SUMMARY

Saccharides and their derivatives being essential components of living systems, perform multiple roles from the storage and transport of energy to participation in the immune system, signaling, cell-cell recognition, molecular and cellular communication, etc. Saccharides are well known to stabilize proteins and lipid bilayers *in vitro* during dehydration. Different theories have been proposed to explain the stabilization effects of saccharides, but the mechanism of protein stabilization is still not clear. Disaccharides (particularly trehalose and sucrose) are well known for their ability to preserve life in cells, organisms, and biomolecules under conditions of extreme drought or low temperatures. Several mechanisms for the antidesiccant properties of saccharides have been proposed, but their protective mechanisms are not well understood.

Due to the recognition of saccharides in various biological processes, the knowledge about hydration characteristics of these compounds in aqueous solutions is very important. Thermodynamic and transport properties are very useful in providing information regarding the solvation behaviour of saccharides and play pivotal role in the study of protein stabilization, antidesiccation, taste qualities, feasibility and optimization of industrial processes.

Saccharides readily undergo isomerization and conformational changes, their complexation with metal ions result in the formation of a mixture of products, although interactions involved are weak, but selective. For example, the addition of disodium tetraborate (borax) to aqueous solutions of saccharides and other polyols results in various complexes between the borate ion and the saccharides. But, their binding preferences, structural and stereochemical features and stabilities of the complexes formed are not well understood. The literature survey shows that studies on saccharides in organic salts are missing.

Therefore, in the present work, the apparent molar volumes, $V_{2,\phi}$, apparent molar isentropic compressibilities, $K_{s,2,\phi}$ and viscosities, $\eta$ of selected monosaccharides: D(+)xylose, D(−)-arabinose, D(−)-ribose, L(−)-sorbose, D(−)-fructose, D(+)galactose, D(+)glucose, and D(+)-mannose, disaccharides: D(+)cellobiose, sucrose, D(+)melibiose, D(+)lactose monohydrate, D(+)trehalose dihydrate and D(+)maltose monohydrate, trisaccharides: D(+)melezitose and D(+)raffinose pentahydrate have been determined in
water and in aqueous solutions of various co-solutes (additives); sodium acetate trihydrate, disodium tetraborate decahydrate, and sodium gluconate at \( T = (288.15, 298.15, 308.15, \text{ and } 318.15) \) K. The vibrating-tube digital densimeter (Model DMA 60/602, Anton Paar, Austria), multifrequency ultrasonic interferometer (M-81 Mittal Enterprises, India) and Ubbelohde type capillary viscometer were used for density, speed of sound and efflux time measurements, respectively. Further, these data were utilized to evaluate the standard partial molar volumes, \( V_2^* \), isentropic compressibilities, \( K_{s,2}^* \) at infinite dilution, viscosity \( B \)-coefficients and the corresponding transfer parameters (\( \Delta V_2^* \), \( \Delta K_{s,2}^* \) and \( \Delta B \)), the apparent specific volumes (ASV), apparent specific isentropic compressibilities (ASIC), hydration numbers (\( n_H \)), expansion coefficients (\( \partial V_2^*/\partial T \)P) and the second derivatives (\( \partial^2 V_2^*/\partial T^2 \)P), the \( dB/dT \) coefficients, pair and triplet interaction coefficients and activation parameters (\( \Delta \mu_2^* \#, \Delta H_2^# \# \) and \( \Delta S_2^# \# \)) of viscous flow.

The standard partial molar volumes, \( V_2^* \) and the standard partial molar volumes of transfer, \( \Delta V_2^* \) of various saccharides in \( m_B \) (molality of sodium acetate) = (0.5, 1.0, 2.0, and 3.0) mol kg\(^{-1}\) aqueous sodium acetate solutions at \( T = (288.15, 298.15, 308.15, \text{ and } 318.15) \) K generally increase with their complexity from mono- to di- to tri-saccharides. An increase in the \( \Delta V_2^* \) values from aldopentoses to aldo/ketohexoses, may be due to the additional –CHO group. Among monosaccharides, the lower values for L(\( – \))-sorbose and D(\( – \))-fructose (ketohexoses) than aldohexoses, may be due to the different nature of the keto (\( \text{>C}=\text{O} \)) and aldehyde (–CHO) groups. The steric strain due to the presence of five-membered rings in cases of L(\( – \))-sorbose and D(\( – \))-fructose may be responsible for the lower \( \Delta V_2^* \) values and this effect seems to be more in the case of L(\( – \))-sorbos.

