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Volumetric and Adiabatic Compressibility Behaviour of Aqueous Haemoglobin Solution in Presence of Sugar

Naseem Ahmed & Sami Ullah Bhat

P.G. Department of Chemistry Govt. P.G. College Rajouri (J&K), India
Email: drnaseem123@gmail, profsamibhat92@gmail.com

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Abstract.

Measurements of density, ultrasonic velocity were made for investigating the intermolecular interactions. As D- glucose-Haemoglobin–water systems. These systems were chosen due to their vital role in the life of living organisms for such studies. Using density and ultrasonic velocity data, various derived parameters such as adiabatic compressibility (β_s), compressibility lowering ($\Delta\beta_s$), relative change in compressibility ($\Delta\beta_s/\beta_{0)}$, apparent molal volume (ϕ_v), apparent molal adiabatic compressibility (ϕ_k), and partial molal volume (ϕ_v^0), of aqueous Haemoglobin solutions in presence of sugar were obtained for each of the concentrations studied in the temperature range: 303.15-328.15k. Temperature dependence of these data suggests the presence of solute-solute, and solute- solvent interactions as well as on the extent of salvation of the protein molecules in presence of sugar. 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 sugar, we can say that the extent of denaturation of protein is reduced and its stabilization was taken place.

Keywords: Density; ultrasonic Velocity; & adiabatic compressibility.

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 age. [1].The denaturation and reactions of proteins under the high static pressure or ultracentrifugal force have been a matter of concern for many investigators.[2-5]in such works, the compressibility of native proteins in solution has been an indispensable quantity to analyze; 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 a function of pressure by the direct densimetric method [6] or ultracentrifuge, [7]which uses sound velocity measurement with an ultrasonic interferometer.[8-9] 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 date [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]

- The constitutive volume estimated as the sum of the constitutive atomic or group volumes.
- The volume of the cavity or void in the molecule due to imperfect atomic packing.
- The volume change due to salvation or hydration

Since the constitutive atomic volume should be approximated as incompressible, compressibility date 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 date should present important information to the understanding of the mechanisms of pressure induced denaturation or reaction of proteins. Sugar solutions have large effects on the structure and properties of proteins including, their solubility and denaturation, etc. In literature, there are reports about the effect of sugars on the stability of proteins and enzymes,[13-20] which has also been explained by their effect on the structure of water[21-22]. Despite some information available on the stability of proteins by sugar, the volumetric and compressibility behavior of aqueous hemoglobin in presence of sugar has not been studied so far. Ultrasonic velocity as such does not provide any information about the nature and the relative strength of the various interactions but its derived parameters, viz., adiabatic compressibility (βs) , compressibility lowering ($\Delta\beta s$) and relative change ($\Delta\beta s / \beta^{o}$) in adiabatic compressibility, provide an information about the type and extent of intermolecular / interionic interactions among the components of a mixture. In the present work, the ultrasonic velocities and densities of aqueous solutions of hemoglobin in presence of sugar have been

measured as functions of concentration of sugar (keeping the concentration of aqueous hemoglobin solution constant) and temperature, and the parameters mentioned above have been calculated in an attempt to investigate the stabilization of hemoglobin in presence of different sugars.

EXPERIMENTAL:

MATERIALS AND METHODS:

Hemoglobin obtained from SIGMA- ALDRICH CHEMIE Gmbh steinheim Germany was used for sample preparation. D - Glucose, used were obtained perm (Qualigens Co-, India) were extra pure and used without further purification. However, before use, they are dried over P_2o_5 in desiccators. Hemoglobin solution was taken as solvent for the preparation of maltose solution of different concentration (The concentration of Haemoglobin remained unchanged) the solution were prepared by weight with laboratory double distilled water.

Density measurement: - The density of solvents and solutions were measured over the temperature range 303.15K-323.15K ($\pm$ 0.1K) using a 8ml pyknometer. The accuracy in the measurement was about $\pm$ 0.4mg/ml .The pyknometer was immersed in a thermostated paraffin bath to maintain the temperature of the system

Ultrasonic velocity measurement: - The ultrasonic velocity in protein solutions was measured by a single frequency (4 MHZ) Ultrasonic interferometer (Mittal's model M-82) which consists of two main parts: 1) The measuring cell 2) The high frequency generator.

