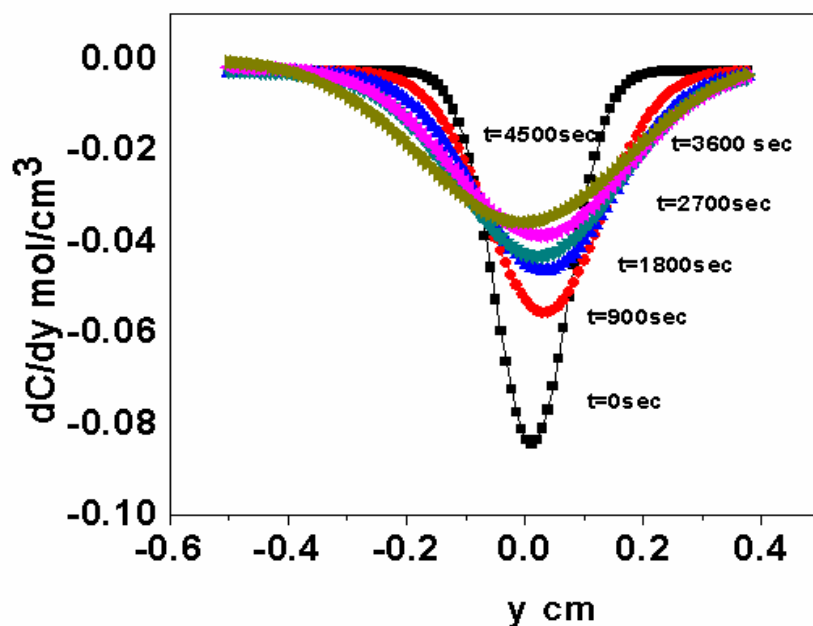


Fig. 2. Variation in concentration profile at various time intervals. From $t=0\text{sec}$ to $t=9000\text{sec}$

The FWHM of these curves were calculated and plotted against time to get a straight line whose slope is used to calculate D (Figure 3).

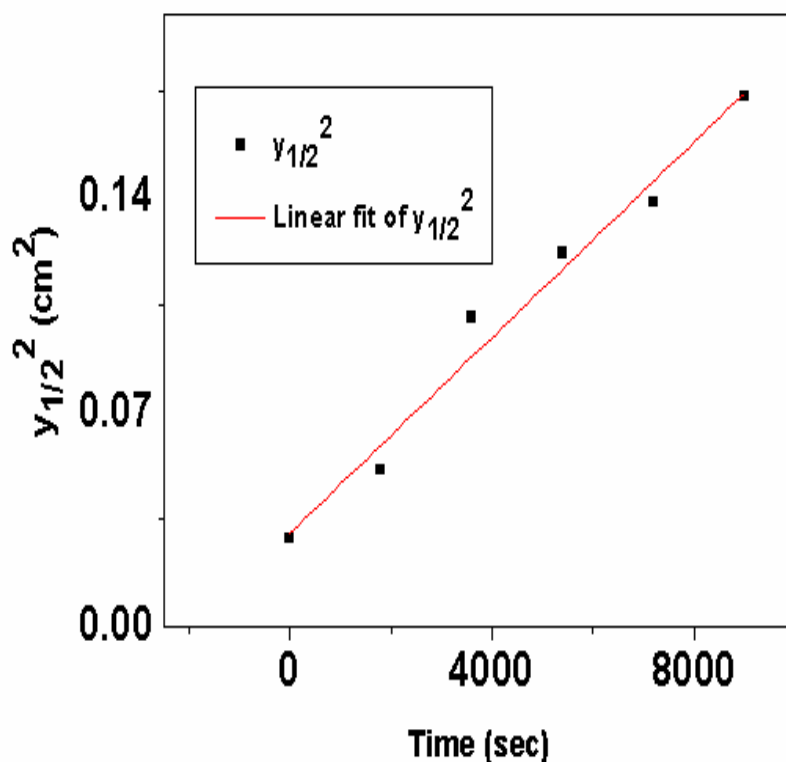


Fig. 3. Square of FWHM $Y_{1/2}^2$ vs time

The variation of Z deflection with time found to decrease with time. It is plotted in Figure 4a) and area of the profile at various time intervals plotted in Figure 4b)

