Effect of Disaccharides on Ion Properties in Milk-Based Systems

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The mean spherical approximation (MSA) theory is used to explain the impact of sugars on ion properties in milk-based systems by taking into account electrostatic interactions and volume exclusion effects. This study first focuses on the changes in Ca^{2+} activity and pH in a solution consisting of $CaCl_2$, KCl, and K_3citrate, as a function of sucrose concentration. MSA model calculations were compared with experimental results, and the model satisfactorily describes the ion properties. The excluded volume effects appear to account for a considerable increase in activity coefficient of the ions. This offers a sufficient explanation for the increase in Ca^{2+} activity and the decrease in pH in milk-based systems with added disaccharides. In addition, hydration of milk proteins seems to enhance ion pair formation in milk. All disaccharides lead to similar modification of the thermodynamic properties of milklike systems, confirming that the observed effects are primarily due to volume exclusion effects.

KEYWORDS: Sucrose; lactose; maltose; trehalose; calcium; pH; activity coefficient; milk; mean spherical approximation

INTRODUCTION

Sugars are commonly added to food to contribute to physicochemical, sensorial, and microbial properties, such as controlling viscosity and texture, adding bulk, retaining moisture, reducing water activity, and improving softness (*1, 2*). Studies show that sugars affect protein properties such as thermal stability, conformation, self-association, surface activity, and hydration (*3−5*). Studies involving sugars and caseins suggest that addition of sugars leads to preferential hydration of the caseins (*6, 7*). The alteration of physicochemical properties of casein micelles may have effects on the ionic equilibria, thereby changing the stability of milk products enriched with sugars, such as dairy desserts and sweetened condensed milk, which may contain sugar content up to 40%. Therefore, it is of great practical importance and relevance to study the effects of the addition of sugars on the ion properties in milk-based systems.

In milk, dynamic ion equilibria exist between the dissolved salts in milk serum and colloidal calcium phosphate in casein micelles. Ion equilibria play an essential role in the conformational stability of casein micelles (*8*). It has been found that addition of sucrose or glucose ranging from 0 to 6.5 mol/kg of water has an impact on the thermodynamic properties of ions in solution, such as a decrease in pH in phosphate buffer solutions with a decrease of 0 and 0.7 pH unit (*9, 10*) and an increase in Ca^{2+} activity in milk (*11*), but an

explanation was not offered. The changes in Ca^{2+} activity and pH are crucial to dairy products during processing such as heat stability and coagulation. As nonelectrolytes, sugars are, in the first instance, not expected to affect the pH and ionic activities. The classical Debye−Hückel (DH) theory is not able to explain the observed phenomena. Addition of sugar can decrease the dielectric permittivity of water, and this causes a decrease in the activity coefficient of the ions in electrolyte solutions according to the DH theory, predicting a decrease in Ca^{2+} activity and H^+ activity (*9, 11*). This is thus inconsistent with the literature results. Moreover, it has been shown that the water activity reduction upon addition of sugars cannot explain the decrease in pH in the buffer solutions (*10*). Additionally, other effects, such as whether addition of sugar affects ionic strength, still remain unclear.

A more recent theory, the mean spherical approximation (MSA), has been developed for the description of thermodynamic properties of electrolyte solutions (*12−14*). In comparison to the DH model, the MSA approach is better suited to describe the nonideality of electrolyte solution in concentrated systems (ionic strength > 0.1 M). In the MSA approach, all the solute species, charged or uncharged, have finite size (*12*). This allows one to take into account the excluded volume effects, e.g. via the hard sphere contribution, which reveals how space can be occupied by spheres (*15*). Also, the decrease in dielectric constant of the solution with increasing concentration of electrolyte or nonelectrolyte is considered. This leads to an increase in the strength of ion−ion interactions with increasing ionic strength (*12*).

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The aim of this paper is to investigate the applicability of the MSA theory to describe ion properties in milk-based systems enriched with disaccharides. For this purpose, we developed a mathematical model based on the MSA to calculate the changes in Ca^{2+} activity and pH as a function of sucrose concentration in a multielectrolyte solution containing $CaCl_2$, KCl, and K_3citrate. To validate the model, experiments were carried out in $CaCl_2$−KCl−K_3citrate solutions with different sucrose concentrations. Subsequently, the MSA theory was used to qualitatively explain the increase in Ca^{2+} activity and decrease in pH in milk-based systems upon addition of sugars. In parallel, the influence of sugars on ion pair formation, conductivity, water activity, and ion equilibria of milk was also studied and compared.

