Volumetric Behavior and Relative Association of Aqueous Hemoglobin Solution in Presence of Different Sugars

Naseem Ahmed[1] and Saeeda Naqvi[2*]

[1]Chemistry Department, Gov. PG College, Rajouri, Jammu India
[2]Chemistry Section, Women's College, Aligarh Muslim University, Aligarh – 202002, India
E-mail: [2]saeedaznaqvi@gmail.com

Abstract—*In the present work, using the density and ultrasonic velocity values of aqueous solutions of hemoglobin in presence of different sugars, viz., D-glucose, D(-)fructose, sucrose and maltose, we have determined apparent molal volume, partial molal volume and relative association as functions of concentration of sugars (keeping the concentration of aqueous hemoglobin solution constant) and temperature. Various parameters which have been derived for the said system have helped in understanding the stabilization of hemoglobin in presence of different sugars.*

Keywords*: Hemoglobin, sugars, apparent molal volume, partial molal volume and relative association.*

1. INTRODUCTION

Compressibility of liquids is an essential physical characteristic reflecting intermolecular interactions and dynamic processes occurring in solutions. Studies of compressibility of aqueous solutions of proteins started a long time ago.

The denaturation and reactions of proteins under the high static pressure or ultracentrifugal force have been a matter of concern for many investigators.[1-3]. In such works, the compressibility of native proteins in solution has been an indispensable quantity to analyse; the protein compressibility in solution has been thus far estimated by following two methods. One is the measurement of the partial molal volume of protein in solution as functions of concentration and temperature by the direct densimetricmethod[4] or ultracentrifuge,[5] which uses sound velocity measurement with an ultrasonic interferometer.The compressibility obtained by this technique is adiabatic. An important result in these compressibility studies is that globular proteins have a positive compressibility while the constitutive amino acids have negative ones due to the hydration effect. This result suggests that the compressibility of the protein interior is very large. However, it is difficult to understand the compressibility of proteins on a molecular level, as few compressibility data[6-9] have been reported, probably due to technical difficulties. The partial molal volume of a protein in solution is known to result from three contributions.[10]

i. The constitutive volume estimated as the sum of the constitutive atomic or group volumes.
ii. The volume of the cavity or void in the molecule due to imperfect atomic packing.
iii. The volume change due to solvation or hydration

Since the constitutive atomic volume should be approximated as incompressible, compressibility data of globular proteins in water will produce useful information on the internal structure and the hydration structure of protein, which are still obscure. Furthermore, such compressibility data should present important information to the understanding of the mechanisms of denaturation or reactions of proteins.

The contributions of both cavity and hydration to the compressibility have been quantitatively analyzed for some globular proteins with appropriate assumptions for the hydration term[11]. Recently, we have found that the compressibility or the volume fluctuation of proteins rather sensitively depends on their structural characteristics, such as hydrophobicity, secondary structure, or amino acid composition.[12] However, most compressibility studies have been performed under a fixed condition of temperature and solvent composition. For a full understanding of the volume-structure relationship of globular proteins, further detailed investigations are required on the effect of temperature on the compressibility, since such data could be useful for deriving the partial molal volume of a protein as functions of temperature and pressure.

2. EXPERIMENTAL

Hemoglobin obtained from SIGMA-ALDRICH CHEMIE GmbhSteinheim, Germany, was used for sample preparation. Sugars viz. D-glucose, D(-)fructose, sucrose and maltose used were obtained from Qualigens fine chemicals (A

DivisionofGlaxo Smith Kline Pharmaceuticals Limited, Mumbai). The solutions were prepared by weight with laboratory double distilled water.

A pyknometer consisting of a small bulb with flat bottom (~ 8ml capacity) and graduated stem was used for the density measurement. Each mark on the stem of the pyknometer was calibrated using double distilled water. For the measurements of density, a thermostated paraffin bath was used to maintain a uniform temperature.