The measuring cell is double walled cell water is circulated through the two tubes in the double walled cell to maintain constant temperature of the liquid during the experiment. There is a quartz crystal plate fixed the experiment. There is a quartz crystal plate fixed at the bottom of the cell and on the top of it is a movable metallic plate. This metallic plate is attached to the micrometer screw held on the top of the cell. The cell is connected to the high frequency generator by a co-axial cable. The high frequency generator excites the quartz plate of the cell to generate ultrasonic waves in the liquid filled in the cell. These waves are reflected back by the movable metallic plate kept parallel to the quartz plate. As a result standing waves are produced. This acoustic resonance gives rise to a maximum of anode current. If the distance between the two plates is exactly a whole multiply of sound wavelength or one- half of the wavelength the current becomes maximum. The changes in current are recorded by a micro ammeter provided with the high frequency generator. The micrometer screw is slowly raised to record the maximum current. The wavelength is determined by recording the total distance moved by the micrometer for 20 maxima of the anode current. The distance (d) this moved by the micrometer gives the value of wavelength (λ) from the relation.

$$d = n\lambda/2$$

Where 'n' is the number of maxima in the current knowing the values of frequency (v) and wavelength (λ) the ultrasonic velocity (u) in the solution is obtained from the relation .

$$u = v\lambda$$

The accuracy in the measurement was found to be $\pm$ 0.3 m/s

Result and Discussion:

The density date of aqueous hemoglobin solutions with sugars viz. D-glucose, are listed in Table–1 and Fig 1 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.

Table 1: Densities ρ (gm cm^2) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molality mol. Kg^{-1}						
0.02	1.1506	3.5192	4.6901	2.6794	2.3710	1.0217
0.04	5.6301	7.1287	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

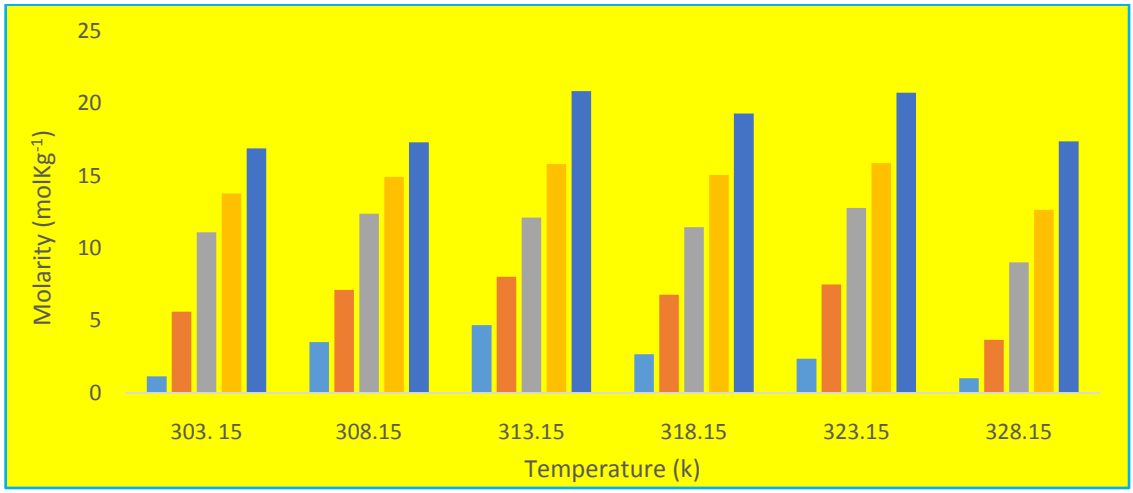

Fig 1: Densities ρ (gm cm^2) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

The ultrasonic velocities of aqueous hemoglobin solution with sugar are listed in Table-2 and Fig 2 at several temperatures. Ultrasonic velocity with increase in temperature as well as concentration of sugar in their respective system. This increase may be attributed to an increase in the intermolecular interactions with increases in temperature and concentration.