MSA THEORY

The MSA theory has been extensively applied to describe the thermodynamic properties of electrolytes and nonelectrolytes (*16*, *17*). MSA theory is used here to learn more about the molar activity coefficient of ion species i (y_i). Generally, the thermodynamic properties can be derived from the excess Helmholtz free energy ΔA^{EX}, which is made up of two contributions:

$$\Delta A^{EX} = \Delta A^{ES} + \Delta A^{HS} \tag{1}$$

ΔA^{ES} is the electrostatic part due to the ionic atmosphere, and ΔA^{HS} is the hard sphere part due to the finite size of the solute species.

The electrostatic contribution to the single ion activity coefficient can be written as (*14*)

$$\ln y_i^{ES} = -\frac{\beta e_0^2}{4\pi\varepsilon_0\varepsilon_S}\left[\frac{\Gamma z_i^2}{1+\Gamma d_i} + \varsigma d_i\left(\frac{2z_i - \varsigma d_i^2}{1+\Gamma d_i} + \frac{\varsigma d_i^2}{3}\right)\right]$$
$$-\frac{\beta\Delta U^{ES}}{\varepsilon_S}\frac{\partial\varepsilon_S}{\partial\rho_i} \tag{2}$$

where e_0 represents the fundamental electronic charge, ε_S the relative permittivity of solution, ε_0 the permittivity of free space, and $\beta = 1/kT$. The parameters d_i and ρ_i represent the effective diameter and number density of certain solute species, respectively. Γ is the MSA screening parameter, which replaces the screening parameter κ in the Debye−Hückel theory. Γ becomes 0.5κ in the limit of zero ionic strength. The estimation of the model parameter ς and screening parameter Γ have been described by Tikanen and Fawcett (*14*). ΔU^{ES} is the excess internal energy due to ion−ion interactions. Once the screening parameter Γ has been determined, the values of ς and ΔU^{ES} are found by solving a series of equations described by Tikanen and Fawcett (*14*).

In the $CaCl_2$−KCl−K_3citrate solution with various sucrose contents, the only species changing the solution permittivity is sucrose. As a result, the value of $\partial\varepsilon_s/\partial\rho_i$ is assumed to be zero for the estimation of $\ln y_i^{ES}$ for the calcium and hydrogen ion species. The solution permittivity depends on the concentration of the solutes. **Table 1** gives the tabulated permittivity of solution for different concentrations of sucrose (*18*). In addition to sucrose, the electrolytes $CaCl_2$, KCl, and K_3citrate contribute to a decrease in permittivity of the solution. This means that the $CaCl_2$−KCl−K_3citrate solution enriched with sucrose should have lower permittivity than the aqueous solution containing only sucrose. The permittivity of the $CaCl_2$−KCl−K_3citrate

Table 1. Relative Permittivity (ε_S) at 25 °C of Aqueous $CaCl_2$−KCl−K_3-citrate Solution Enriched with Sucrose

sucrose (%) (w/w)	sucrose (mol/L)	ε_S of sucrose soln [a]	est ε_S of the $CaCl_2$−KCl−K_3citrate−sucrose soln [b]
0	0	78.45	77.44
10	0.304	76.19	75.18
20	0.632	73.65	72.64
30	0.988	70.86	69.85
40	1.376	67.72	66.71
50	1.798	64.2	63.19

[a] Data were taken from ref *18*. [b] Used in the MSA model calculation and estimated from eq 3 as explained in the text.

solution on the basis of the relationship (*14*) is

$$\varepsilon_S = \varepsilon_{S0} - \delta_S c_e + b_S c_e^{3/2} \tag{3}$$

where ε_{S0} is the relative permittivity of pure water (78.45 at 25 °C), ε_S is the relative permittivity of the solution, c_e is the electrolyte concentration, δ_S is the dielectric decrement for the given electrolyte, and b_S is a parameter describing the nonlinear part of this dependence. Due to the lack of information about δ_S and b_S of calcium and citrate ions, we assume that the decrease in permittivity of pure water is completely caused by KCl. δ_S and b_S for KCl solution are 14.7 L mol^{-1} and 3.0 $L^{3/2}$ $mol^{-3/2}$, respectively (*14*). The concentration of KCl was estimated to be 0.073 mol/kg, representing the same ionic strength of the $CaCl_2$−KCl−K_3citrate solution, which corresponds to a decrease in permittivity of water by approximately 1.01. The resulting permittivities are shown in **Table 1**.

