Effect of lactose on the viscometric behaviour of L-threonine in aqueous solutions over the temperature range of (293.15 to 313.15) K

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Abstract: The experimental data for viscosity of L-threonine (0.025 to 0.2 mol kg^{-1}) in aqueous lactose (0 to 6 mass % of lactose in water) has been obtained at temperatures of (293.15, 298.15, 303.15, 308.15 and 313.15) K. Viscosity *B*-coefficients, viscosity *B*-coefficients of transfer (B_{tr}), variation of *B* with temperature (*dB*/*dT*) and solvation behavior (B/V°_{ϕ}) of L-threonine have been determined using viscosity data. The results have been interpreted in terms of various molecular interactions prevalent in these systems and moreover, it has been seen that the L-threonine acts as structure maker in all the aqueous-lactose solutions of study.

Key words*:* Viscosity, L-threonine, Lactose, Viscosity B-coefficients, Activation parameters

1. Introduction

Saccharides are of arch significance due to its wide distribution in a number of living organisms and are located with a gigantic range of complexity ranging from monosaccharides to polysaccharides. Saccharides are substantial as they have the hydroxyl (–OH) rich periphery, coordinating ability, stereospecificity, homochirality etc. [1–5]. Thermal stability of proteins or the extent of their denaturation can be increased using saccharides by adding other reagents.

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During lyophilisation properties, disaccharides are used as cryoprotectants versus the destabilizing and degradation of enzymes and drugs. Water structuring characteristics of the solutes are exploited by the organisms in a lot of ways, so as to correct the viscosity of cellular fluids and to protect against freezing and dehydration [6,7]. Being essential part of many biomacromolecules like glycoproteins, glycolipids, nucleic acids, etc., saccharides involve in a number of biologically significant processes such as fertilization, development, pathogenesis, etc. [8,9].

Storage of biomedical materials is also possible by using saccharides [10]. The solution properties of saccharides are of substantive interest for a lot of aspects in many applications and in fundamental research as well [11,12]. It has been seen that saccharides in general, and disaccharides (trehalose, sucrose and lactose) in particular can stabilize unstable biomolecules in aqua solutions by a blend of both kinetic and specific effects [6]. Like saccharides, proteins are too vital class of compounds in the processes of all living organisms. Amino acids are the low molecular weight compounds which are regarded as the model components of protein [13-18]. Amino acids can be used for studying the expected impact of solvation and conformation of proteins [19, 20]. The complex mechanism involved in conformational and configurational factors for determining the structure of proteins in saccharide solutions makes the study of protein-saccharide

interactions extremely difficult. Thus, one applicable way is to study the basic components of proteins i.e., amino acids in water and mixed aqueous solutions of saccharides. The *B* coefficients calculated using the Jones–Dole equation and obtained from viscosity values are considered as a very important parameter to describe the kosmotropic and chaotropic (structure maker or structure breaker) nature of solute in various solvents. So, by the study of transport properties of amino acids in the aqueous-saccharides solutions, one can get some very fruitful information about the various molecular interactions in solutions, which are further useful in understanding the mechanism involved in the protein stabilization [21].

Although, a elaborative and detailed study of transport properties of amino acids has been known [22-26], however, rheological studies of the interactions between amino acids and saccharides in the water are meager [27-30]. So, this compelled us to probe the viscometric behaviour of L-threonine with aqueous-lactose solutions. The present paper is continuation of our previous work on amino acid in saccharides [31]. In this work, we have obtained the viscosity values of L-threonine in the concentration range (0.025 to 0.2 mol kg^{-1}) in water and various aqueous-lactose (0 to 6 mass % of lactose in water) solutions at 293.15, 298.15, 303.15, 308.15 and 313.15 K. Using the determined viscosity data, Falkenhagen and Jones-Dole coefficients, viscosity *B*-coefficients of transfer and solvation behaviour have been evaluated. Moreover, the data has been interpreted in terms of various molecular interactions present in the system and moreover, it has been found out that L-threonine act as structure maker in several aqueous-lactose solutions.

