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The Volumes of Hydrated Glucose, Sucrose and Raffinose Molecules, and the Osmotic Pressures of These Aqueous Saccharide Solutions as Measured by the Freezing-Point-Depression Method

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The osmotic pressures of aqueous saccharide solutions were calculated from the data obtained by means of the freezing-point-depression method. Thus, the water activity and the osmotic pressure of D-glucose, sucrose, and raffinose solutions were measured by the freezing-point-depression method. For dilute aqueous saccharide solutions, the freezing-point depression was proportional to their molal concentrations. However, the higher the molal concentration, the larger the freezing-point depression observed became than those to be expected from the molal concentration dependent on the size of the saccharide molecules. The osmotic pressures calculated from the data of the freezing-point-depression method were compared with those measured with semi-permeable membranes by Morse and his colleagues. To obtain the apparent volumes of hydrated D-glucose, sucrose, and raffinose molecules, their partial molal volumes were measured, together with the partial molal volumes of the water of their solutions. The partial molal volumes of the water were verified to be equal to the molar volumes of pure water up to the highest concentrations examined. The apparent volumes of the hydrated D-glucose, sucrose, and raffinose molecules were calculated to be 0.1865, 0.3525, and 0.5105 nm^3 respectively.

To physiologists who study water relations in plant and animal cells, and membrane phenomena, it has become common knowledge that the osmotic pressure inside and outside a cell[1–5] and the ionic component[6–8] both have important effects on its activities. Thus, various aqueous electrolyte solutions, such as KCl, NaCl, CsCl, RbCl, $RbNO_3$, RbF, $CaCl_2$, $Ca(NO_3)_2$, and $MgSO_4$,[7,9–12] have been used to control the ionic condition and the osmotic pressure inside and outside cells. Various saccharides, such as glucose, sucrose,[13,14] and raffinose, and their derivatives, such as mannitol,[2,15,16] and sorbitol,[17] have also been used.

Although we have very detailed data about the freezing-point depression of simple aqueous electrolyte solutions,[18] we have only a few data about the osmotic pressure of saccharide and its derivative solutions.[18–20]

In this paper, the water activity, the osmotic pressure, the activity coefficient of the water, and the osmotic coefficients of aqueous D-glucose, sucrose and raffinose solutions, as calculated from the freezing-point-depression data and the partial molal volume of the water as well as the partial molal volumes of these saccharides, are reported as functions of their concentration. The values of the partial molal volume thus obtained were then used to calculate the volume of a hydrated saccharide molecule.

Materials and Methods

The D-glucose, sucrose, and raffinose used were of a reagent grade from Nakarai Chemicals, Ltd., and Wako Pure Chemical Industries, Ltd. The raffinose, a hydrate, had been kept in a petri dish for a few months so as to make it in equilibrium with the water in the air. These saccharides were not further purified by the method of recrystalization from their hot concentrated aqueous solutions by adding alcohol because our preliminary test using the atomic-absorption method had shown that the saccharides were fairly free from contaminating electrolytes, such as K^+, Na^+, Ca^{2+}, and Mg^{2+}. On the other hand, ethyl alcohol which had been stored in a glass bottle for a long period contained a small amount of Na^+.

The water used had been distilled from a deionized supply. The air dissolved in the distilled water was not completely removed except when the effect of the dissolved air was examined. The air dissolved in the aqueous solutions was not a serious problem in measuring their density with the mechanically oscillating densimeter except in winter. The effect of the dissolved air in the solutions will be discussed later from the biological point of view.

The freezing-point depression was measured with an osmometer (Advanced Instruments, Inc.; Type 3W) which had been calibrated with standard aqueous NaCl solutions whose freezing-point depressions are listed in "Handbook of Physics and Chemistry".[21] The osmometer was equipped with a thermister to detect the freezing point of the aqueous solution to be examined and a cooling bath in which a glass tube containing the aqueous solution was designed so as to be cooled gently.

Calculation of osmotic pressure from the data measured with the osmometer: The osmometer expresses the freezing-point depression $\theta(=T^{\circ}-T)$ in terms of mosmol, where T° and T are the freezing points of pure water and the aqueous solution to be tested respectively. 1.0000 mosmol is defined as the molal concentration of an ideal aqueous solution, whose freezing-point depression θ is 1.86×10^{-3} °C.

The equation used to express precisely the freezing-point depression is as follows (cf. Appendix):

$$\Delta_{\mathrm{fus}}Hm_{(1)}(T^{\circ})\left(\frac{1}{T}-\frac{1}{T^{\circ}}\right) - \frac{1}{T}\int_{T}^{T^{\circ}}\{\overset{\mathrm{l}}{Cp},m_{(1)}(T'')-\overset{\mathrm{s}}{Cp},m_{(1)}(T'')\}\mathrm{d}T'' + \int_{T}^{T^{\circ}}\frac{\overset{\mathrm{l}}{Cp},m_{(1)}(T'')-\overset{\mathrm{s}}{Cp},m_{(1)}(T'')}{T''}\mathrm{d}T''$$

$$= -R\ln x_1 f_1 \qquad (1)$$

$$= -\phi R\ln x_1 \qquad (2)$$

where $\overset{\mathrm{l}}{Cp},m_{(1)}$ (T), $\overset{\mathrm{s}}{Cp},m_{(1)}$ (T), x_1, f_1, and ϕ are the molar heat capacities at a constant pressure of liquid and solid water as functions of the temperature; the mole fraction of water; the activity coefficient of water, and the osmotic coefficient of the solution respectively.