Table 2: Ultrasonic velocities, U, (m. s^1) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molalitymol. Kg^{-1}						
0.02	1517.4	1527.4	1535.8	1543.0	1548.2	1553.6
0.04	1519.3	1528.8	1537.0	1544.8	1550.8	1554.1
0.06	1522.6	1531.8	1539.1	1547.2	1553.7	1557.2
0.08	1523.3	1532.4	1540.6	1548.8	1554.9	1558.8
0.10	1524.8	1533.2	1543.5	1550.9	1557.5	1561.3

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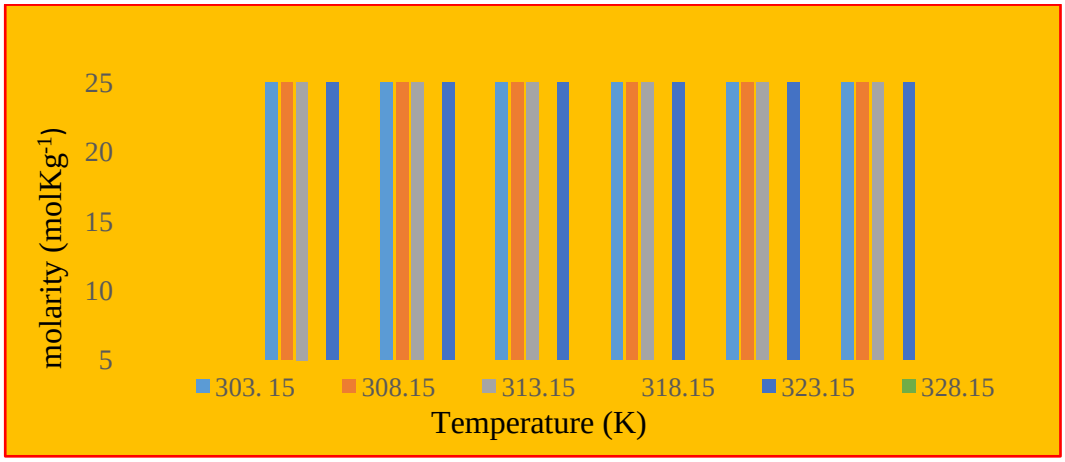

Fig 2: Ultrasonic velocities, U, (m. s^1) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

The adiabatic compressibility, (βs) is calculated employing the date of sound velocity,u, and the density'd' using the following Laplace equation

$$\beta s = 1/ud \qquad \text{..... (i)}$$

The adiabatic compressibility, βs, obtained from equation (i) decreases with increase in temperature Table -3 and Fig 3. The decrease in compressibility with increase in thermal breaking of the solvent components, which, in turn results in greater attractive force among the molecules of a solution. Decrease in the (βs) values with increase in composition is due to greater attractive force among the molecules of a liquid.

Table 3: Adiabatic Compressibility (βs X 10^{-7}, cm^2 $dyne^{-1}$) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molalitymol. Kg^{-1}						
0.02	4.3301	4.2821	4.2439	4.2128	4.1930	4.1723
0.04	4.3107	4.2666	4.2297	4.1954	4.1714	4.1612
0.06	4.2869	4.2440	4.2122	4.1757	4.1492	4.1388
0.08	4.2753	4.2331	4.1965	4.1605	4.1361	4.1237
0.10	4.2618	4.2228	4.1749	4.1426	4.1185	4.1039