2. Experimental design

L-threonine (mass fraction purity >99.8%) obtained from Sigma Aldrich, India, was used after keeping over anhydrous calcium chloride in a vacuum desiccator overnight at room temperature while α-lactose monohydrate (Sigma Aldrich, India, mass fraction purity >99.8%) which was used as such. The solutions were prepared freshly by mass using Mettler balance (Model AE 240) with a precision of ±0.01 mg in doubly distilled- deionised water. The stock solutions of 2% lactose, 4% lactose and 6% lactose in water were used as the solvent to prepare L-threonine solution of eight different molalities (ranging from 0.0 to 0.2) mol kg^{-1}. All the solutions were prepared with precaution and stored in special airtight bottles to avoid the exposure to air and evaporation. The viscosity of the solutions was measured by using Ubbelohde type suspended level viscometer, calibrated at 298.15 K with distilled water and pure methanol. In order to avoid the thermal fluctuation of solutions in viscometer, the solution was allowed to stand for about half an hour in a thermostatic water bath. The time of flow was noticed using an electronic watch with the resolution of 0.01s. The average of at least five readings reproducible within ±0.1 s was used with sufficient care to minimize the evaporation loss. The uncertainty in measurements of viscosity was within ±1 × 10^{-6} N s m^{-2}. The temperature of the sample solution was maintained to an accuracy of ±0.02 K using an electronic controlled thermostatic water bath (Model: TIC-4000N, Thermotech, India).

3. Results and discussion

Viscometric studies

The experimental viscosity data of L-threonine in the pure water and aqueous solutions of lactose (0 to 6 mass % of lactose in water) as a function of molalities of L-threonine and temperature (Table, 1) and the viscosities of L-threonine in solutions {representative 3-D plot (Fig.,1) of viscosity, η vs m, molality of L-threonine in 2% aqueous-lactose solutions} increase with increase in the molal concentration of L-threonine but decrease with the rise of temperature.

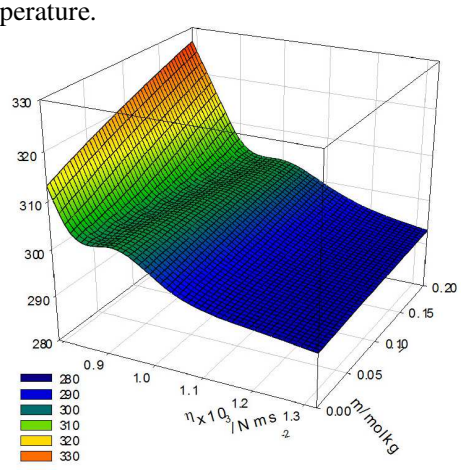

Figure 1. Variation of viscosity (η) with molality (m) for L-threonine in 6% aqueous-lactose solution at (293.15, 298.15, 303.15, 308.15 and 313.15) K.

Table 1. Viscosity (η) and reduced viscosity $\{(\eta_r-1)/m^{1/2}\}$ of solutions of L-threonine in water, aqueous–lactose solutions as function of molality of L-threonine at different temperatures