The unrestricted hard sphere contribution to $\ln y_i$ for unequally sized solute spheres is written as (*14*)

$$\ln y_i^{HS} = \left(\gamma_i - 1 - \frac{2\eta_i}{\eta}y_3\right)\ln(1-\eta) + \frac{\eta}{(1-\eta)^2}\left[3(1-\alpha_i)\right.$$
$$\left. + \gamma_i + \frac{3\eta}{2}(\alpha_i - \beta_i - \gamma_i - 1)\right] + \frac{\eta}{(1-\eta)^3}\left\{\eta\left[5y_3 - \frac{9}{2}y_1 - 2\right.\right.$$
$$\left.\left. + \eta\left(\frac{3}{2}y_1 - 3y_2 - 4y_3 + 1\right)\right] - 2y_3 + 4\right\} \tag{4}$$

$$\eta_i = \frac{\pi}{6}\rho d_i^3$$

$\eta = \sum_i \frac{\rho_i}{\rho}\eta_i, \rho = \sum_i \rho_i$ The parameters introduced in eq 4 were described by Tikanen and Fawcett (*14*).

Combination of eqs 2 and 4 yields the expression for the single ion molar activity coefficient:

$$\ln y_i = \ln y_i^{ES} + \ln y_i^{HS} \tag{5}$$

In addition, cations and anions can associate to form ion pairs, which contribute to the single ion activity coefficient. Only 1:1 ion pair formation is taken into account. The association constant (K_{ass}) can be written as

$$K_{ass} = \frac{a_{ip}}{a_+ a_-} = \frac{(1-\alpha)}{\alpha^2 c}\frac{y_{ip}}{y_+ y_-} \tag{6}$$

where a_{ip}, a_+, and a_- are the activities of the ion pair, cation, and anion, respectively. $1 - \alpha$ is the fraction of cations forming ion pairs, c is the molar concentration of the electrolyte, and y_+, y_-, and y_{ip} represent the molar activity coefficients of the cation, anion, and ion pair, respectively.

Table 2. Overview of the Experimentally Studied Systems

sugar	amt, % (w/w)	analyses[a]			DMT[c]		
		$CaCl_2$−KCl−K_3citrate [b]	SMUF	skim milk	$CaCl_2$−KCl−K_3citrate	SMUF	skim milk
none	0	x	x	x	x	x	x
sucrose	5	nd[d]	x	x	nd	nd	nd
	10	x	x	x	nd	x	x
	20	x	x	x	x	x	x
	30	x	x	x	nd	x	x
	40	x	x	x	nd	nd	nd
	50	x	nd	nd	nd	nd	nd
lactose	5	nd	x	x	nd	nd	nd
	10	nd	x	x	nd	x	x
	15	nd	x	x	nd	nd	nd
maltose	5	nd	x	x	nd	nd	nd
	10	nd	x	x	nd	x	x
	15	nd	x	x	nd	nd	nd
	20	nd	x	x	nd	nd	nd
trehalose	5	nd	x	nd	nd	nd	nd
	10	nd	x	nd	nd	x	nd
	15	nd	x	nd	nd	nd	nd

[a] pH, Ca^{2+} activity (Ca-ISE), conductivity, and water activity measurements. [b] The MSA theory was applied to calculate the effect of sucrose on Ca^{2+} and H^+ ion activity in the system $CaCl_2$−KCl−K_3citrate. [c] The Donnan membrane technique (DMT) was used to determine the concentrations of free Ca^{2+} and Mg^{2+} ions. [d] nd = not determined.

