Asian Journal of Chemistry Vol. 22, No. 7 (2010), 5413-5418

Molecular Interaction Studies of Galactose in Aqueous α-Amylase Solution at 298 K

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The ultrasonic velocity, density and viscosity at 303 K have been measured in the ternary system of galactose with α-amylase in aqueous medium. The acoustical parameters such as adiabatic compressibility, free length, free volume and internal pressure are calculated. The results are interpreted in terms of molecular interaction between the components of the mixtures. The existence of weak interactions is confirmed by the observed excess values.

Key Words: Ultrasonic velocity, Acoustic parameters, α-Amylase, Galactose.

INTRODUCTION

Ultrasonic analysis of biological specimen had their beginning at the end of first world war. There has been substantial work on tissue studies in recent past, especially by Dunn and his group[1]. Survey of literature[2-4] reveals that there has been five broad divisions of bio-acoustical studies of which the present work deals with the characterization of the specimen using the sound velocity. The magnitude of density as well as the velocity of sound in human body fluids or constituents is of vital importance for carrying out acoustical analysis of human system or organs[5-9] since sudden excess or reduction of velocity of the wave indicates some abnormality[10,11].

The carbohydrate splitting enzymes must break down the linkages in order to form simple products[12], these are mostly α-amylases, found both in the salivary and in the pancreatic juice[13]. It is also activated by chloride with the help of Ca^{2+} ions. Another type of amylase recognized as β-amylase, acts only at the terminal reducing end of a polyglucan chain found in plants. Animal amylases including those present in human tissues are α-amylases. They attack α-1,4-linkages in a random manner anywhere along the polyglucan chain.

Large polysaccharide molecule is thus rapidly broken down into small units, as lactose and some galactose units. As both lactose and galactose are reducing sugars, the course of the hydrolytic reaction is paralleled by an increase in soluble reducing materials. However the breaking of a carbohydrate complex molecule or its subunit by hydrated amylase is found to be better than that by a pure amylase molecule, as

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the formation of mixture shows precipitation in the second case[13]. If the addition of enzyme to galactose to split then the components of the medium may have large interactions, as the medium is having more number of independent constituents. To ascertain this situation, a systematic study is required. The amylase activity in blood serum and urine are largely of use in the diagnosis of the pancreas and in the investigation of pancreatic function[14]. Hence, in order to investigate the actual picture in the breaking mechanism of the polysaccharide by hydrated amylase enzyme, the ultrasonic technique has been employed for the first time. The present work deals with the measurements of sound velocity, density and viscosity in the ternary system of amylase in the aqueous solution at room temperature.

Sample preparation: A 6 % standard solution of amylase was prepared initially. From the stock solutions, various dilutions (0.5-6 %) were obtained by adopting dilution procedure[15]. Molar solutions of galactose, from 0.06 m from 0.10 m, in steps of 0.01 m were prepared by weight. All the solutions are left for 2 h and complete solubility is found. Then mixtures of amylase and galactose were made in three different proportions as 90:10, 50:50 and 10:90.

EXPERIMENTAL

The ultrasonic velocity in the liquid mixtures have been measured using an ultrasonic interferometer (Mittal type) working at 2 MHz frequency with accuracy ± 0.1 ms^{-1}. The density and viscosity are measured using a Pycknometer and an Ostwald's viscometer of accuracy of ± 0.1 kg m^{-3} and 0.001 mNs m^{-2}, respectively.

Using the measured data, the acoustical parameters such as adiabatic compressibility (β), free length (L_f), free volume (V_f) and internal pressure (π_i) and their excess parameters have been calculated using the following expressions.

$$\beta = 1/U^2\rho \quad (1)$$

$$L_f = k_T(b)^{1/2} \quad (2)$$

$$V_f = (M_{eff}U/\eta k)^{3/2} \quad (3)$$

$$\pi_i = bRT(k\eta/U)^{1/2}/(\rho^{2/3}/M^{7/6}) \quad (4)$$

$$A^E = A_{expt} - A_{id} \quad (5)$$

and

$$A_{id} = \Sigma X_i A_i \quad (6)$$

The α-amylase and galactose, supplied by SD fine Chem. have been taken in the forms of solutions. Double distilled water is used throughout the work.

RESULTS AND DISCUSSION

Table-1 shows the measured value of ultrasonic velocity, density and viscosity for the experimental mixtures. The calculated values such as adiabatic compressibility (β), free length (L_f), free volume (V_f) and internal pressure (π_i) are given in Table-2 and the excess parameters of these values are shown in Figs. 1-4.