Actually, however, we are at present forced to adopt the following equation to calculate the water activity because of the lack of precise values of $\overset{\mathrm{l}}{Cp},m_{(1)}$ (T) and $\overset{\mathrm{s}}{Cp},m_{(1)}$ (T) in Eqs. 1 and 2 of the aqueous solutions to be examined (cf. Dorsey[22]). That is :

$$\Delta_{\mathrm{fus}}Hm_{(1)}(T^{\circ})\left(\frac{1}{T}-\frac{1}{T^{\circ}}\right) = -R\ln x_1 f_1 \qquad (3)$$

$$= -\phi R\ln x_1. \qquad (4)$$

It will be shown in the Discussion section that Eq. 3 or 4 gives values of the water activity of, at least, sucrose solutions fairly consistent with those obtained with semi-permeable membranes. Thus, from Eqs. 3 and 4, $\ln x_1 f_1$, f_1, and ϕ are calculated after T has been calculated from both T^0 and θ.

The osmotic pressure π which should be generated between an aqueous solution of the mole fraction x_1 and pure water $x_1=1$ across a semi-permeable membrane is defined as the osmotic pressure of the solution and is expressed by this equation:[23,24]

$$\Pi = -\frac{RT\ln x_1 f_1}{Vm_{(1)}(T,0)\left\{1-\frac{1}{2}\kappa_1(P'+P'')\right\}} \qquad (5)$$

or by:

$$\Pi = -\frac{\phi RT\ln x_1}{Vm_{(1)}(T,0)\left\{1-\frac{1}{2}\kappa_1(P'+P'')\right\}} \qquad (6)$$

where $Vm_{(1)}$ $(T,0)$ is the molar volume of water extrapolated to zero pressure at the temperature T K; κ_1, the compressibility of water ($4.6\times10^{-5}\,\mathrm{atm}^{-1}$ or $4.5\times10^{-10}\,\mathrm{Pa}^{-1}$ at 298.15K[22]); and P' and P'', the pressures of the pure-water phase and the aqueous-solution phase respectively.

Since, in ordinary solutions used in physiology, $1\gg1/2\kappa_1(P'+P'')$ and T=17—25 °C, we obtain:

$$\Pi = -\frac{TR}{Vm_{(1)}(298.15,0)}\ln x_1 f_1 \qquad (7)$$

where $Vm_{(1)}$ (298.15,0) is the molar volume of water extrapolated to zero pressure at 25 °C: approximately $1.8075\times10^{-2}\,\mathrm{dm^3\,mol^{-1}}$. Therefore, in order to relate the results obtained from the freezing-point-depression method to the osmotic pressure, it is essential to calculate the value of $\ln x_1 f_1$ or $\phi\ln x_1$ from θ, T°, and T.

Partial molal volume of the water: In order to estimate the effect of saccharides on the water structures in their aqueous solutions, the partial molal volume of the water of the aqueous saccharide solutions were determined as functions of their concentrations. The partial molal volume of the water $Vm_{(1)}$ of an aqueous solution was determined as an intersect of the linear curve obtained by plotting the mean molar volume $V_{1,2}$ against the mole fraction of saccharide x_2 with the $V_{1,2}$-axis. $V_{1,2}$ was expressed by the mole fraction of the water x_1 and the solute x_2, the partial molal volume of water $Vm_{(1)}$ and a saccharide $Vm_{(2)}$ as :[24,25]

$$V_{1,2} = Vm_{(1)}x_1 + Vm_{(2)}x_2 \qquad (8)$$

In this study, $V_{1,2}$ was calculated from the density ρ of the solution by means of this equation:

$$V_{1,2} = \frac{1}{\rho}\,\frac{1.0000+M_2m}{55.5093+m} \qquad (9)$$

where M_2 and m are the molecular weight of the solute and the molal concentration of its aqueous solution and where 55.5093 moles is the mole number of 1.0000 kg of water respectively. In the calculation of the molal concentration of each aqueous solution, the buoyancy due to air was corrected.

The density of the saccharide solutions was measured with a mechanically oscillating densimeter (Shibayama SS-D-200) at 25.00±0.01 °C. The effects of the viscosity of the solution on the density measurement with the densimeter[26] was negligibly small up to the highest concentrations of the solutions tested in this study.

Results

Table 1 shows the freezing-point depression of D-glucose, sucrose, and raffinose solutions and the osmotic pressure calculated from the data of the freezing-point depression. They are averages of 4—9 measurements and are expressed in terms of mosmol; atm and Pa are, respectively, functions of the molal and molar concentrations. The activity coefficient f_1 and the osmotic coefficient ϕ, which were determined by means of Eqs. 3 and 4, are also shown in Table 1.

At higher concentration than about 420 mmol kg^{-1}, raffinose was precipitated when ice was formed by agitation in its supercooled aqueous solution. In concentrated D-glucose and sucrose solutions, the formation of ice seemed to occur more slowly by the same agitation in supercooled states.

Table 1. Freezing-Point Depression, Water Activity in Terms of $-\ln x_1 f_1$, Osmotic Pressure Π, Activity Coefficient of Water f_1, and Osmotic Coefficient ϕ of Aqueous Saccharide Solutions