The MSA model for the description of the thermodynamic properties of electrolyte solutions has been developed at the so-called McMillan−Mayer (MM) level (*19*). Briefly, the MM framework considers the solvent as a continuum, not as consisting of discrete particles/molecules, and the solvent is characterized only by its permittivity (*15*). This means that only solute−solute interaction potentials are considered in the MM framework. The ion activity coefficients calculated by MSA cannot be directly compared to experimental values, which are described in the Lewis−Randall (LR) framework unless a conversion is made from the MM level to the LR level. The LR description of a solution considers the excess Gibbs energy, the energy state function defined with temperature (*T*), pressure (*P*), and number of moles (*n*) as the independent variables (which are the three natural variables in a chemical experiment) (*15*). The effect of the conversion was negligible at relatively low concentrations, but it became significant in highly concentrated electrolyte solutions, typically above 1−2 mol/kg of water (*20*). In this study, the concentrations of the salts were far below 1 mol/kg of water. However, there was a large amount of sucrose present in some of the systems. Therefore, the conversion of ion activity coefficient would be needed so that the modeled activity coefficient could be compared to the experimental results. The way to do this conversion has been described by Simonin (*17*). Briefly the first step is to make a link between the osmotic coefficient at the MM and LR level (Eq 7) and then convert the molar activity coefficient from the MM to LR level (Eqs 8 and 9):

$$\ln a_W = -\phi^{LR} M_W \sum_{j=1}^{j=i} m_j \tag{7}$$

where ϕ^{LR} is the osmotic coefficient at the LR level. Equation 7 is taken from the literature (*21*). a_W is the water activity. Water activity data at different concentrations of sucrose were obtained from Robinson and Stokes (*22*). M_W is the molecular weight of water, and m_j is the molal concentration of the solute.

$$\phi^{LR} = \phi^{MM}(1 - CV_{\pm}) \tag{8}$$

$$\ln y_i^{LR} = \ln y_i^{MM} - \phi^{MM} CV_i \tag{9}$$

where ϕ^{MM} is the osmotic coefficient at the MM level. C is the total solute concentration (mol/L). $V_{\pm}$ is the mean partial molal volume of the solute, and V_i is the partial molal volume of species i. y_i^{MM} and y_i^{LR} are the molar activity coefficients at the MM and LR levels, respectively. y_i^{MM} is calculated by eq 5.

The next step is to convert the molar activity coefficient to molal activity coefficient within the LR level:

$$y_i^{LR} = \gamma_i^{LR} V d_W \tag{10}$$

γ_i^{LR} is the molal activity coefficient of species i at the LR level. V is the volume of the solution per mass solvent in the LR system. d_W is the density of water. The obtained values of γ_i^{LR} for Ca^{2+} ions were compared to the experimental activity coefficients of SMUF and skim milk enriched with sugars.

MATERIALS AND METHODS

Materials. All the salts (>99.5% purity) used were obtained from Fluka (The Netherlands). Sucrose, maltose hydrate, and trehalose dihydrate (HPLC grade) were obtained from Sigma (The Netherlands). Lactose hydrate (HPLC grade) was purchased from Merck (Germany). The low heat pasteurized skim milk powder (Nilac) was obtained from NIZO (The Netherlands).

Experimental Setup. Table 2 shows the experimental setup. Three systems of increasing complexity were employed: (i) a solution consisting of $CaCl_2$ (5 mM), KCl (55 mM), and K_3citrate (4 mM); (ii) lactose-free simulated milk ultrafiltrate (SMUF); (iii) reconstituted skim milk. SMUF was prepared according to the method of Jenness and Koops (*23*). The reconstituted skim milk was prepared from low heat skim milk powder as described by Gao et al. (*24*). Four disaccharides were separately added to each system, except for the $CaCl_2$−KCl−K_3citrate system, where only sucrose was used. Prior to analyses, the systems with added lactose and maltose were kept at 25 °C for 24 h to allow the reducing sugars to reach their mutarotation equilibrium. The systems with added sucrose and trehalose were directly measured. Physicochemical properties, pH, Ca^{2+} activity, water activity, conductivity, and Ca^{2+} concentration of selected samples were measured as described in Methods. Moreover, the MSA model was used to calculate the pH and Ca^{2+} activity in the $CaCl_2$−KCl−K_3citrate solution with various sucrose contents.