m (mol kg^{-1})	T (K) 293.15		298.15		303.15		308.15		313.15	
	$\eta \times 10^3$ (N s m^{-2})	$(\eta_r-1)/m^{1/2}$ (mol$^{-1/2}$ kg$^{1/2}$)	$\eta \times 10^3$ (N s m^{-2})	$(\eta_r-1)/m^{1/2}$ (mol$^{-1/2}$ kg$^{1/2}$)	$\eta \times 10^3$ (N s m^{-2})	$(\eta_r-1)/m^{1/2}$ (mol$^{-1/2}$ kg$^{1/2}$)	$\eta \times 10^3$ (N s m^{-2})	$(\eta_r-1)/m^{1/2}$ (mol$^{-1/2}$ kg$^{1/2}$)	$\eta \times 10^3$ (N s m^{-2})	$(\eta_r-1)/m^{1/2}$ (mol$^{-1/2}$ kg$^{1/2}$)
L-threonine + water										
0.000	1.0019		0.8903		0.7973		0.7190		0.6526	
0.025	1.0010	0.0492	0.8981	0.0554	0.8035	0.0489	0.7245	0.0486	0.6569	0.0422
0.050	1.0187	0.0751	0.9058	0.0780	0.8098	0.0700	0.7301	0.0688	0.6613	0.0596
0.075	1.0273	0.0925	0.9131	0.0934	0.8160	0.0854	0.7355	0.0839	0.6652	0.0707
0.100	1.0356	0.1063	0.9201	0.1058	0.8219	0.0976	0.7408	0.0959	0.6698	0.0835
0.125	1.0440	0.1189	0.9278	0.1190	0.8283	0.1100	0.7460	0.1061	0.6741	0.0932
0.150	1.0526	0.1306	0.9356	0.1313	0.8345	0.1206	0.7514	0.1164	0.6791	0.1049
0.175	1.0615	0.1423	0.9434	0.1425	0.8413	0.1318	0.7570	0.1262	0.6832	0.1121
0.200	1.0699	0.1518	0.9509	0.1521	0.8470	0.1393	0.7620	0.1338	0.6877	0.1204
L-threonine + 2% aqueous-lactose										
0.000	1.1704		0.9729		0.8430		0.7774		0.7765	
0.025	1.1840	0.0735	0.9833	0.0677	0.8518	0.0663	0.7848	0.0601	0.7828	0.0513
0.050	1.1968	0.1009	0.9933	0.0940	0.8605	0.0930	0.7925	0.0869	0.7898	0.0764
0.075	1.2100	0.1236	1.0038	0.1159	0.8696	0.1153	0.7997	0.1046	0.7957	0.0901
0.100	1.2235	0.1434	1.0140	0.1335	0.8783	0.1325	0.8072	0.1210	0.8027	0.1067
0.125	1.2370	0.1610	1.0250	0.1516	0.8870	0.1477	0.8152	0.1375	0.8095	0.1202
0.150	1.2503	0.1762	1.0360	0.1675	0.8960	0.1622	0.8222	0.1488	0.8166	0.1332
0.175	1.2638	0.1908	1.0461	0.1798	0.9047	0.1749	0.8299	0.1615	0.8237	0.1453
0.200	1.2777	0.2049	1.0568	0.1927	0.9128	0.1850	0.8375	0.1729	0.8303	0.1548
L-threonine + 4% aqueous-lactose										
0.000	1.1856		1.0147		0.9101		0.8098		0.7887	
0.025	1.2039	0.0975	1.0276	0.0802	0.9217	0.0804	0.8191	0.0729	0.7977	0.0720
0.050	1.2222	0.1379	1.0408	0.1152	0.9335	0.1149	0.8284	0.1026	0.8066	0.1014
0.075	1.2405	0.1689	1.0541	0.1419	0.9453	0.1412	0.8374	0.1245	0.8155	0.1241
0.100	1.2574	0.1916	1.0666	0.1618	0.9570	0.1631	0.8471	0.1456	0.8238	0.1405
0.125	1.2733	0.2093	1.0814	0.1858	0.9676	0.1788	0.8561	0.1618	0.8320	0.1553
0.150	1.2917	0.2310	1.0947	0.2035	0.9798	0.1977	0.8664	0.1805	0.8411	0.1715
0.175	1.3088	0.2485	1.1076	0.2188	0.9907	0.2117	0.8759	0.1952	0.8501	0.1861
0.200	1.3233	0.2598	1.1215	0.2353	1.0030	0.2283	0.8856	0.2093	0.8591	0.1996
L-threonine + 6% aqueous-lactose										
0.000	1.2455		1.0405		0.9167		0.8608		0.8737	
0.025	1.2667	0.1076	1.0565	0.0971	0.9299	0.0910	0.8716	0.0796	0.8841	0.0749
0.050	1.2868	0.1483	1.0718	0.1347	0.9442	0.1340	0.8833	0.1171	0.8943	0.1056
0.075	1.3074	0.1813	1.0884	0.1681	0.9573	0.1617	0.8952	0.1460	0.90478	0.1299
0.100	1.3279	0.2092	1.1050	0.1960	0.9710	0.1875	0.9063	0.1672	0.9152	0.1503
0.125	1.3491	0.2352	1.1217	0.2208	0.9843	0.2085	0.9176	0.1865	0.9263	0.1702
0.150	1.3704	0.2588	1.1378	0.2414	0.9979	0.2287	0.9295	0.2060	0.9374	0.1883
0.175	1.3903	0.2779	1.1533	0.2592	1.0121	0.2489	0.9418	0.2248	0.9489	0.2057
0.200	1.4111	0.2973	1.1695	0.2773	1.0244	0.2628	0.9533	0.2402	0.9590	0.2183

This is also true for the other viscosity results of L-threonine in water and other aqueous-lactose solutions. Lowering of viscosity as a result of increase in temperature may be due to the accelerated molecular motion in the system. The viscosity values have been fitted using the Jones-Dole equation [32] which explains the relative viscosities in the following form:

$$\eta_r = \frac{\eta}{\eta_o} = 1 + Am^{1/2} + Bm \quad \text{..... 1}$$

where η_r is the relative viscosity, η_o and η are the viscosities of solvent (aqueous-lactose) and solution, respectively. *A* is a constant arising from the ion-ion or solute–solute interactions and is called Falkenhagen coefficient, while *B* is the Jones-Dole coefficient, which measures the size, shape, charge and structural effects induced by solute-solvent interactions [33,34]. Jones-Dole *B* coefficient can be explained in terms of competition among the special viscosity effects at a given concentration [35] and is regarded as an important parameter to study solute-solvent interactions in the systems. The *A*-coefficients for L-threonine are found to be much smaller in magnitude as compared to *B*-coefficients and hence, can be considered negligible in case of non-electrolytes [36]. The positive viscosity *B*-coefficients (given in Table 2) for L-threonine are indicative of strong solute-solvent interactions over the weak solute-solute interactions, since L-threonine is strongly hydrated solute and therefore, shows a larger change in the viscosity of the solution with concentration.