According to the cosphere overlap model, the positive \( \Delta V_2^* \) values obtained for most of the saccharides studied suggest that the hydrophilic-ionic interactions predominate over the hydrophobic-ionic interactions. Furthermore, the systematic increase in \( \Delta V_2^* \) values with concentration of sodium acetate in almost all the cases points towards the strengthening of the hydrophilic-ionic interactions over the whole concentration range. However, the negative \( \Delta V_2^* \) values observed for L(\( – \))-sorbose, D(\( + \))-xylose and D(\( – \))-fructose, suggest the predominance of hydrophobic-ionic interactions. The negative contribution to the \( \Delta V_2^* \) from hydrophobic-ionic interactions decreases and that the positive contribution from hydrophilic-ionic interactions increases with the rise of temperature. Thus, there is a strong competition between various interactions occurring in these solutions, depending upon the nature of the saccharide, concentration of the co-solute and temperature of the solution.
From volumetric studies, it has been reported that sodium acetate behaves as a structure breaker in water. Due to the stronger interactions between saccharides and Na⁺/CH₃COO⁻ ions, their effect on the water structure is decreased. Dehydration of ions (Na⁺ and CH₃COO⁻) and solute (saccharide) molecules may also make positive contributions to the Δ₁V₂⁺ values. According to Shahidi’s equation, the standard partial molar volume of a saccharide molecule at infinite dilution can be represented as:

\[ V_2 = V_{v.w} + V_{\text{void}} - V_{\text{shrinkage}} \]

if we assume that \( V_{v.w} \) (van der Waal’s volume) and \( V_{\text{void}} \) (associated void/empty volume) have the same magnitudes in water and aqueous sodium acetate solutions, the positive Δ₁V₂⁺ values obtained for most of the saccharides can be attributed to the decrease in the volume of shrinkage in aqueous sodium acetate solutions, whereas reverse will be true for the negative Δ₁V₂⁺ values. Due to saccharide (solute) and sodium acetate (co-solute) interactions, the partial molar volumes of saccharides are modulated as the effect of saccharides and sodium acetate on the structure of water is altered.

The Δ₁V₂⁺ values of saccharides in \( m_B = (0.05 \text{ and } 0.10) \) mol·kg⁻¹ at \( T = 288.15 \) K and in \( m_B = (0.05, 0.10, 0.12, \text{ and } 0.15) \) mol·kg⁻¹ aqueous disodium tetraborate solutions at \( T = (298.15, 308.15, \text{ and } 318.15) \) K decrease with the complexity of saccharides indicating that the monosaccharide-disodium tetraborate interactions are stronger than di- and tri-saccharides-disodium tetraborate interactions. The significant positive Δ₁V₂⁺ values indicate that hydrophilic-ionic interactions predominate over the hydrophobic-ionic interactions, suggesting the complex formation between the borate (B₄O₇²⁻) ion and saccharides. It has been reported that, in aqueous disodium tetraborate solutions, the Na⁺ ion being small in size acts as a structure maker, whereas the B₄O₇²⁻ ion is considered as a strong structure breaker. The stronger interactions between the saccharides and (Na⁺/B₄O₇²⁻) cause the reduction of electrostriction of H₂O molecules in the vicinity of saccharide molecules and consequently contribute positive to Δ₁V₂⁺. As the concentration of disodium tetraborate increases, these interactions will become stronger and therefore transfer volumes increase. The Δ₁V₂⁺ values for saccharides in disodium tetraborate have been compared with those in other co-solutes, which follow the order: Na₂B₄O₇ > Na₂SO₄ (only disaccharides) > NaCl (at 298.15 K) > NaOOCCH₃. It suggests that strong interactions exist among saccharide molecules and B₄O₇²⁻ anions in comparison to SO₄²⁻, Cl⁻ and CH₃COO⁻ anions as cation (Na⁺) is same in these electrolytes.