Methods. *pH Measurement.* The pH values of selected samples were measured at 25 °C using a pH meter (Orion 720A+, Thermo, Beverly, MA) equipped with a Ross pH electrode (Orion 8172BNWP, Thermo, Beverly, MA). The pH electrode was calibrated at 25 °C using two buffer solutions (Thermo, Beverly, MA) having pH values of 4.00 and 7.00.

Calcium Activity Measurement. Ionic calcium activity was determined as described by Gao et al. (*24*) using an Orion 720A+ meter equipped with

an Orion 9720 calcium ion selective electrode (Ca-ISE) (Thermo, Beverly, MA).

Donnan Membrane Technique (DMT). The Donnan membrane technique (DMT) was applied to determine the concentrations of free Ca^{2+} and Mg^{2+} ions in the selected samples (**Table 2**). The details of the DMT methodology were described by Gao et al. (*24*) and Temminghoff et al. (*25*). Briefly, the sample solution, also named the donor, is separated from the acceptor solution by a cation exchange membrane (BDH Laboratory Supplies, Poole, U.K.). The negative electric potential of the membrane allows cations to pass through and restricts anions from crossing the membrane. Within a certain time, a Donnan equilibrium is reached for the free metal ion activities between donor and acceptor. If both sides have the same ionic strength, the free metal concentration in the donor is equal to that in the acceptor. In this research, the acceptor solution had the same amount and type of sugar and the same ionic strength as the donor solution did. This eliminated the differences in osmotic pressure and excluded volume between the donor and the acceptor. Samples taken from both sides were measured by an inductively coupled plasma atomic emission spectrometer (ICP-AES) (Varian, Mulgrave, Australia).

Conductivity. Conductivities were measured to check for ion pair formation upon addition of sugar. Sugar molecules can bind free water molecules, leading to a decrease in water activity. Addition of sugar molecules to electrolyte solutions may even cause dehydration of ions due to strong hydration of sugar, implying that ion pair formation may be promoted. This would then affect the conductivity of the electrolyte solution.

Conductivity (*G*) can be expressed as (*26*)

$$G = zuvcF \tag{11}$$

$$u = \frac{ze_0}{6\pi\eta_s a} \tag{12}$$

By combining eqs 11 and 12

$$G = \frac{z^2 e_0 vcF}{6\pi r} \times \frac{1}{\eta_s} \tag{13}$$

where *z* is the charge of the ion, *u* is the mobility of the ion, *vc* is the molar concentration of the ion, *F* is Faraday's constant, *r* is the radius of the ion, and η_s is the viscosity of the solution.

Adding sugar increases the viscosity of electrolyte solutions, which in itself causes a decrease in conductivity. Equation 13 is therefore split into two parts. The left part accounts for the ion pair formation, and the right part represents the influence of viscosity on conductivity. When a linear relationship is obtained between *G* and $1/\eta_s$, the left part apparently remains constant, meaning that ion pair formation would be insignificant. The viscosity data of a series of sucrose solutions were obtained from the literature (*27*). The viscosity of milk samples enriched with sucrose was measured as described in Viscosity.

The conductivities of selected samples were measured using a conductivity meter (WTW Inolab Cond Level 2, Weilheim, Germany) equipped with a conductivity electrode (WTW TetraCon 325, Weilheim, Germany). The conductivity probe was calibrated at 25 °C using a 0.01 M KCl solution having conductivity values ranging between 1411 and 1423 μS/cm.

Viscosity. The viscosity of skim milk samples enriched with sucrose was determined by using a Anton Paar MCR 300 rheometer and a double gap geometry (DG 26.7 mm). The shear rate ranged from 0.01 to 500 s^{-1}. The viscosity was determined between the shear rates 10 and 500 s^{-1}. The measurements were made in duplicate. The temperature of the samples was 25 °C.

Water Activity. The water activity of selected samples (**Table 2**) was measured using an AquaLab CX3 instrument (Decagon Devices, Pullman, WA).