Concentration		Freezing-point depression		$-\ln x_1 f_1$	Π		f_1	ϕ
m/mol kg^{-1}	c/mol dm^{-3}	mosmol	θ/°C		$\times 10^5$Pa	atm		
				D-Glucose				
0.04984	0.04942	49.8	0.0926	8.9749×10^{-4}	1.231	1.215	1.000	0.9997
0.05077	0.05034	50.7	0.0943	9.1397	1.253	1.237	1.000	0.9992
0.10021	0.09881	101.0	0.1879	1.8218×10^{-3}	2.499	2.466	1.000	1.010
0.10078	0.09937	101.4	0.1879	1.8218	2.499	2.466	1.000	1.004
0.14994	0.14702	151.1	0.2810	2.7254	3.738	3.690	1.000	1.009
0.15149	0.14852	152.2	0.2831	2.7457	3.766	3.717	1.000	1.007
0.19955	0.19462	200.9	0.3737	3.6257	4.973	4.909	1.000	1.010
0.19992	0.19502	201.5	0.3748	3.6364	4.987	4.923	1.000	1.011
0.25077	0.24320	251.9	0.4685	4.5470	6.236	6.156	1.000	1.008
0.25110	0.24350	253.3	0.4711	4.5723	6.271	6.190	0.9999	1.013
0.29816	0.28766	301.1	0.5600	5.4369	7.457	7.361	0.9999	1.015
0.30039	0.28976	303.5	0.5645	5.4807	7.517	7.420	0.9999	1.015
0.39874	0.38059	404.2	0.7518	7.3041	10.017	9.889	0.9999	1.020
0.39919	0.38098	404.6	0.7526	7.3119	10.028	9.899	0.9999	1.020
0.59871	0.55939	612.7	1.1396	1.10876×10^{-2}	15.206	15.011	0.9996	1.033
0.59939	0.55997	614.1	1.1422	1.11130	15.241	15.045	0.9996	1.034
0.79965	0.73166	824.7	1.5339	1.49456	20.497	20.234	0.9994	1.045
0.80065	0.73246	825.0	1.5345	1.49514	20.505	20.242	0.9994	1.044
0.99847	0.89515	1036.3	1.9275	1.88079	25.794	25.463	0.9990	1.055
1.00029	0.89661	1040.4	1.9351	1.88825	25.897	25.564	0.9990	1.057
1.19751	1.05235	1251.8	2.3283	2.27523	31.205	30.804	0.9986	1.066
1.19822	1.05286	1254.4	2.3332	2.28006	31.271	30.870	0.9986	1.067
1.39665	1.20343	1474.6	2.7428	2.68440	36.817	36.344	0.9980	1.080
1.39818	1.20459	1475.6	2.7446	2.68617	36.841	36.368	0.9980	1.079
1.59715	1.34972	1697.7	3.1577	3.09521	42.451	41.906	0.9974	1.091
1.59734	1.34985	1692.0	3.1471	3.08470	42.307	41.764	0.9975	1.087
1.79670	1.48987	1910.8	3.5541	3.48889	47.850	47.236	0.9970	1.095
1.79870	1.49098	1907.7	3.5483	3.48312	47.771	47.158	0.9971	1.092
				Sucrose				
0.03001	—	30.5	0.0567	5.4908×10^{-4}	0.753	0.743	1.000	1.016
0.03007	—	30.5	0.0568	5.5083	0.755	0.746	1.000	1.017
0.05000	0.04912	50.6	0.0941	9.1222	1.251	1.235	1.000	1.013
0.05008	—	50.8	0.0944	9.1494	1.255	1.239	1.000	1.014
0.09982	0.09688	101.1	0.1880	1.82323×10^{-3}	2.501	2.468	1.000	1.014
0.10000	0.09732	100.7	0.1873	1.81557	2.490	2.458	1.000	1.008
0.14985	0.14507	151.7	0.2822	2.73720	3.754	3.706	1.000	1.015
0.15003	0.14527	151.7	0.2821	2.73574	3.752	3.704	1.000	1.013
0.19997	0.19093	202.9	0.3774	3.66151	5.022	4.957	0.9999	1.018
0.20002	0.19102	203.7	0.3789	3.67599	5.042	4.977	0.9999	1.022
0.24983	0.23644	255.5	0.4752	4.61243	6.326	6.245	0.9999	1.027
0.25001	0.23688	255.4	0.4750	4.60970	6.322	6.241	0.9999	1.025
0.29971	0.28154	307.8	0.5725	5.55858	7.624	7.526	0.9998	1.032
0.30008	0.28166	308.1	0.5730	5.56344	7.630	7.532	0.9998	1.031
0.35009	0.32499	360.9	0.6713	6.52040	8.943	8.828	0.9998	1.037
0.35021	0.32512	358.8	0.6673	6.48145	8.889	8.775	0.9998	1.030
0.39551	0.36391	410.9	0.7643	7.42563	10.184	10.053	0.9997	1.045
0.40050	0.36814	416.0	0.7737	7.51790	10.311	10.178	0.9997	1.045
0.44952	0.40958	467.8	0.8701	8.45666	11.598	11.449	0.9996	1.048
0.45012	0.41008	469.7	0.8736	8.49167	11.646	11.497	0.9996	1.050
0.49661	0.45061	521.6	0.9703	9.43405	12.939	12.773	0.9995	1.059
0.49773	0.45085	522.3	0.9714	9.44518	12.954	12.773	0.9995	1.058

Table 1. (Continued)