The Δ₁V₂⁺ values of saccharides in \( m_B = (0.25, 0.50, 1.00 \text{ and } 1.50) \) mol·kg⁻¹ aqueous sodium gluconate solutions at \( T = (288.15, 298.15, 308.15, \text{ and } 318.15) \) K are negative for all the studied saccharides, and overall their magnitudes decrease with co-solute concentration.
The magnitudes of $\Delta V_2^*$ values decrease in the following order: tri- > di- > monosaccharides, which indicates that trisaccharide-sodium gluconate interactions are stronger. The significant negative $\Delta V_2^*$ values observed in all the cases indicate that the overlap of hydration co-spheres of hydrophobic (alkyl R groups of saccharides) parts and ionic (Na$^{+}$/C$_6$H$_{11}$O$_7^-$) species predominate, suggesting strong hydrophobic effect of gluconate (C$_6$H$_{11}$O$_7^-$) anion on the transfer values of studied saccharides. This decrease in $\Delta V_2^*$ values with concentration of sodium gluconate indicates the strengthening of the hydrophobic-ionic interactions. The $\Delta V_2^*$ values for saccharides in sodium gluconate as compared to other studied sodium salts follow the order: Na$_2$B$_4$O$_7$ > Na$_2$SO$_4$ (only disaccharides) > NaCl (at 298.15 K) > NaOOCCH$_3$ > NaC$_6$H$_{11}$O$_7^-$. It suggests that sodium gluconate-saccharide interaction is the weakest as compared to other electrolytes and this may be due to the strong hydrophobic effect of C$_6$H$_{11}$O$_7^-$ anions in comparison to B$_4$O$_7^{2-}$, SO$_4^{2-}$, Cl$^-$ and CH$_3$COO$^-$ anions as cation (Na$^+$) is same in these electrolytes.

Apparent specific volumes, ASVs (calculated from apparent molar volumes) of saccharides in sodium gluconate fall in sweet taste range (0.52-0.71) cm$^3$g$^{-1}$, except for aldopenoses (D(+)-xylose, D(−)-arabinose, and D(−)-ribose) which become sour (0.33-0.52) cm$^3$g$^{-1}$ at higher concentration of sodium gluconate ($\approx$1.5 mol kg$^{-1}$). In presence of sodium acetate, the ASV values of studied saccharides increase with co-solute concentration and fall in sweet taste range. ASVs of monosaccharides in aqueous disodium tetraborate solutions shift towards the bitter taste range (0.71-0.93) cm$^3$g$^{-1}$, whereas ASVs of di- and trisaccharides remain in sweet taste range. This variation in ASVs of studied saccharides in presence of various co-solutes (additives) as compared to their values in water, suggests that with increase in size of anion (as cation, Na$^+$ is same in these salts), the taste quality of studied saccharides deviates from sweet taste. Borate anion (B$_4$O$_7^{2-}$) makes some monosaccharides bitter, gluconate anion (C$_6$H$_{11}$O$_7^-$) makes few monosaccharides sour in taste, whereas acetate anion (CH$_3$COO$^-$) does not have any appreciable effect on the taste quality of studied saccharides.

The expansion coefficients ($\partial V_2^*/\partial T$)$_p$ and the second derivatives ($\partial^2 V_2^*/\partial T^2$)$_p$ have been calculated by fitting the $V_2^*$ data into the following equation: $V_2^* = v_0 + v_1T + v_2T^2$, where $v_0$, $v_1$ and $v_2$ are constants. The ($\partial V_2^*/\partial T$)$_p$ values are positive and their magnitudes increase with concentration of co-solute and temperature of the solutions, except in few cases. By using the relation suggested by the Hepler: ($\partial C_{p,2}^*/\partial T$)$_T$ = $-T$ ($\partial^2 V_2^*/\partial T^2$)$_p$, the positive ($\partial^2 V_2^*/\partial T^2$)$_p$ values obtained for most of the saccharides in aqueous co-solutes
sodium acetate, disodium tetraborate and sodium gluconate) solutions suggest that these behave as structure makers, whereas negative \( \langle \partial^2 V^2 / \partial T^2 \rangle_p \) values obtained for few saccharides indicate that these act as structure breakers. However, this classification has its own limitation.