Ultrasonic velocity in solutions was measured by determining the wavelength of sound in this media using a multi-frequency ultrasonic interferometer model M-82 (Mittal Enterprises, India) working at 2MHz. The temperature of the solution was controlled bycirculating water bath through the jacket of a double walled cell from a constant temperature controlled to $\pm$ 0.03K

3. RESULTS AND DISCUSSIONS

In order to check the effect of sugars on the stability of hemoglobin we have determined various parameters as function of temperature and concentration. The density data[6] of aqueous hemoglobin solutions with different sugars viz. D-glucose, D(-)fructose, sucrose and maltose are listed in Table-1 (a-d), as functions of concentration and temperature. The density values have been found to exhibit the usual decrease with an increase in temperature and increase with increase in concentration.

The ultrasonic velocities of aqueous hemoglobin solution with different sugars have been determined at several temperatures and concentrations. They are foundto increase with increase in temperature as well as concentration of different sugars in their respective systems. This increase may be attributed to an increase in the intermolecular interactions with increases in temperature and concentration[6].

The adiabatic compressibility, $\Delta\beta_s$ is calculated employing the data of sound velocity, u, and the density'd' using the following Laplace equation.

$$\beta_s = 1/u^2 d \qquad \text{(i)}$$

It is found to decrease with increase in temperature and concentration[6]. The decrease in compressibility with increase in thermal breaking of the solvent components, which, in turn results in greater attractive forces among the molecules of a solution. Decrease in the $\Delta\beta_s$ values with increase in composition is due to greater attractive forces among the molecules of a liquid.

4. TABLES

Table- 1 (a) Densities ρ (gm cm^{-3}) of D-Glucose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0030	1.0010	0.9990	0.9970	0.9950	0.9930
0.04	1.0050	1.0028	1.0008	0.9988	0.9968	0.9950
0.06	1.0062	1.0042	1.0022	1.0004	0.9984	0.9964
0.08	1.0080	1.0060	1.0040	1.0020	1.0000	0.9980
0.10	1.0092	1.0074	1.0054	1.0036	1.0016	0.9996

Table- 1 (b) Densities ρ (gm cm^{-3}) of D(-)Fructose-Hemoglobin-Water System as Functions of concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0018	0.9998	0.9976	0.9956	0.9936	0.9914
0.04	1.0040	1.0018	0.9998	0.9976	0.9954	0.9934
0.06	1.0056	1.0036	1.0016	0.9994	0.9974	0.9952
0.08	1.0078	1.0056	1.0036	1.0014	0.9994	0.9974
0.10	1.0092	1.0072	1.0052	1.0032	1.0012	0.9990

Table- 1 (c) Densities ρ (gm cm^{-3}) of Sucrose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0046	1.0028	1.0008	0.9990	0.9972	0.9952
0.04	1.0064	1.0046	1.0026	1.0008	0.9988	0.9970
0.06	1.0082	1.0062	1.0044	1.0024	1.0006	0.9986
0.08	1.0098	1.0080	1.0060	1.0040	1.0020	1.0002
0.10	1.0118	1.0098	1.0078	1.0058	1.0038	1.0018

Table- 1 (d) Densities ρ (gm cm^{-3}) of Maltose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0054	1.0038	1.0022	1.0006	0.9980	0.9974
0.04	1.0066	1.0050	1.0034	1.0018	1.0002	0.9986
0.06	1.0086	1.0068	1.0052	1.0034	1.0018	1.0000
0.08	1.0102	1.0084	1.0068	1.0050	1.0034	1.0016
0.10	1.0122	1.0104	1.0086	1.0068	1.0050	1.0032

The experimentally determined adiabatic compressibility of a protein would mainly consist of two contributions, volume of the cavity and hydration. The volume of the cavity in a protein molecule is generated by imperfect atomic packing, and change in volume occurs due to solvation or hydration. Increased pressure may squeeze cavity in the protein molecules and force water into the cavity. Thus, the positive β_s values observed can be ascribed to the large cavity effect overcoming the hydration effect. At low temperature, however, the hydration effect would oppositely overcome the cavity effect due to the increased amount of hydration. The temperature for β_s=0 can be regarded as a compensation temperature for both factors, the packing state in the protein molecule and the protein-solvent interaction.

Relative change in adiabatic compressibility is calculated

by using the equation

$$\beta_r = \Delta\beta_s / \beta^0 \ldots \quad \ldots\ldots \text{(ii)}$$

These values have been found to increase with increase in concentration and no regular trend is observed with temperature. It is noteworthy that table 2(a-d) shows linear relationship between the relative change in adiabatic compressibility and the solute concentration.