Table 2. Viscosity *B*-coefficients, viscosity *B*-coefficients of transfer (B_{tr}) of L-threonine in aqueous-lactose solutions at different temperatures and temperature coefficients (*dB/dT*)

System	*T* (K)					*dB/dT* (dm^3 mol^{-1} K^{-1})
	293.15	298.15	303.15	308.15	313.15	
	Viscosity *B*-coefficients (dm^3 mol^{-1})/ Viscosity *B*-coefficients of transfer (dm^3 mol^{-1})					
L-threonine + water	0.351 (±0.004)	0.333 (±0.004)	0.314 (±0.003)	0.294 (±0.002)	0.272 (±0.005)	-0.004 (±0.001)
L-threonine + 2% aqueous-lactose	0.457 (±0.004) /0.106	0.437 (±0.005) /0.104	0.414 (±0.003) /0.100	0.388 (±0.003) /0.094	0.358 (±0.005) /0.086	-0.005 (±0.001)
L-threonine + 4% aqueous-lactose	0.564 (±0.001) /0.213	0.536 (±0.005) /0.203	0.506 (±0.005) /0.192	0.473(±0.005) /0.179	0.436 (±0.005) /0.164	-0.006 (±0.001)
L-threonine + 6% aqueous-lactose	0.661 (±0.005) /0.310	0.631 (±0.006) /0.298	0.594 (±0.006) /0.280	0.552 (±0.005) /0.258	0.502 (±0.006) /0.230	-0.008 (±0.001)

The values *B*-coefficients are estimated using a plot between $(\eta_r\text{-}1)/m^{1/2}$ and $m^{1/2}$ by least squares analysis method, found to be linear at all concentrations and temperatures. The values of reduced viscosity, $(\eta_r\text{-}1)/m^{1/2}$ for L-threonine are given in Table 1 and plotted in Fig.2 (L-threonine in 2% aqueous-lactose solutions).

As mentioned earlier, the viscosity *B*-coefficients provide useful information regarding the solvation of the solute and its effects on the structure of solvent in the surrounding of the solute molecules. An analysis of Table 2 reveals that the values of viscosity *B*-coefficients for L-threonine in water agree very well with available literature value [33]. The observed viscosity *B*-coefficients for L-threonine in water and aqueous-lactose solutions are positive indicating that the solute-solvent interactions are strong at all the studied molalities and temperatures. Viscosity *B*-coefficients for L-threonine in aqueous-lactose solutions are larger than in water indicating that in the presence of solute (L-threonine), the structure of solution gets strengthened. However, as for as the dependence of *B*-coefficient values on solvent systems, it has been seen that it increase with increase in the mass% of aqueous-lactose solutions. An increase in viscosity *B*-coefficient values as we go from lower mass% to higher mass% (as shown in Table 2) may be owing to the formation of structure that allows the solute to act on solvent and reinforce its structure by hydrogen bonding [37].