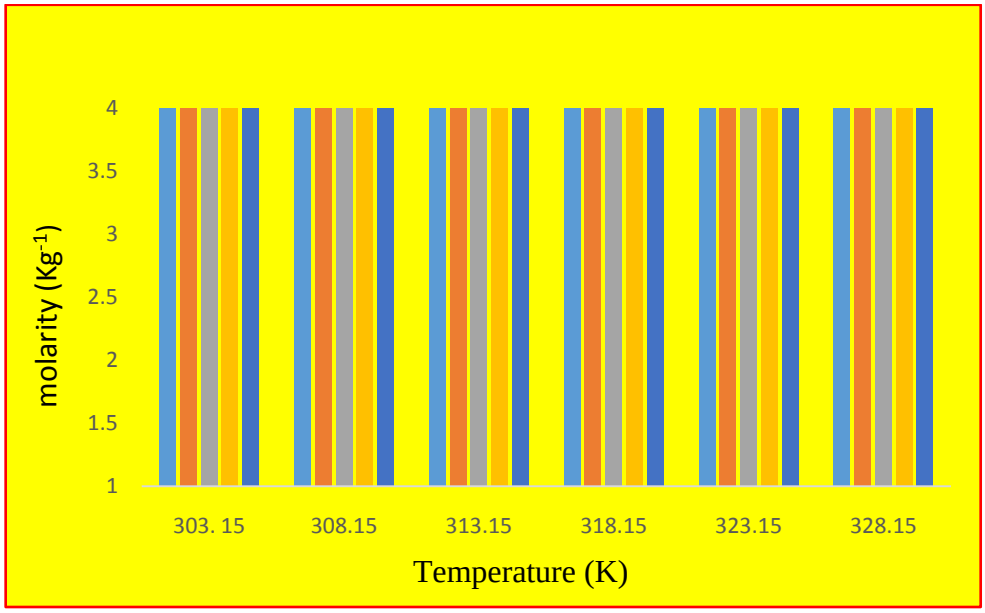

Fig 3: Adiabatic Compressibility (βs X 10^{-7}, cm^2 $dyne^{-1}$) of D – Glucose – Haemoglobin

– Water System as Functions of concentration and temperature

The experimentally determined adiabatic compressibility of a protein would mainly consist of two contributions, volume of the cavity and hydration [11]. The volume of the cavity in a protein molecule is generated by imperfect atomic packing, and change in volume occurs due to salvation or hydration. Increased pressure may squeeze cavity in the protein molecules and force water into the cavity [23]. Thus, the positive (βs) 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 βs =0 can be regarded as a compensation temperature for both factors, the packing state in the protein molecule and the protein – solvent interaction.

The compressibility lowering $\Delta\beta_s$values [24] have been obtained suing the following equation

$$\Delta\beta_s = \beta_o - \beta_s. \quad \text{..... (ii)}$$

Where β_o and β_s are the adiabatic compressibility values of solvent and solutions,

respectively. The Δβs values listed in Table – 4 and Fig 4 show an increase with increase in concentration of sugar and do not show definite trend with temperature.

Table 4: Compressibility lowering (Δs X 10^{-7}, cm^2 $dyne^{-1}$) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molalitymol. Kg^{-1}						
0.02	0.0050	0.0151	0.0200	0.0113	0.0100	0.0043
0.04	0.0244	0.0306	0.0342	0.0287	0.0316	0.0153
0.06	0.0481	0.0533	0.0517	0.0484	0.0538	0.0377
0.08	0.0598	0.0642	0.0674	0.0637	0.0668	0.0528
0.10	0.0733	0.0745	0.0890	0.0816	0.0872	0.0726