Table-2 (a) Relative Change in Adiabatic Compressibility$\Delta\beta_s / \beta^0 \times 10^{-3}$) of D-Glucose-Hemoglobin-Water Systemas Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.1506	1.1506	4.6901	2.6794	2.3710	1.0217
0.04	5.6301	5.6301	8.0309	6.7952	7.5088	3.6712
0.06	11.1165	12.3928	12.1180	11.4577	12.7951	9.0283
0.08	13.7893	14.9316	15.8085	15.0743	15.8953	12.6471
0.10	16.8990	17.3269	20.8685	19.3057	20.7450	17.3819

Table- 2 (b) Relative Change in Adiabatic Compressibility$\Delta\beta_s / \beta^0 \times 10^{-3}$) of D(-) Fructose-Hemoglobin-WaterSystem as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.7970	1.5389	1.9442	1.2769	1.3524	1.3638
0.04	4.3775	7.8257	7.8135	5.7292	5.7284	3.4775
0.06	9.2254	10.6400	10.4979	9.8288	8.8731	7.6732
0.08	13.7231	15.0540	15.0325	14.4841	12.7665	12.1800
0.10	17.0280	17.9007	19.9118	18.9149	16.5689	16.1617

Table- 2 (c) Relative Change in Adiabatic Compressibility$\Delta\beta_s / \beta^0 \times 10^{-3}$) of Sucrose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	6.5424	7.9076	8.1601	6.4797	5.5999	4.6400
0.04	11.0530	12.1412	12.3837	11.4696	11.2823	10.1333
0.06	15.14.89	16.1550	16.9662	15.3402	16.3588	13.6201
0.08	20.4406	20.8517	20.3103	19.1907	19.107	17.7197
0.10	24.8050	25.7720	25.4746	23.5925	23.5925	20.9200

Table- 2 (d) Relative Change in Adiabatic Compressibility$\Delta\beta_s / \beta^0 \times 10^{-3}$) of Maltose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	11.3688	11.2248	8.5138	6.1388	8.8300	7.7219
0.04	13.7130	13.9515	13.4276	13.0943	13.0478	10.4491
0.06	17.9405	19.0459	17.7485	17.0832	17.2843	15.8849
0.08	20.8284	22.7682	20.7080	20.4193	21.6225	18.4641
0.10	25.1904	27.7419	26.3735	24.3110	24.7572	23.2868

The apparent molal volumes of the aqueous hemoglobin solution with different sugars have been determined from the density of the solution using the equation.

$$\phi_v = \frac{M}{d} + \frac{(d_0 - d)10^3}{mdd_0} \quad \ldots\ldots \text{(iii)}$$

Where d_0 is the density of water, m is the molality; M is the molecular weight of the solute. The apparent molal volume (ϕ_v) for the systems under study[6], show that ϕ_v values of glucose at all temperatures are smaller than those of fructose, though the difference is not very large. No definite trend is seen in versus concentration for D(-) fructose. The higher, values in the case of sucrose suggest that it is comparatively more hydrated, since sucrose is made up of glucose and fructose. The apparent molal volume has been found to vary with concentration according to the following equation

$$\phi_v = \phi_v^o + S_v m \quad \ldots\ldots \text{(iv)}$$

where$\phi_v°$, the apparent molal volume at infinite dilution is referred to as partial molal volume as shown in Table-3(a-b). It is a measure of the solute-solvent interaction. It is obtained at each temperature from the linear fitting of (ϕ_v), with m using the least square method. S_v is the experimental slope and is a measure of the solute-solute interaction.