The perusal of Table-2 shows that the sound velocity decreases as the molarity of galactose decreases in all the proportions. Further, for a given molarity, velocity is found to be maximum for 90:10 and least for 10:90 combinations that suggested

TABLE-1
ULTRASONIC VELOCITY (U), VISCOSITY (η), DENSITY (ρ) OF (AMYLASE + GALACTOSE) IN WATER AT 298 K

M	U (ms^{-1})	ρ (Kgm^{-3})	$\eta \times 10^{-3}$ (Nsm^{-2})
	90:10		
0.06	1522.3	1017.2	1.0000
0.07	1524.3	1017.7	1.0195
0.08	1526.0	1018.2	1.0408
0.09	1528.2	1018.8	1.0521
0.10	1530.2	1019.3	1.0881
	50:50		
0.06	1519.5	1010.4	0.9890
0.07	1521.1	1011.5	1.0057
0.08	1522.6	1012.6	1.0239
0.09	1524.4	1013.4	1.0421
0.10	1526.2	1014.4	1.0619
	10:90		
0.06	1512.8	1005.0	0.9499
0.07	1514.2	1005.7	0.9567
0.08	1515.3	1006.4	0.9653
0.09	1517.0	1007.2	0.9770
0.10	1519.0	1007.9	0.9901

TABLE-2
ADIABATIC COMPRESSIBILITY (β), FREE LENGTH (L_f), FREE VOLUME (V_f) AND INTERNAL PRESSURE (π_i) FOR VARIOUS MOLARITY OF (AMYLASE + GALACTOSE) IN WATER AT 298 K

M	$\beta \times 10^{-10}$ ($N^{-1}m^2$)	$L_f \times 10^{-11}$ (M)	$V_f \times 10^8$ (m^3mol^{-1})	$\pi_i \times 10^6$ (Nm^{-2})
		90:10		
0.06	4.2421	4.1096	1.7670	2695
0.07	4.2289	4.1032	1.7206	2740
0.08	4.2174	4.0976	1.6711	2747
0.09	4.2027	4.0905	1.6484	2761
0.10	4.1897	4.0841	1.5706	2806
		50:50		
0.06	4.2563	4.2863	1.7348	2739
0.07	4.2826	4.2726	1.6966	2760
0.08	4.2599	4.2599	1.6559	2783
0.09	4.2465	4.2465	1.6176	2804
0.10	4.2322	4.2322	1.5771	2829
		10:90		
0.06	4.3479	4.1605	1.7729	2748
0.07	4.3368	4.1552	1.7601	2754
0.08	4.3274	4.1507	1.7425	2762
0.09	4.3143	4.1444	1.7180	2774
0.10	4.2999	4.1375	1.6901	2787