The Jones-Dole viscosity \( B \)-coefficient has significance in the description and understanding of ionic processes. It is an empirical term which measures the size, shape and charge effects as well as the structural effect induced by solute-solvent interactions. The \( B \)-coefficient values of saccharides studied in water and aqueous co-solute solutions are positive and fairly large and their magnitudes increase from mono- to di- to tri-saccharides, which can be explained on the basis of size and shape effects of saccharide molecules. The \( dB/dT \) parameter is better indicative in measuring the structure-making or -breaking ability than sign of the \( B \)-coefficient. The \( dB/dT \) values are negative and their magnitudes increase with concentration of co-solute, which again suggests an enhancement of structural order in these solutions.

The viscosity \( B \)-coefficients of transfer, \( \Delta B \) of saccharides from water to aqueous solutions of sodium acetate are positive and increase systematically with complexity of the saccharides, suggesting an overall structural increase in the solution. The comparison of \( \Delta B \) values of saccharides in aqueous sodium acetate solutions with those in the presence of other electrolytes decrease in the order: \((\text{NH}_4)_2\text{SO}_4 > \text{Na}_2\text{SO}_4 > \text{NaOOCCH}_3 > \text{KCl}\). This shows that 1:2 electrolytes influence the transfer values to greater extent than 1:1 electrolytes. According to Hofmeister series, the stabilizing ability of anions is in the order: \( \text{SO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- \) and the stabilizing ability of cations (\( \text{NH}_4^+, \text{Na}^+, \text{K}^+ \)) is almost the same. Therefore, the higher values of \( \Delta B \) for D(+)lactose monohydrate and D(+)maltose monohydrate in aqueous solutions of (NH\(_4\))\(_2\)SO\(_4\) and Na\(_2\)SO\(_4\) than in aqueous solutions of NaOOCCH\(_3\) and KCl may be due to the cumulative effects of higher ionic strength of (NH\(_4\))\(_2\)SO\(_4\) and Na\(_2\)SO\(_4\) (1:2 electrolyte) than NaOOCCH\(_3\) and KCl (1:1 electrolyte) and specificity of the ions (\( \text{NH}_4^+/\text{Na}^+/\text{K}^+/\text{SO}_4^{2-}/\text{CH}_3\text{COO}^-/\text{Cl}^- \)). The \( \Delta B \) values for saccharides in the presently studied co-solutes follow the order: \( \text{Na}_2\text{B}_4\text{O}_7 > \text{NaC}_6\text{H}_{11}\text{O}_7 > \text{NaOOCCH}_3 \). This suggests that solute-co-solute interactions are stronger between saccharide molecules and \( \text{B}_4\text{O}_7^{2-} \) anions in comparison with \( \text{CH}_3\text{COO}^- \) and \( \text{C}_6\text{H}_7\text{O}_{11}^- \) anions, which is also observed from volumetric studies.
According to Feakins et al., the activation Gibbs free energy, $\Delta \mu^{\#}_2$ for viscous flow of the saccharides in aqueous and mixed aqueous solutions is related to the viscosity $B$-coefficient as follows: $\Delta \mu^{\#}_2 = \Delta \mu^{\#}_1 + (RT/V_1 \phi) [1000B - (V_1 \phi - V_2)]$. High positive values of $\Delta \mu^{\#}_2$ and the activation parameter of transfer, $\Delta G^{\#}_2(1\rightarrow1')$ suggest that the formation of the transition state is less favoured for the systems studied. Activation entropy, $\Delta S^{\#}_2$ and enthalpy, $\Delta H^{\#}_2$ for viscous flow of saccharides have also been calculated.

Standard partial molar isentropic compressibility of transfer, $\Delta K^{s,2}_s$ for studied saccharides are positive and increase with concentration of co-solutes, indicating that the compressibility of solution increases in the presence of co-solutes (except in the presence of sodium gluconate). The positive $\Delta K^{s,2}_s$ values again suggest that the hydrophilic-ionic interactions predominate over the hydrophobic-ionic interactions. Due to these interactions, the less compressible water present in the hydration shells of saccharide molecules comes out into the bulk water, thus exhibiting positive $\Delta K^{s,2}_s$ values. The decrease in $\Delta K^{s,2}_s$ values of saccharides with co-solute concentration in the presence of sodium gluconate indicates the dominance of hydrophobic-ionic interactions, which may be due to strong hydrophobic effect of gluconate anion ($C_6H_7O_11^-$) as mentioned earlier.