Concentration		Freezing-point depression		$-\ln x_1 f_1$	Π		f_1	ϕ
m/mol kg^{-1}	c/mol dm^{-3}	mosmol	θ/°C		$\times 10^5$Pa	atm		
0.54993	0.49115	582.7	1.0839	1.05431×10^{-2}	14.460	14.274	0.9993	1.069
0.55039	0.49150	583.0	1.0844	1.05480	14.467	14.281	0.9993	1.069
0.59637	0.52796	634.3	1.1797	1.14796	15.744	15.542	0.9992	1.074
0.59671	0.52821	635.5	1.1820	1.15024	15.776	15.573	0.9992	1.075
0.64803	0.56814	694.7	1.2921	1.25783	17.251	17.030	0.9990	1.083
0.64903	0.56891	694.6	1.2910	1.25773	17.250	17.028	0.9991	1.081
0.70095	0.60857	754.3	1.4031	1.36642	18.740	18.500	0.9989	1.088
0.70099	0.60854	755.3	1.4049	1.36818	18.765	18.524	0.9989	1.090
0.74781	0.64375	809.1	1.5050	1.46622	20.109	19.851	0.9987	1.095
0.74817	0.64394	811.7	1.5097	1.47088	20.173	19.914	0.9987	1.098
0.79922	0.68144	872.5	1.6229	1.58176	21.694	21.415	0.9985	1.106
0.79931	0.68156	871.9	1.6216	1.58056	21.677	21.399	0.9985	1.105
0.84769	0.71643	931.9	1.7333	1.69004	23.179	22.881	0.9983	1.115
0.84869	0.71721	932.6	1.7346	1.69136	23.197	22.899	0.9983	1.114
0.89920	0.75302	990.4	1.8422	1.79701	24.646	24.330	0.9981	1.118
0.89937	0.75313	991.8	1.8447	1.79941	24.679	24.362	0.9981	1.119
0.94906	0.78769	1052.3	1.9573	1.91010	26.197	25.861	0.9979	1.126
0.94915	0.78774	1051.7	1.9561	1.90890	26.181	25.845	0.9979	1.125
0.99965	0.82239	1114.4	2.0727	2.02358	27.753	27.397	0.9976	1.133
1.00005	0.82263	1116.3	2.0762	2.02702	27.801	27.444	0.9976	1.135
1.19662	0.95164	1374.0	2.5555	2.49940	34.279	33.839	0.9964	1.171
1.19743	0.95179	1367.1	2.5427	2.48673	34.106	33.668	0.9965	1.165
				Raffinose				
0.03000	—	30.2	0.0562	5.4433×10^{-4}	0.747	0.737	1.000	1.007
0.03002	—	30.3	0.0564	5.4617	0.749	0.739	1.000	1.010
0.04990	—	50.8	0.0945	9.1581	1.256	1.240	1.000	1.019
0.04997	—	50.6	0.0941	9.1222	1.251	1.235	1.000	1.013
0.10004	—	102.4	0.1905	1.84661×10^{-1}	2.533	2.500	1.000	1.025
0.10007	—	102.0	0.1897	1.83944	2.523	2.490	1.000	1.021
0.14990	—	154.2	0.2868	2.78176	3.815	3.766	0.9999	1.031
0.14990	—	153.6	0.2857	2.77099	3.800	3.750	0.9999	1.027
0.19989	—	208.0	0.3869	3.75372	5.148	5.082	0.9998	1.044
0.20003	—	207.7	0.3863	3.74827	5.141	5.075	0.9999	1.042
0.24973	—	260.7	0.4849	4.70645	6.455	6.372	0.9998	1.048
0.24983	—	262.0	0.4873	4.72997	6.487	6.404	0.9998	1.053
0.29994	—	318.1	0.5917	5.74502	7.879	7.778	0.9996	1.066
0.29997	—	318.5	0.5924	5.75220	7.889	7.788	0.9996	1.067
0.34989	—	375.4	0.6982	6.78243	9.302	9.183	0.9995	1.079
0.35000	—	375.4	0.6982	6.78243	9.302	9.183	0.9995	1.079
0.39986	—	436.5	0.8119	7.88968	10.821	10.682	0.9993	1.099
0.39992	—	436.1	0.8112	7.88246	10.811	10.672	0.9993	1.098

The molal concentrations at which the osmolality of the solutions became larger than their molal concentration by 3% were about 800 mmol kg^{-1} for D-glucose, about 350 mmol kg^{-1} for sucrose, and about 150 mmol kg^{-1} for raffinose. In the dilute ideal aqueous solutions, plotting the osmolality against its molal concentration gives a linear curve. Actually, however, the degree of the deviation of the osmolality from the molal concentration depended on the molecular weight or the molecular volume. That is, the larger the molecular weight or the molecular volume, the larger the deviation became at the same concentration.

Table 2 shows the mean molar volumes $V_{1,2}$ of D-glucose, sucrose, and raffinose solutions at 25 °C. In order to obtain the partial molal volumes of water of the saccharide solutions, their $V_{1,2}$ values were plotted against the mole fraction of the saccharide x_2. All of them gave linear curves up to the highest concentrations examined, and all of the curves intersected with the $V_{1,2}$-axis at 0.01807 dm^3 mol^{-1}. Figure 1 shows plots of $V_{1,2}$ of the aqueous sucrose solution against x_2

Table 2. Mean Molar Volumes $V_{1,2}$ of Glucose, Sucrose, and Raffinose Aqueous Solutions as Functions of the Molal Concentrations m and Mole Fractions of Saccharides x_2

Concentration		Mean molar volume
m/mol kg^{-1}	x_2	$V_{1,2}$/dm^3 mol^{-1}
	D-Glucose	
0.05077	9.1433×10^{-4}	0.01815
0.10021	18.029	0.01824
0.14994	26.950	0.01832
0.19955	35.835	0.01840
0.25077	44.991	0.01849
0.30039	53.846	0.01857
0.39919	71.429	0.01874
0.59871	106.75	0.01907
0.80065	142.24	0.01941
0.99847	176.77	0.01974
1.19751	211.26	0.02007
1.39665	245.53	0.02039
1.59734	279.79	0.02072
1.79870	313.99	0.02105
	Sucrose	
0.04978	8.9635×10^{-4}	0.01824
0.09899	17.809	0.01841
0.15032	27.020	0.01859
0.19965	35.854	0.01876
0.24964	44.791	0.01894
0.30028	53.830	0.01911
0.35021	62.724	0.01928
0.39551	70.779	0.01944
0.44987	80.428	0.01963
0.49999	89.310	0.01980
0.55039	98.224	0.01998
0.59671	106.40	0.02013
0.64803	115.45	0.02031
0.70099	124.77	0.02049
0.74817	133.05	0.02065
0.79931	142.02	0.02083
0.84868	150.65	0.02100
0.89921	159.48	0.02117
0.94906	168.17	0.02134
1.00004	177.05	0.02151
1.19743	211.26	0.02219
	Raffinose	
0.049899	8.9854×10^{-4}	0.01833
0.099881	17.970	0.01859
0.15043	27.039	0.01885
0.20010	35.935	0.01911
0.24984	44.829	0.01937
0.29989	53.760	0.01963
0.35004	62.693	0.01988
0.40033	71.637	0.02014