Table- 3 (a) Partialmolal volume,ϕ_v^o(cm^3 .mol^{-1}) of D-Glucose-Hemoglobin-Water System as Functions of Concentration and Temperature

Temp. (K)	D-Glucose		D(-)Fructose	
	ϕvo	Sv	ϕvo	Sv
303.15	23.88	696.05	86.04	-15.50
308.15	26.92	653.75	88.29	-28.10
313.15	26.66	656.35	96.35	-129.05
318.15	36.78	545.20	109.55	-235.80
323.15	26.29	641.35	101.90	-168.20
328.15	33.80	575.05	120.29	-344.85

Table- 3 (b) Partial molal volume,ϕ_v^o(cm^3 .mol^{-1}) of D(-) Fructose-Hemoglobin-Water System as Functions of Concentration and Temperature

Temp. (K)	D-Glucose		D(-)Fructose	
	ϕvo	Sv	ϕvo	Sv
303.15	110.34	1281.6	94.69	1638.4
308.15	138.33	1399.5	73.72	1862.2
313.15	98.88	1404.9	52.18	2074.9
318.15	98.22	1443.2	41.13	2208.3
323.15	78.86	1644.4	49.45	2012.6
328.15	86.51	1569.4	46.42	2045.4

The values of relative association (RA) parameter have been computed using the following equation.

$$RA = d/d_o[u_o/u]^{1/3} \text{--} \quad \text{------ (v)}$$

where'd' and 'd°' are the densities of the solution -and solvent, respectively, while U and U° are their corresponding ultrasonic velocities. Relative association values listed in Table-4.(a-d) show an increasing trend with increase in concentration of different sugars; the corresponding variation with temperature seems insignificantly small over a range of 303.15-328.15 K. The relative association (RA) is influenced by two factors:

i. The breaking up of solvent molecules on addition of solute to it.

ii. The solvation of solutes those are simultaneously present.

The former results in decrease and the latter in increase of association in a system. RA increases with increase in concentration of sugar, which suggests that solvation of the solute molecules predominates over the breaking up of the solvent molecules.

Table 4 (a) Relative Association (RA) of D-Glucose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0033	1.0029	1.0027	1.0028	1.0031	1.0031
0.04	1.0049	1.0044	1.0043	1.0043	1.0043	1.0050
0.06	1.0054	1.0052	1.0052	1.0053	1.0054	1.0058
0.08	1.0072	1.0068	1.0067	1.0066	1.0067	1.0071
0.10	1.0079	1.0081	1.0075	1.0078	1.0078	1.0081

Table 4 (b) Relative Association (RA) of D(-) Fructose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0018	1.0018	1.0015	1.0004	1.0017	1.0016
0.04	1.0039	1.0031	1.0031	1.0030	1.0030	1.0032
0.06	1.0050	1.0048	1.0048	1.0045	1.0049	1.0046
0.08	1.0068	1.0063	1.0064	1.0060	1.0066	1.0064
0.10	1.0079	1.0077	1.0074	1.0074	1.0080	1.0076

Table 4 (c) Relative Association (RA) of Sucrose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0043	1.0043	1.0043	1.0045	1.0052	1.0052
0.04	1.0056	1.0057	1.0052	1.0058	1.0061	1.0063
0.06	1.0076	1.0069	1.0070	1.0070	1.0073	1.0076
0.08	1.0080	1.0082	1.0077	1.0086	1.0084	1.0088
0.10	1.0095	1.0094	1.0095	1.0096	1.0098	1.0101

Table 4 (d) Relative Association (RA) of Maltose-Hemoglobin-Water System as Functions of Concentration and Temperature

Molality mol kg-1 / Temp. (K)	303.15	308.15	313.15	318.15	323.15	328.15
0.02	1.0044	1.0049	1.0058	1.0065	1.0056	1.0073
0.04	1.0054	1.0058	1.0064	1.0067	1.0074	1.0081
0.06	1.0068	1.0069	1.0078	1.0079	1.0086	1.0089
0.08	1.0084	1.0083	1.0091	1.0092	1.0097	1.0103
0.10	1.0099	1.0098	1.0103	1.0106	1.0108	1.0115

5. CONCLUSION

It has been observed that after the addition of different sugars to the hemoglobin solution there is an increase in the values of apparent molal volumes and decrease in compressibility of the solutions. This may be attributed to the fact that the addition of sugars to the protein increases thehydrophobic, electrostatic and hydrogen-bonding interactions giving rise to the compact form of protein. Therefore, by observing a decrease in the compressibility of the solution and the increase in the apparent molal volume of the protein after the addition of sugars, we can say that the extent of denaturation of protein is reduced and its stabilization has taken place.

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