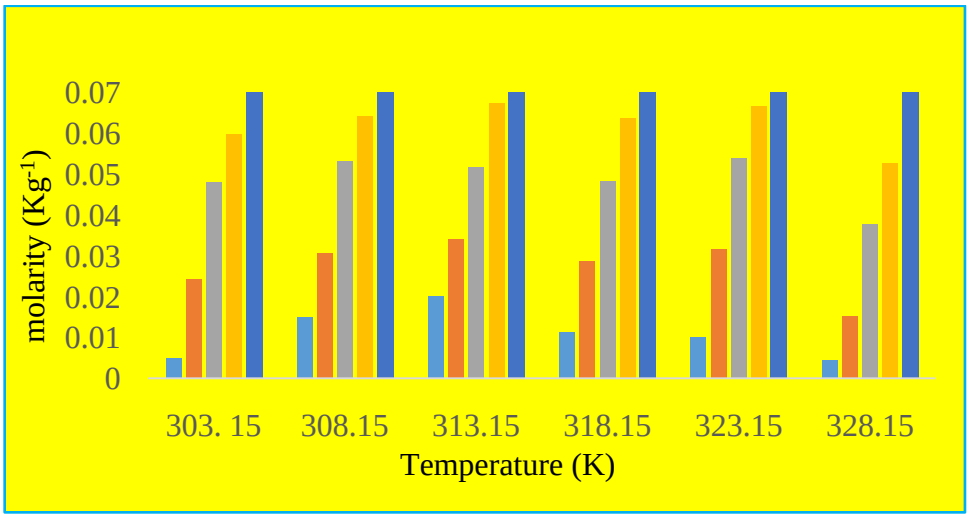

Fig 4: Compressibility lowering (Δs X 10^{-7}, cm^2 $dyne^{-1}$) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Relative change in adiabatic compressibility is calculated by using the equation.

$$\beta_r = \Delta\beta_s / \beta_o \quad \text{..... (iii)}$$

Table 5: Relative Change in Adiabatic Compressibility ($\Delta\beta s/\beta^o$ X 10^{-3}, cm^2 $dyne^{-1}$) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molalitymol. Kg^{-1}						
0.02	1.1506	3.5192	4.6901	2.6794	2.3710	1.0217
0.04	5.6301	7.1287	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

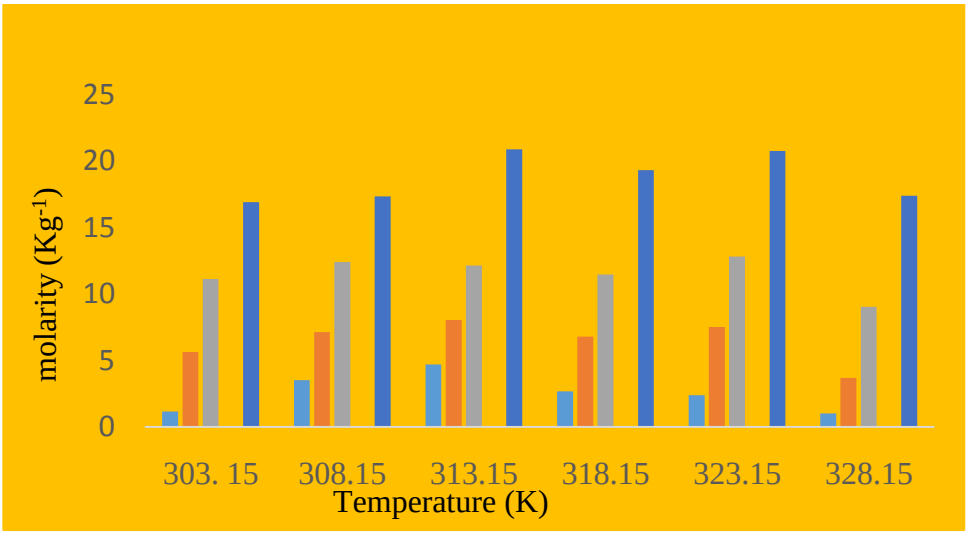

Fig 5: Relative Change in Adiabatic Compressibility ($\Delta\beta s/\beta^o$ X 10^{-3}, cm^2 $dyne^{-1}$) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

These values have been found to increase with increase in concentration and no regular trend is observed with temperature. The shows linear relationship between the relative charge in adiabatic compressibility and the solute concentration.

The apparent molal volumes ϕ_v, and the adiabatic apparent molal adiabatic compressibility $\phi_{k(s)}$ values of the hemoglobin solution with sugar have been determined from the density, d, and adiabatic compressibility β_s. of the solution using the equations.

$$\phi_v = \frac{M}{d} + \frac{(d_0 - d)10^3}{mdd_0} \qquad \text{.....(iv)}$$

and

$$\Phi_{k(s)} = \frac{1000(\beta_s d^0 - \beta_{s^0} d)}{mdd_0} + \frac{\beta_s M}{d} \qquad \text{.....(v)}$$

Where d_o is the density of water, m is the molality; M is the molecular weight of the solute. The apparent molal volumes for the systems from 303.15 to 328.15 K are presented in Table-5 and Fig 5 The apparent molal volume (ϕ_v) for the system under study, show that ϕ_v values of glucose is not very large. The apparent molal volume has been found to vary with concentration at constant temperature as envisaged by the equation [26].