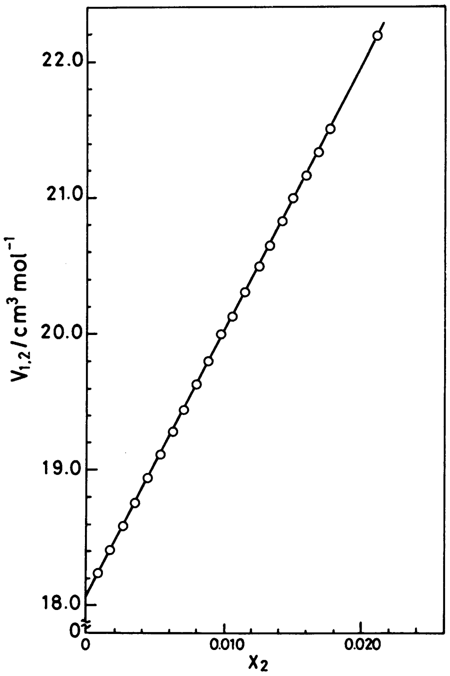

Fig. 1. Mean molar volume of aqueous sucrose solution $V_{1,2}$ as a function of sucrose mole fraction x_2.

as an example. The value of 0.01807 dm^3 mol^{-1} is almost equal to the molar volume of pure water, 0.0180685 dm^3 mol^{-1}, at 25 °C. This fact suggests that the aqueous saccharide solutions consist of a water phase which has the same property as bulky pure water and that of hydrated saccharide molecules scattered in the water phase, at least up to the highest concentrations examined in this study.

The partial molal volume $Vm_{(2)}$ of the 2 solute was calculated from the density ρ and the molar concentration c of the solution as follows. Assuming that the aqueous saccharide solution is composed of pure water and hydrated saccharide molecules, its density ρ is expressed by this equation:

$$\rho = \frac{M_2c+\rho_1(1.0000-Vm_{(2)}c)}{1.0000}\text{kg dm}^{-3} \tag{10}$$

where M_2 and ρ_1 are the molecular weight of saccharide and the density of pure water respectively. By re-arranging Eq. 10, we obtain:

$$\rho = (M_2-\rho_1Vm_{(2)})c + \rho_1. \tag{11}$$

Thus, if $Vm_{(2)}$ is constant, plots of the density ρ against c should give a linear curve whose slope is $(M_2-\rho_1Vm_{(2)})$ and $Vm_{(2)}$ can be calculated from the slope of this linear curve. After a linear relationship between ρ and c has been ascertained, the partial molal volume of a saccharide $Vm_{(2)}$ at each concentration c can also be calculated from the values of ρ, ρ_1, c, and M_2.

Table 3 shows the densities ρ of the D-glucose,

Table 3. Densities of Aqueous Saccharide Solutions ρ, Partial Molal Volumes of Saccharides $Vm_{(2)}$, Apparent Hydrated Volumes v_2° and Equivalent Radii r_2 of a Hydrated Saccharide Molecule at 25.00±0.01 °C

Concentration		Density	Partial molal volume	Apparent hydrated volume	Equivalent radius
m/mol kg^{-1}	c/mol dm^{-3}	ρ/kg dm^{-3}	$Vm_{(2)}$/dm^3 mol^{-1}	v_2°/nm^3	r_2/nm
		D-Glucose			
0.04984	0.04942	1.0005	0.1111	0.1845	0.353
0.05077	0.05034	1.0005	0.1123	0.1865	0.354
0.10021	0.09881	1.0038	0.1124	0.1866	0.354
0.10078	0.09937	1.0039	0.1118	0.1856	0.354
0.14994	0.14702	1.0071	0.1123	0.1865	0.354
0.15149	0.14852	1.0072	0.1123	0.1865	0.354
0.19955	0.19462	1.0104	0.1120	0.1860	0.354
0.19992	0.19502	1.0104	0.1121	0.1861	0.354
0.25077	0.24320	1.0137	0.1121	0.1861	0.354
0.25110	0.24350	1.0137	0.1122	0.1863	0.354
0.29816	0.28766	1.0167	0.1122	0.1863	0.354
0.30039	0.28976	1.0167	0.1120	0.1860	0.354
0.39874	0.38059	1.0231	0.1121	0.1861	0.354
0.39919	0.38098	1.0231	0.1122	0.1863	0.354
0.59871	0.55939	1.0352	0.1123	0.1865	0.354
0.59939	0.55997	1.0352	0.1124	0.1866	0.354
0.79965	0.73166	1.0469	0.1124	0.1866	0.354
0.80065	0.73246	1.0469	0.1125	0.1868	0.355
0.99847	0.89515	1.0579	0.1125	0.1868	0.355
1.00029	0.89661	1.0580	0.1125	0.1868	0.355
1.19751	1.05235	1.0685	0.1126	0.1870	0.355
1.19822	1.05286	1.0685	0.1127	0.1871	0.355
1.39665	1.20343	1.0786	0.1127	0.1871	0.355
1.39818	1.20459	1.0787	0.1127	0.1871	0.355
1.59715	1.34972	1.0884	0.1128	0.1873	0.355
1.59734	1.34985	1.0884	0.1128	0.1873	0.355
1.79670	1.48987	1.0977	0.1130	0.1876	0.355
1.79870	1.49098	1.0978	0.1129	0.1875	0.355
		Sucrose			
0.05000	0.04912	1.0035	0.2120	0.3520	0.438
0.09982	0.09668	1.0098	0.2113	0.3508	0.438
0.10000	0.09732	1.0099	0.2111	0.3505	0.437
0.14985	0.14507	1.0161	0.2117	0.3515	0.438
0.15003	0.14527	1.0162	0.2112	0.3507	0.437
0.19997	0.19093	1.0221	0.2118	0.3517	0.438
0.20002	0.19102	1.0222	0.2114	0.3510	0.438
0.24983	0.23644	1.0281	0.2117	0.3515	0.438
0.25001	0.23688	1.0282	0.2115	0.3512	0.438
0.29971	0.28154	1.0340	0.2118	0.3517	0.438
0.30008	0.28166	1.0340	0.2118	0.3517	0.438
0.35009	0.32499	1.0391	0.2121	0.3522	0.438
0.35021	0.32512	1.0397	0.2118	0.3517	0.438
0.39551	0.36391	1.0447	0.2120	0.3520	0.438
0.40050	0.36814	1.0453	0.2119	0.3518	0.438
0.44952	0.40958	1.0507	0.2120	0.3520	0.438
0.45012	0.41008	1.0508	0.2119	0.3518	0.438
0.49661	0.45061	1.0561	0.2119	0.3518	0.438
0.49773	0.45085	1.0561	0.2120	0.3520	0.438
0.54993	0.49115	1.0613	0.2122	0.3523	0.438
0.55039	0.49150	1.0613	0.2122	0.3523	0.438
0.59637	0.52796	1.0661	0.2122	0.3523	0.438
0.59671	0.52821	1.0661	0.2122	0.3523	0.438