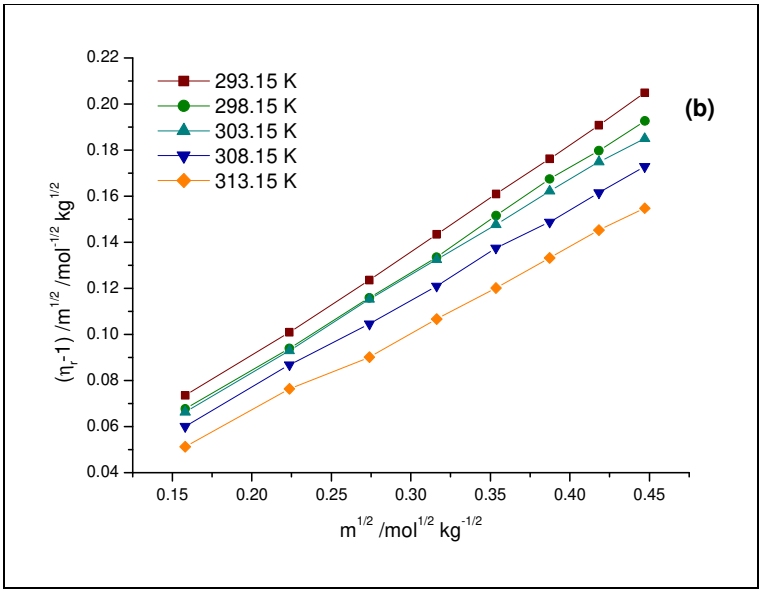

Figure 2. Variation of reduced viscosity $\{(\eta_r-1)/m^{1/2}\}$ with molality $(m^{1/2})$ of L-threonine in 2% lactose at different temperatures.

Thus, the inference drawn from viscosity *B*-coefficients supports the behaviour of an existence of strong solute-solvent interactions in comparison to solute-solute interactions which further advocates the existence of strong ionic-hydrophillic and hydrophilic-hydrophillic interactions in the current systems. The viscosity *B*-coefficients of L-threonine decrease with an increase in temperature implying that hydration effects are sensitive to the temperature variations. An important information regarding structure-making or structure-breaking capability of the solute in solvent system is well documented by considering the temperature derivative of the viscosity *B*-coefficients, *dB/dT* [32]. Our result of L-threonine shows that *dB/dT* is negative which suggests that L-threonine acts as structure maker in all mass% of aqueous-lactose solutions. The structure-making ability of L-threonine in aqueous-glucose solutions and in aqueous-sucrose solutions has also been observed by Nain *et al.* [33] and Xiofen *et al.* [40].

The viscosity *B*-coefficients of transfer, B_{tr}, of L-threonine from water to aqueous-lactose solutions has been evaluated using the relation:

$$B_{tr} = B_{aq\text{-}saccharides} - B_{water} \quad \text{.....(2)}$$

where B_{water} is the viscosity *B*-coefficients of L-threonine in water. B_{tr} values are calculated by taking the difference between viscosity *B*-coefficients in aqueous-lactose solutions and water for each studied aqueous-lactose solutions. The B_{tr} values are reported in Table 2. The increase in B_{tr} values with the increase in concentration is due to interactions of lactose with either R groups or charged centers of L-threonine. In other words, the main contribution to viscosity *B*-coefficients of transfer values comes from the interactions between charged centers of L-threonine and lactose molecules, rather than from interactions between R groups of L-threonine and lactose molecules. With increasing temperature, B_{tr} values show a decreasing trend in all systems of study. Similar results are available in literature [41].

The ratio of *B*-coeffcient to apparent molar volume (B/V°_{ϕ}) at infinite dilution can be used to determine the solvation. These values are major pointer for solvated and unsolvated solute species. For an unsolvated spherical species, the value of B/V°_{ϕ} lies between 0 and 2.5 [38,39]. A higher value is pointer of solvated spherical solute molecules. The higher B/V°_{ϕ} values (Table 3) for the evaluated L-threonine in various aqueous-lactose solutions relative to water, show that the L-threonine are largely solvated in the presence of lactose. The values of B/V°_{ϕ} in case of L-threonine decreases with temperature which may be attributed to the competition among various interactions operating in these systems.

Table 3. Ratio of viscosity *B*-coefficient to limiting apparent molar volume (B/V°_{ϕ}) values, for L-threonine in water and aqueous-lactose solutions at different temperatures

System	*T* (K)				
	293.15	**298.15**	**303.15**	**308.15**	**313.15**
	B/V°_{ϕ}				
L-threonine + water	4.58	4.32	4.06	3.78	3.48
L-threonine + 2% aqueous-lactose	5.88	5.61	5.31	4.96	4.57
L-threonine + 4% aqueous-lactose	7.21	6.83	6.44	6.01	5.53
L-threonine + 6% aqueous-lactose	8.39	7.99	7.50	6.96	6.32

Conclusions

The viscosity of solutions of L-threonine in water and in aqueous-lactose solutions (0 to 6 mass % of lactose in water) are determined at different temperatures. From the experimental viscosity results, Jones-Dole coefficient, *dB/dT*, B_{tr}, solvation and the various activation parameters are evaluated. The positive viscosity *B*-coefficient and B_{tr} values are indicative of strong amino acid-saccharide-water interactions than amino acid-amino acid interactions. The negative values of *dB/dT* have been argued for the structure-making behaviour of L-threonine in aqueous-lactose solutions.

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