Table 6: Apparent Molal Volume, ϕv (cm^3 mol^{-1}) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molalitymol. Kg^{-1}						
0.02	30.0698	29.8296	29.5872	39.4617	29.0958	39.0478
0.04	54.8856	59.752	59.6304	64.5666	59.3823	59.2687
0.06	76.3533	76.2991	76.2431	76.1934	72.7495	76.0779
0.08	79.5238	79.4827	79.4406	81.9274	79.3536	81.8588
0.10	87.3563	85.3401	85.3218	85.3045	83.2526	85.2638

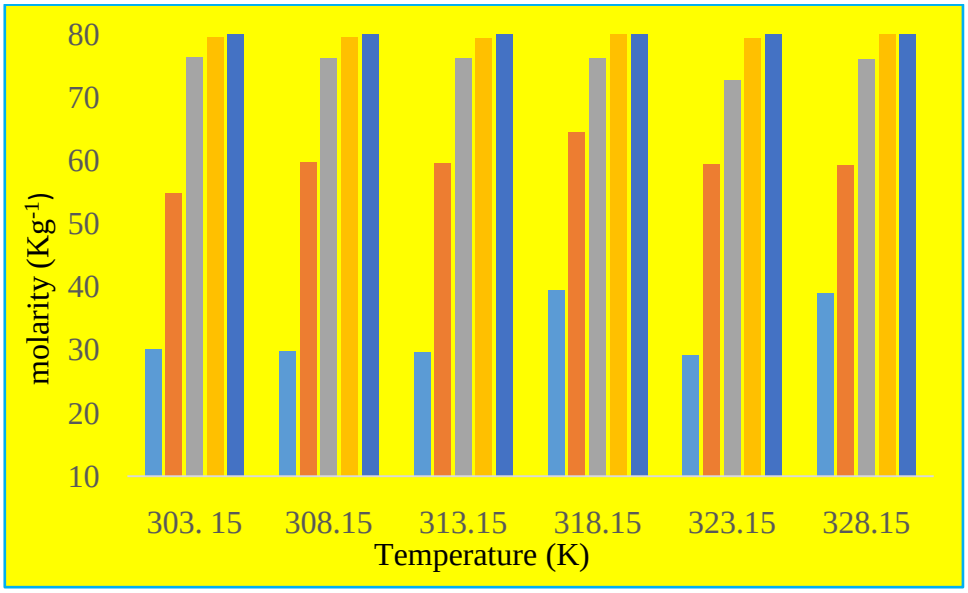

Table 6: Apparent Molal Volume,ϕv (cm^3 mol^{-1}) of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

$$\phi_v=\phi_v + S_v m \text{ ……..(viii)}$$

Where ϕ_v , the apparent molal volume at infinite dilution is referred to as partial molal volume as shown in Table- 6 and Fig 6. 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. Some typical plots of ϕ_v versus m have been displayed in Fig. A.

It has been observed that after the addition of sugar 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 the hydrophobic, 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 sugar, we can say that the extent of denaturation of protein is reduced and its stabilization has taken place.

Proteins are stabilized by a combination of hydrogen- bonding interactions, electrostatic interactions and hydrophobic interaction. In some proteins there is an additional contribution from cross- linking, metal complexing and specific binding of ions and cofactors. In discussing the effect of sugar on the stability of hemoglobin, we have to consider the effect of sugar on these various forces and interactions. In aqueous solutions of protein, there is a cooperative hydrogen – bonded structure. [21] When sugar is added to the protein solution, the OH groups of sugar may also compete for hydrogen-bonding [21]now we have to consider the respective interactions between protein, water and additive (sugar) molecules. The additive interacting more strongly with protein than with water will tend to stabilizer the denatured stated by the formation of protein additive complexes. They will, therefore, have a denaturing effect. However, additives interacting more strongly with water molecules than with protein will favour the stabilization of protein molecules [27].