Table 3. (Continued)

Concentration		Density	Partial molal volume	Apparent hydrated volume	Equivalent radius
m/mol kg^{-1}	c/mol dm^{-1}	ρ/kg dm^{-3}	$Vm_{(2)}$/dm^3 mol^{-1}	c_2°/nm^3	r_2/nm^3
0.64803	0.56814	1.0713	0.2123	0.3525	0.438
0.64903	0.56891	1.0714	0.2123	0.3525	0.438
0.70095	0.60857	1.0766	0.2122	0.3523	0.438
0.70099	0.60854	1.0765	0.2124	0.3527	0.438
0.74781	0.64375	1.0813	0.2121	0.3522	0.438
0.74817	0.64394	1.0812	0.2123	0.3525	0.438
0.79922	0.68144	1.0860	0.2124	0.3527	0.438
0.79931	0.68156	1.0861	0.2123	0.3525	0.438
0.84769	0.71643	1.0905	0.2125	0.3528	0.438
0.84869	0.71721	1.0907	0.2124	0.3527	0.438
0.89920	0.75302	1.0953	0.2125	0.3528	0.438
0.89937	0.75313	1.0953	0.2125	0.3528	0.438
0.94906	0.78769	1.0997	0.2126	0.3530	0.438
0.94915	0.78774	1.0997	0.2126	0.3530	0.438
0.99965	0.82239	1.1043	0.2125	0.3528	0.438
1.00005	0.82263	1.1043	0.2126	0.3530	0.438
1.19662	0.95164	1.1208	0.2129	0.3535	0.439
1.19743	0.95179	1.1208	0.2129	0.3535	0.439
			Raffinose		
0.049899	0.049004	1.0068	0.3068	0.5094	0.495
0.099881	0.096629	1.0162	0.3074	0.5104	0.496
0.15043	0.14337	1.0255	0.3070	0.5098	0.496
0.20010	0.18797	1.0343	0.3074	0.5104	0.496
0.24984	0.23136	1.0428	0.3077	0.5109	0.496
0.29989	0.27381	1.0512	0.3076	0.5107	0.496
0.35004	0.31515	1.0594	0.3076	0.5107	0.496
0.40033	0.35545	1.0673	0.3078	0.5111	0.496

sucrose, and raffinose solutions as functions of the molal m and molar c concentrations. When the ρ values are plotted against c, they give straight curves in all the concentrations up to the highest in the freezing-point-depression experiments. The partial molal volumes of D-glucose, sucrose, and raffinose, as calculated from their slopes, were almost constant from diluted up to concentrated concentrations; they were 0.1123, 0.2123, and 0.3075 dm^3 mol^{-1} respectively. The values of the density ρ and the molal volume $Vm_{(2)}$ of the saccharides examined were almost coincident with those reported by Shahidi et al.[27]

Since the partial molal volume of the saccharide is constant up to an infinitely diluted concentration where the partial molal volume of the water is equal to the molar volume of pure water and where solute-solute interactions can be disregarded, it is safe to say that the partial molal volume of the saccharide is equal to the volume of the apparent volume of a hydrated saccharide molecule multiplied by the Avogadro number. Thus, the apparent volume of a hydrated saccharide molecule v_2° was obtained by dividing $Vm_{(2)}$ by the Avogadro number N_A, i.e.,

$$v_2^\circ = Vm_{(2)}/N_A. \tag{12}$$

The equivalent radius of a hydrated saccharide molecule r_2 was calculated as follows. Assuming that the hydrated saccharide molecule is spherical,

$$v_2^\circ = \frac{4}{3}\pi r_2^3. \tag{13}$$

Thus,

$$r_2 = \sqrt[3]{\frac{3}{4\pi} v_2^\circ}. \tag{14}$$

The calculated results are also shown in Table 3.

The apparent volume of one hydrated D-glucose, sucrose, or raffinose molecule was obtained as 0.1865, 0.3525, or 0.5105 nm^3 respectively. These values indicate that the apparent volume of the hydrated saccharide molecule is approximately proportional to the molecular weight.

Discussion

The present studies have made it clear that (1) the osmolality and the freezing-point depression of aqueous saccharide solutions are proportional to the molal concentration at dilute concentrations, although they become larger than the values of the molal concentration at higher concentrations; (2) the degree of the deviation depends on the molecular weight or the molecular volume of the solute; (3) the activity coefficients of the water of aqueous saccharide solutions at the freezing point are almost unity up to 1.0 mol kg^{-1} for D-glucose, up to 0.65 mol kg^{-1} for sucrose, and up to 0.4 mol kg^{-1} for raffinose, while the osmotic coefficients of aqueous saccharide solutions tend to increase with an increase in the saccharide concentration; (4) the addition of saccharide molecules to the aqueous saccharide solution has little effect on the partial molal volume of the water, which is almost equal to the molar volume of pure water, at least up to the highest concentrations examined, and (5) the volume of a hydrated saccharide molecule is proportional to the molecular weight.