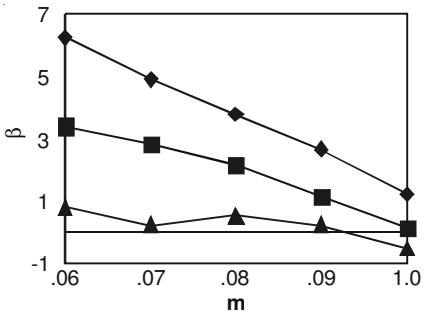

Fig. 1. Molarity *versus* excess adiabatic compressibility

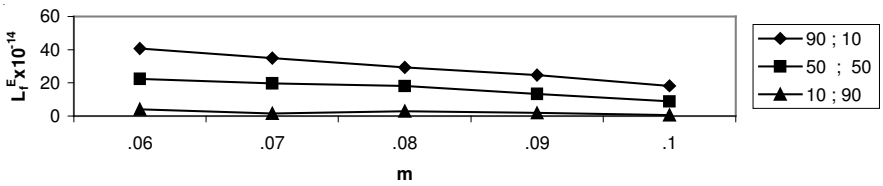

Fig. 2. Molarity *versus* excess free length

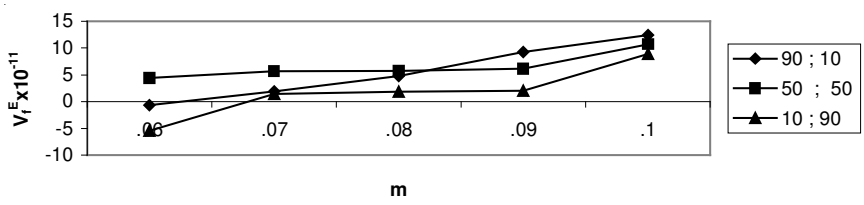

Fig. 3. Molarity *versus* excess free volume

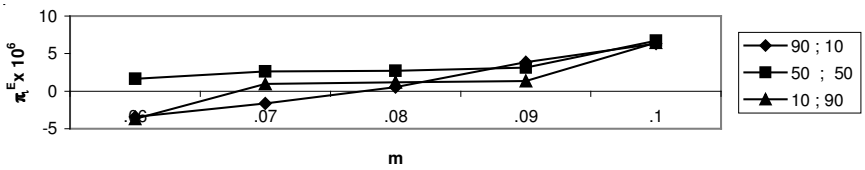

Fig. 4. Molarity *versus* excess internal pressure

that the sound velocity is highly influenced by amylase as well as galactose. However, the non-linear variation of sound velocity with the molarity of galactose indicates the presence of interaction between the components of the mixture[16].

The galactose molecules are having very low molecular weight and the molarity taken up for the present work is also small. Further the heavier molecule amylase is dispersed in a comparatively larger volume of water, the density variation is not much pronounced and mostly it closes to that of water.

The amylase normally occurring in human plasma is small molecules with molecular weight nearly 55000. The enzyme is so small enough that it can pass

through the glomeruli of the kidneys[13], which are 200 μ in diameter. Hence the available surface area of amylase molecule is smaller than the galactose molecule. This is reflected in the rapid decrease of viscosity (η) with the reduction of amylase proportion.

Galactose molecule possess isomerism and they are having either aldehyde/ ketone as their active group[17]. The increased trend of sound velocity with increase in molarity of galactose indicates the existence of strong interaction. Further, this trend predicts some formation of structure between the solute particles and solvent. Such type of complex formation tendency in some sugar solutions were found by Syal *et al.*[18]. These are again confirmed by the reverse trend of adiabatic compressibility (β) as expected.

The variation of free length (L_f) values as found in Table-2 shows the closer approach of component molecules with increase in molarity of galactose as well as amylase percentage. The amylase molecules can break the lengthier chain of carbohydrate at selected sites as stated earlier. However, the final unit obtained is galactose and it can be rarely subdivided by the amylase enzyme[19]. Hence, the addition of galactose or amylase increases the number of components in the medium. These are indicated by the variation of free volume with the molarity of galactose as well as with variation of amylase. The same is further supported by the reverse trend shown by internal pressure.

As galactose molarity is increased or amylase proportion is increased, it is expected that splitting will be enhanced. However, all the splitted components are held together by the existing adhesive forces between the components, that leads to increasing trend of internal pressure (π_i) with galactose molarity. The changes of cohesive force to adhesive forces are noticed by Palaniappan *et al.*[20] in some ternary system. This confirms the existence of strong interaction between the components of the mixture.

In order to confirm the interactions and their strength, excess value of these parameters have been calculated and are shown Figs. 1-4. The trend observed in excess β^E and L_f^E is same and are positive. In 90:10 and 50:50, these parameters show a monotonous decrease with respect to increasing molarity of galactose. The monotonous trend and positive β_E (or L_f^E) shows that the components are far away from each other, as molarity of galactose is increased. This set away approach arises due to the strong repulsion type adhesive nature of the components of the mixture. It is to be noted that the addition of either galactose or amylase will increase the activation of the enzyme and so more number of splitted carbohydrate components can be seen in the system. However in 10:90 combination at 0.08 m, the β^E shows a peak. This minimum value of experimental β appears as amylase gains all its enzymatic activitiy (above critical acitivity)[19] and hence galactose-galactose interaction predominates all other and so experimental β^E and L_f^E reaches minimum. However, as galactose molarity is further increased, this critical condition is lost and so again β_E (and L_f^E) decreases. However as regards the excess free

volume and excess internal pressure, for 90:10 and 10:90 combinations, the values changes from negative to positive whereas for 50:50, they remain positive. Further in all the cases, an increasing trend is observed with increasing molarity. The observed trend suggests that the electrostatic coulomb force that governs the components of the mixture make them to be well separated from each other. However, the columbic attractions are also significant evidenced by the change of sign. This type of environment where in the free charges or radicals have a key role is an indication for the existence of strong interaction[21-23]. Further, the addition of galactose leads to a gradual development adhesive forces rather that the cohesion and this confirms our view obtained earlier.

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(*Received*: 12 October 2009; *Accepted*: 26 March 2010) AJC-8568