Table 7: Apparent Molal Adiabatic Compressibility (ϕk X 10^{-5}, cm^3 mol^{-1} dyn^{-1} of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

Temp. (k)	303. 15	308.15	313.15	318.15	323.15	328.15
Molalitymol. Kg^{-1}						
0.02	-1.1920	-6.2993	-8.7835	-4.0296	-3.8027	-5.2544
0.04	-3.7358	-5.1245	-6.0730	-4.5090	-5.4763	-1.4049
0.06	-4.7586	-5.6555	-5.4347	-4.9319	-6.0166	-3.1980
0.08	-4.0724	-4.6721	-5.1259	-4.5974	-5.1361	-3.2924
0.10	-3.6029	-3.8570	-5.3717	-4.6688	-5.3628	-3.8323

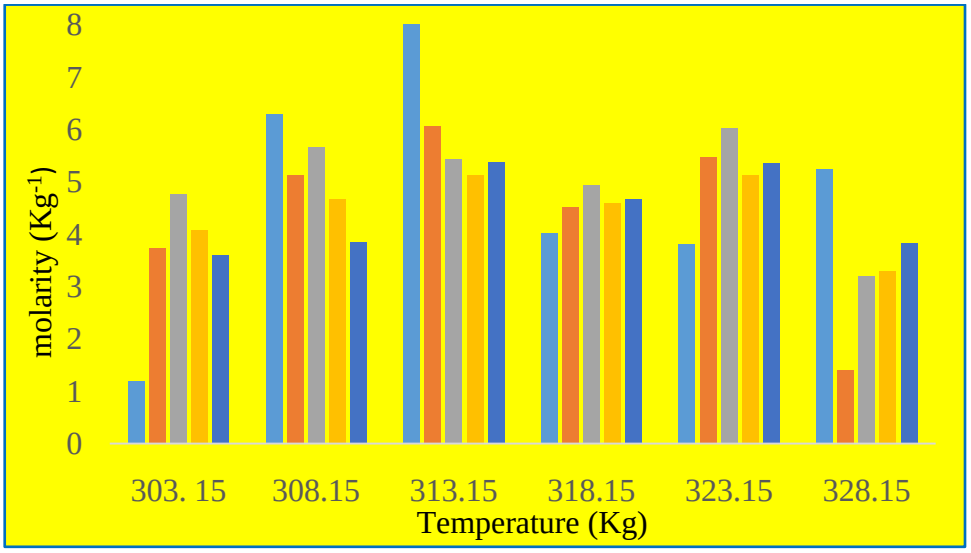

Fig 7: Apparent Molal Adiabatic Compressibility (ϕk X 10^{-5}, cm^3 mol^{-1} dyn^{-1} of D – Glucose – Haemoglobin – Water System as Functions of concentration and temperature

The apparent molal adiabatic compressibility (ϕ_k) values of aqueous solutions of hemoglobin with sugar at 303.15K-328.15K are evaluated as shown in Table-7 and Fig 7and are found to be negative at all temperatures, which can be explained by postulating that polar OH groups of sugar interact with surrounding solvent water through dipole-dipole interaction in such a way that the surrounding water loses its own compressibility to a certain extent, and degree of organization of water molecules increases by forming clusters in vicinity of protein, so it will limit the denaturation of protein. The unfavorable (or polar) environment produced by sugar molecules will increase the hydrophobic interaction in proteins thus increasing its stabilization.

Conclusion:

Measurements of density, ultrasonic velocity were made for investigating the intermolecular interactions. As D- glucose-Haemoglobin–water systems. These systems were chosen due to their vital role in the life of living organisms for such studies. Using density and ultrasonic velocity data, various derived parameters such as adiabatic compressibility (β_s), compressibility lowering ($\Delta\beta_s$), relative change in compressibility ($\Delta\beta_s/\beta_{0)}$, apparent molal volume (ϕ_v), apparent molal adiabatic compressibility (ϕ_k), and partial molal volume (ϕ_v^0), of aqueous Haemoglobin solutions in presence of sugar were obtained for each of the concentrations studied in the temperature range: 303.15-328.15k.The unfavorable (or polar) environment produced by sugar molecules will increase the hydrophobic interaction in proteins thus increasing its stabilization.

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