The fact that the addition of saccharide molecules to water or to its aqueous solution has little effect on the partial molal volume of the water suggests that their addition induces insignificant changes in the water structure, except in the vicinity of the solute molecules of the aqueous solution. Further, this suggests that we may ignore the effects of the saccharide molecules on the structure of the water phase surrounding the cell membrane, especially in the neighborhood of the inlet or outlet of the water-filled pores[28] and ion pores.

Miyajima et al.,[29] measuring the excess partial molar entropy, one of the thermodynamic quantities, of water for D-glucose, D-mannose, and D-galactose, reported that the ordering of water molecules occurred by the dissolution of these monosaccharides. Judging from the present results, their results may reflect an ordering of the water structure in the vicinity of the solute molecule as a result of hydration. However, this discrepancy between the present results and Miyajima et al.'s cannot be explained until an analysis of water structure is done by some other methods, such as X-ray diffraction or neutron scattering, that will enable us to detect some actual changes in the water structure.

Since the $R\ln x_1$ term is derived from the entropy of mixing $\Delta S_{1,2}$ on the basis of statistical mechanics,[25,30] the type of its expression should be different depending on the model adopted to evaluate the entropy of mixing. From primary statistical mechanics, the second term of the chemical potential equation, expressed as $R\ln x_1$, is derived based on the well-known lattice model under the assumptions that the solvent and the solute are nearly equal in size and that the distance between the lattice is constant, though arbitrary.

Based on the lattice model, some modified equations have been proposed to explain the entropy change of the mixing of solutions composed of solute and solvent molecules of different sizes.[31–35] However, since the present author thought of those models as less actual, he omitted the analysis of the molecular-weight or molecular-size dependence of $R\ln x_1$ as a function of the concentration.

Beginning in 1902[36] Morse and his colleagues extensively studied the osmotic pressure of rather dilute sucrose solutions using an apparatus of their own design, equipped with a semi-permeable membrane formed from both potassium ferocyanide and copper sulphate solutions. Since no one else has ever measured the osmotic pressure of dilute aqueous saccharide solutions using semi-permeable membranes, the present author has shown their results[37] in Table 4 to compare them with his own values calculated from the freezing-point-depression method. The osmotic pressure calculated coincides well with those directly measured by Morse et al. at 20 °C in the range of lower concentrations, while at higher concentrations the present results coincide well with those measured previously at 25 °C. However, the present results give values a little higher than Morse et al.'s at 0 °C. These small differences between the present results and Morse et al.'s might come from the difference between Eq. 1 and Eq. 3, used to calculate the water activity using the freezing-point-depression method. This problem should be checked when the molar-heat capacities of water and ice are precisely measured.

The equivalent radii of the hydrated saccharide molecules obtained by the present author are smaller

Table 4. The Osmotic Pressure of Sucrose Solutions as Functions of the Temperature (Morse et al.[37])

Concentration m	Osmotic pressure/atm					
	Temperature/°C					
	0	5	10	15	20	25
0.1	2.462	2.452	2.498	2.541	2.590	2.634
0.2	4.722	4.818	4.893	4.985	5.064	5.148
0.3	7.085	7.198	7.334	7.476	7.605	7.729
0.4	9.442	9.608	9.790	9.949	10.137	10.296
0.5	11.895	12.100	12.297	12.549	12.748	12.943
0.6	14.381	14.605	14.855	15.144	15.388	15.624
0.7	16.886	17.206	17.503	17.815	18.128	18.434
0.8	19.476	19.822	20.161	20.535	20.905	21.254
0.9	22.118	22.478	22.884	23.305	23.717	24.126
1.0	24.825	25.283	25.693	26.189	26.638	27.053

than those adopted by Durbin[38] to estimate the equivalent pore radius of the cellulose membrane. Hereafter, however, the equivalent radii of the hydrated saccharide molecules obtained by the present author will be used to estimate the equivalent pore radius of the membrane through which water and solute molecules pass, for the present author's procedure to obtain them seems to be more reasonable.

In studies of water and solute flows across such permeable membranes as the cell wall[39] and water and permeable-solute flows across the cell membrane,[40–42] not only the equivalent radius but also the properties of the surfaces of the saccharide molecules[43] and the permeable molecules are important, for solute–solute, solute–solvent, and solvent–solvent interactions at the inlet of the water-filled pores, those in the water-filled pores and/or ion pores, and solute-solvent-surface molecules of the water-filled pore and/or ion pore interactions at the inlet, and those in the water-filled pores and/or ion pores may have much to do with the membrane phenomena.[28,44]

The freezing point of the water containing dissolved air was lower than that of the air-free water by 0.36 mosmol, or 0.0007 °C on the average. However, under physiological conditions, the solutions inside (cytoplasm and vacuolar sap) and outside a cell can be considered to contain nearly equal amounts of such gases as N_2, O_2, and CO_2. Therefore, the effect of the dissolved air on the difference in the osmotic pressure between inside and outside the cell is not only practically, but also theoretically, insignificant.

Appendix

A Precise Equation of Freezing-Point Depression. The actual phenomena occurring in freezing-point depression in an aqueous solution consist of an unstable state caused by the creation of a chemical potential difference between the liquid water and the ice of the aqueous solution by supercooling up to a certain temperature T' K below the freezing point of the aqueous solution to be tested, T K, and pure water, T° K, a non-equilibrium process, which is followed by an equilibrium state. During the non-equilibrium process, ice grows and the temperature of the aqueous mixture containing the ice rises to T K from T' K, which is dependent on the water activity of the aqueous solution: this nullifies the difference in the chemical potential. The difference in temperature between T° and T is the freezing-point depression.