The hydration numbers, $n_H$ of saccharides were calculated by using the method reported by Millero et al.: $n_H = -[K^{s,2}_s\text{(elect)}/K^{s,2}_s V_1]$, where $K^s$ is the compressibility, $V_1$ is the molar volume of bulk water/solvent and $K^{s,2}_s\text{(elect)}$ is the electrostriction partial molar compressibility. Overall, the $n_H$ values are less in aqueous co-solute solutions as compared to their values in water and the values decrease as a function of co-solute concentration. This indicates an increase in solute-co-solute interactions and thus reduction in the electrostriction, which shows that co-solute has a dehydration effect on the saccharides.

Apparent specific isentropic compressibility, ASIC for various saccharides has been calculated from apparent molar isentropic compressibility ($K_{s,2,\phi}$) as: $K_{2(s)} = K_{s,2,\phi}/M$, where $M$ is the molar mass of solute. The ASIC values of studied saccharides in water at different temperatures are in sweet taste range ($-3.383 \times 10^{-7}$ to $-2.335 \times 10^{-5}$ cm$^3$ g$^{-1}$ bar), which is also observed from ASV data. In the presence of sodium acetate, the ASIC values of saccharides increase with the concentration of sodium acetate as well as temperature of the solution and falls in sweet taste range, except in cases of few saccharides. In aqueous disodium tetraborate, the ASIC values of all the saccharides fall
sour taste range, whereas in sodium gluconate solutions, the ASIC values fall in sweet taste range. Since the ASIC parameter is more sensitive to hydration characteristics therefore, conclusion drawn from the ASIC parameters may be more realistic.

The interaction coefficients (volumetric and viscometric) have been calculated based on the McMillan-Mayer theory of solutions as: \( \Delta X_2^2 = 2X_{AB}m_B + 3X_{ABB}m_B^2 + \ldots \). In general, the pair interaction coefficients, \( X_{AB} \) are positive and triplet coefficients, \( X_{ABB} \) are negative. The magnitudes of viscometric interaction coefficients decrease with temperature, whereas those for volumetric interaction coefficients increase with temperature in all the cases. The contributions of pair interaction coefficients to transfer values (\( \Delta X_2^2 \)) are generally positive and vary linearly, whereas those of triplet interaction coefficients are negative and their magnitudes vary non-linearly over the whole concentration range of cosolutes. The high positive magnitudes of pair interaction coefficients suggest that saccharide-co-solute interactions are mainly pairwise.

The values of interaction coefficients seem to depend on the stereochemistry of different kinds of –OH groups [equatorial (e), axial (a) and exocyclic (exo)]. In the presence of sodium acetate, the \( V_{AB} \) values are higher for D(–)-arabinose and D(+)-galactose than those for D(+)-xylose and D(+)-glucose, respectively at all temperatures studied. This may arise due to the differences in their dominant conformations (1e2e3e4a, 1e2e3e4e) in water, respectively. Hence, D(+)-xylose/D(+)-glucose fit well into the structure of water than D(–)-arabinose/D(+)galactose. Therefore, dehydration in the cases of D(–)-arabinose/ D(+)galactose [OH(4a)] will be more which contribute positively to \( V_{AB} \). Among disaccharides, the highest values of pair interaction coefficients for D(+)trehalose dihydrate indicate that it interacts more strongly with cosolutes (sodium acetate and disodium tetraborate), which is again an indication that D(+)trehalose dihydrate has a peculiar behavior as it is considered to be stronger protective agent. The high magnitudes of pair interaction coefficients in the case of D(+)-maltose monohydrate in comparison to D(+)-cellobiose in the presence of all the three studied additives, strengthens the view that D(+)-maltose monohydrate being more folded has stronger interactions with co-solute molecules than D(+)-cellobiose.

Overall the magnitudes of interaction coefficients of saccharides in studied cosolutes (additives) follow the order: \( \text{Na}_2\text{B}_4\text{O}_{17} > \text{NaOOCCCH}_3 > \text{NaC}_6\text{H}_{11}\text{O}_7 \). This indicates that the solute-co-solute interactions are weak in case of sodium gluconate solutions due to strong hydrophobic effect of gluconate, \( \text{C}_6\text{H}_7\text{O}_{11}^- \) anions.