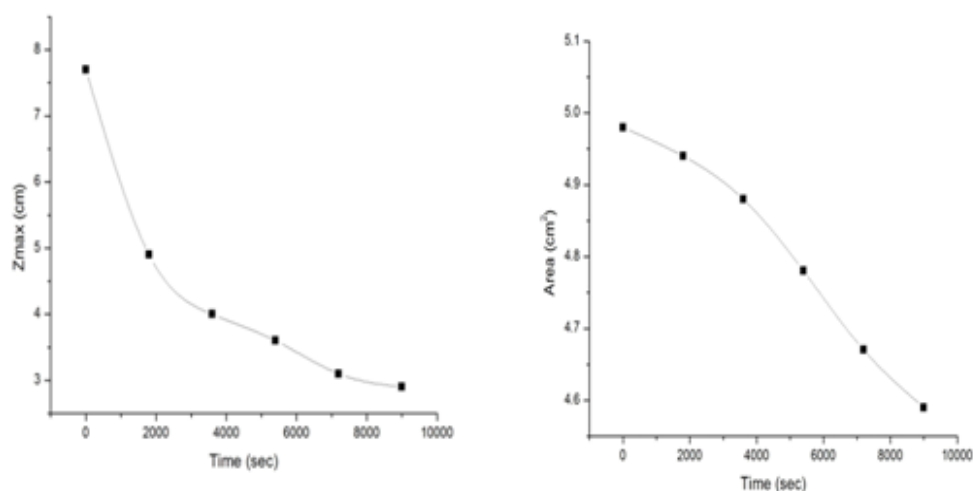


Fig. 4: a) Variation of maximum deflection Z_{max} with time b) Area of deflection pattern with time

Dependence of D on pH: Figure 5 shows the values of D for a solution of concentration 0.15g/ml of BSA as a function of pH for temperature values of 293K and 303K.

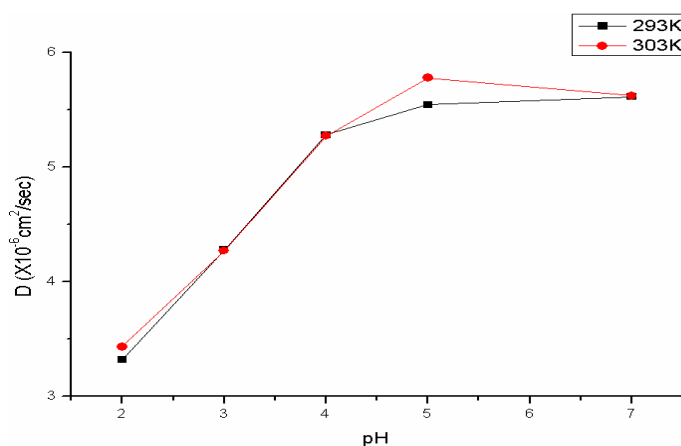


Fig. 5: Variation of D with pH at two different temperatures.

As the pH is decreased below the isoelectric point (below pH5), D decreases at both temperatures. This decrease in D corresponds to the structural change of the protein according to Sogami and Foster (1968). *Dependence of D on Concentration:* The dependence of D on concentration was studied at pH values 3 and 4 at room temperature 303K. (Graph 5).

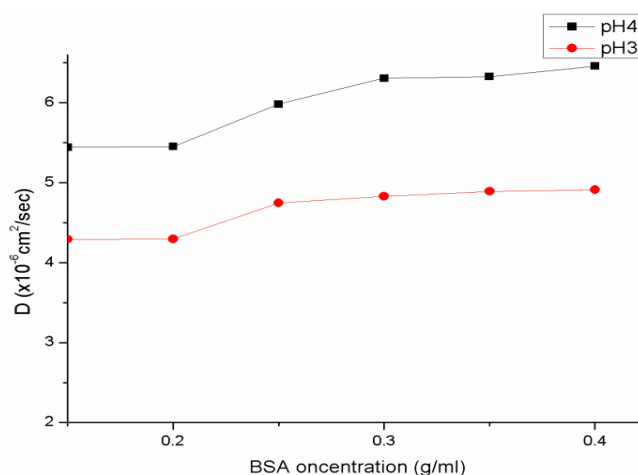


Fig.6: Variation of D with BSA concentration at pH values 3 and 4

D increases with concentration. When the pH is 4, but in pH 3 almost constant value of D is obtained indicating the structural invariance in the regime.

Dependence of D on temperature: Temperature had no significant effect in the D value within the range 293K to 303K, which shows stability of the BSA structure in this temperature range.

Table 1: Diffusion coefficient of BSA at several values of temperature at pH values 3 and 4 at same concentration 0.15g/ml

Temperature(K)	Dx10 ⁻⁶ cm ² /sec
<i>pH3</i>	
293	4.28
295	4.28
297	4.27
299	4.29
301	4.28
303	4.27
<i>pH 4</i>	
293	5.28
295	5.44
297	5.43
299	5.47
301	5.27
303	5.27

IV. CONCLUSION

We have productively tested LBD to demonstrate the sensitivity of BSA structure with pH, temperature and concentration. Diffusion coefficient of BSA in water of the order of 10⁻⁶cm²/sec at various concentrations, temperature and pH is studied with better accuracy using Laser Beam Deflection method. So far structural studies of BSA have been conducted all of which require sophisticated and expensive methodology. Our method indicates that such studies can be done in a modest way. The measured value of diffusion coefficient agrees much with the reported values with other techniques.

Our investigation has important implication for those who conduct diffusion tests with biological particles such as proteins, carbohydrates enzymes and hormones. Taken together these results are expected to improve our understanding and modeling of interactions of protein at various pH, temperature and concentration conditions and thus offer a better way to apply to various biomedical applications.

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