Thus, the difference in the chemical potential between the ice and the liquid water at T' K shouls be calculated as a function of the temperature. To do this, chemical potentials of both the ice and the water must be obtained as functions of the temperature and the water concentration. The chemical potentials of ice $\mu_1^{\circ s}(T)$ and of liquid water $\mu_1^{\circ l}(T)$ are formulated as, respectively:

$$\mu_1^{\circ s}(T) = \overset{s}{Hm}_{(1)}(T) - T\overset{s}{Sm}_{(1)}(T) \tag{1}$$

$$\mu_1^{\circ l}(T) = \overset{l}{Hm}_{(1)}(T) - T\overset{l}{Sm}_{(1)}(T) \tag{2}$$

where $Hm_{(1)}(T)$ and $Sm_{(1)}(T)$ are the partial molar enthalpy and the partial molar entropy of water respectively, and where the suffixes s, l, and 1 repesrent solid(ice) and liquid states, and water, respectively. For an ideal aqueous solution where the mole fraction of water is x_1:

$$\mu_1^{l}(T) = \overset{l}{Hm}_{(1)}(T) - T\overset{l}{Sm}_{(1)}(T) + TR\ln x_1. \tag{3}$$

Here,

$$\overset{s}{Hm}_{(1)}(T) = \int_0^{T} \overset{s}{Cp},m_{(1)}(T'')\mathrm{d}T'' \tag{4}$$

$$\overset{l}{Hm}_{(1)}(T) = \int_0^{T^\circ} \overset{s}{Cp},m_{(1)}(T'')\mathrm{d}T'' + \Delta_{\mathrm{fus}}Hm_{(1)}(T^\circ) - \int_T^{T^\circ} \overset{l}{Cp},m_{(1)}(T'')\mathrm{d}T'', \tag{5}$$

$$\overset{s}{Sm}_{(1)}(T) = \int_0^{T} \overset{s}{Cp},m_{(1)}(T'')\frac{\mathrm{d}T''}{T''}, \tag{6}$$

$$\overset{l}{Sm}_{(1)}(T) = \int_0^{T^\circ} \overset{s}{Cp},m_{(1)}(T'')\frac{\mathrm{d}T''}{T''} + \frac{\Delta_{\mathrm{fus}}Hm_{(1)}(T^\circ)}{T^\circ} - \int_T^{T^\circ} \overset{l}{Cp},m_{(1)}(T'')\frac{\mathrm{d}T''}{T''} \tag{7}$$

where $\overset{s}{Cp},m_{(1)}(T)$, $\overset{l}{Cp},m_{(1)}(T)$, and $\Delta_{\mathrm{fus}}Hm_{(1)}(T)$ are the molar heat capacity of ice(solid) and liquid water as functions of the temperature at a constant pressure, and the molar heat of the fusion of ice at temperature T K, respectively.

In the equilibrium state,

$$\mu_1^{l}(T) = \mu_1^{\circ s}(T). \tag{8}$$

From Eqs. 1 and 3, Eq. 8 becomes:

$$\overset{l}{Hm}_{(1)}(T) - \overset{s}{Hm}_{(1)}(T) = T\{\overset{l}{Sm}_{(1)}(T) - \overset{s}{Sm}_{(1)}(T) - R\ln x_1\}. \tag{9}$$

Since

$$\overset{l}{Hm}_{(1)}(T) - \overset{s}{Hm}_{(1)}(T) = \Delta_{\mathrm{fus}}Hm_{(1)}(T), \tag{10}$$

Eq. 9 becomes:

$$\frac{\Delta_{\mathrm{fus}}Hm_{(1)}(T)}{T} = \overset{l}{Sm}_{(1)}(T) - \overset{s}{Sm}_{(1)}(T) - R\ln x_1. \tag{11}$$

For pure water, we have the same equation as Eq. 11, i.e.;

$$\frac{\Delta_{\mathrm{fus}}Hm_{(1)}(T^\circ)}{T^\circ} = \overset{l}{Sm}_{(1)}(T^\circ) - \overset{s}{Sm}_{(1)}(T^\circ). \tag{12}$$

Thus, from Eqs. 11 and 12, we obtain this equation:

$$\frac{\Delta_{\mathrm{fus}}Hm_{(1)}(T)}{T} - \frac{\Delta_{\mathrm{fus}}Hm_{(1)}(T^\circ)}{T^\circ} + \overset{l}{Sm}_{(1)}(T^\circ) - \overset{l}{Sm}_{(1)}(T) - \overset{s}{Sm}_{(1)}(T^\circ) + \overset{s}{Sm}_{(1)}(T) = -R\ln x_1. \tag{13}$$

Since the molar heat of the fusion of ice at T' K, $\Delta_{\mathrm{fus}}Hm_{(1)}(T')$, is expressed with the molar heat of the fusion of ice at T° K, $\Delta_{\mathrm{fus}}Hm_{(1)}(T^\circ)$, by this equation:

$$\Delta_{\mathrm{fus}}Hm_{(1)}(T') = \Delta_{\mathrm{fus}}Hm_{(1)}(T^\circ) - \int_{T'}^{T^\circ} \{\overset{l}{Cp},m_{(1)}(T'') - \overset{l}{Cp},m_{(1)}(T'')\}\mathrm{d}T'', \tag{14}$$

introducing Eqs. 4, 5, 6, 7, and 14 into Eq. 13 and then rearranging it gives:

$$\Delta_{\mathrm{fus}}Hm_{(1)}(T^{\circ})\left(\frac{1}{T}-\frac{1}{T^{\circ}}\right) - \frac{1}{T}\int_{T}^{T^{\circ}}\{\overset{l}{C}p,m_{(1)}(T'')-\overset{s}{C}p,m_{(1)}(T'')\}\mathrm{d}T'' + \int_{T}^{T^{\circ}}\frac{\overset{l}{C}p,m_{(1)}(T'')-\overset{s}{C}p,m_{(1)}(T'')}{T''}\mathrm{d}T'' = -R\ln x_1. \tag{15}$$

This equation is a theoretically precise expression of the freezing-point depression in ideal aqueous solutions.

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