Ultracentrifugation. The skim milk samples enriched with sugars were ultracentrifuged (at 100 000*g* and 25 °C for 1 h) in a Beckman L-60 ultracentrifuge with a 70 Ti rotor (Beckman Instruments Inc., Brea, CA). The concentrations of total Ca, Mg, K, Na, and P in milk serum were measured by ICP-AES (Varian, Mulgrave, Australia).

RESULTS AND DISCUSSION

Addition of Sucrose to CaCl$_2$−KCl−K$_3$citrate Solution. Figure 1a,c shows that the addition of sucrose (0−50% w/w) causes a considerable increase in Ca^{2+} and H^+ ion activities, respectively, in the CaCl$_2$−KCl−K$_3$citrate solution. The Ca^{2+} activity and pH calculated by the MSA model were in general agreement with the experimental data after using the values of the diameters of cation (d_{cation}), anion (d_{anion}), ion pair (d_{ip}), and sucrose ($d_{sucrose}$) and the association constants (K_{ass}) in **Table 3** and the dielectric permittivity data in **Table 1**. The estimated diameters of the species were chosen on the basis of the agreement between model prediction and experimental results. The diameters for individual ions and ion pairs are in fair agreement with the values from the literature (*14, 19, 28, 29*). However, differences in Ca^{2+} activity and pH between calculation and measurement became larger at higher sucrose concentrations (**Figure 1a,c**). Ions may behave far more nonideally in such highly concentrated systems so that the MSA model may also be no longer applicable. The diameter of hydrated cations decreases as the sucrose concentration increases and may decrease considerably in the high sucrose concentration solutions. Such changes in cation diameter were not taken into account in the MSA model due to the lack of diameter data for cations in sucrose solutions.

The changes in Ca^{2+} activity and pH rely on the free ion concentrations and single ion activity coefficients. **Figure 1b,d** reveals that the increase in activity coefficient is the main contribution to the increase in Ca^{2+} or H^+ activity in the CaCl$_2$−KCl−K$_3$citrate solution enriched with sucrose. The addition of sucrose contributes to the changes in ion activity coefficient in two ways. First, on the one hand, the decrease in permittivity of the solution causes a decrease in the ion activity coefficient due to enhanced electrostatic interaction. On the other hand, the calculated ionic strength decreased from 0.069 to 0.044 mol/L due to an increase in volume (**Figure 1e**), which increased the ion activity coefficient. **Figure 1b,d** shows the summation of the two effects. The calculated y_{Ca}^{ES} and y_H^{ES} values decreased from 0.390 to 0.355 and from 0.782 to 0.764, respectively. Second, the system is occupied by many sucrose molecules, leading to an increase in excluded volume effect. This accounts for the considerable departure from ideality of the ions in the concentrated sucrose solutions. **Figure 1b,d** shows that the calculated y_{Ca}^{HS} and y_H^{HS} values remarkably increase from 1.025 to 3.859 and from 1.022 to 3.298, respectively, as a function of sucrose concentration. By combining the electrostatic and hard sphere contribution, it turns out that the single ion activity coefficient still increases significantly, for y_{Ca} from 0.400 to 1.369 and for y_H from 0.799 to 2.519 (**Figure 1b,d**). Additionally, the molar fraction of free Ca^{2+} ions was calculated by the MSA model (**Figure 1f**), and it was found to slightly increase from 22% to 26% throughout the range of sucrose concentrations. Consequently, the increase in activity coefficient, particularly the excluded volume effect, is the main reason for the increase in ion activity in the CaCl$_2$−KCl−K$_3$citrate solution enriched with sucrose. The MSA theory appears to offer a satisfactory explanation for the observed ion behaviors in electrolyte solutions at a very high content of sucrose. In addition, the effect of using the MM-to-LR conversion (eqs 7−8910), that is from y_{Ca}^{MM} to γ_{Ca}^{LR}, was examined. **Table 4** shows that the difference between y_{Ca}^{MM} and γ_{Ca}^{LR} increases as the concentration of sucrose increases, which indicates that the effect of this conversion is not negligible, particularly at very high sucrose contents. Thus, the values of obtained γ_{Ca}^{LR} were used to compare with experimental molal Ca^{2+} activity coefficients of milk-based systems in the presence of sugars. In the following section, MSA theory was

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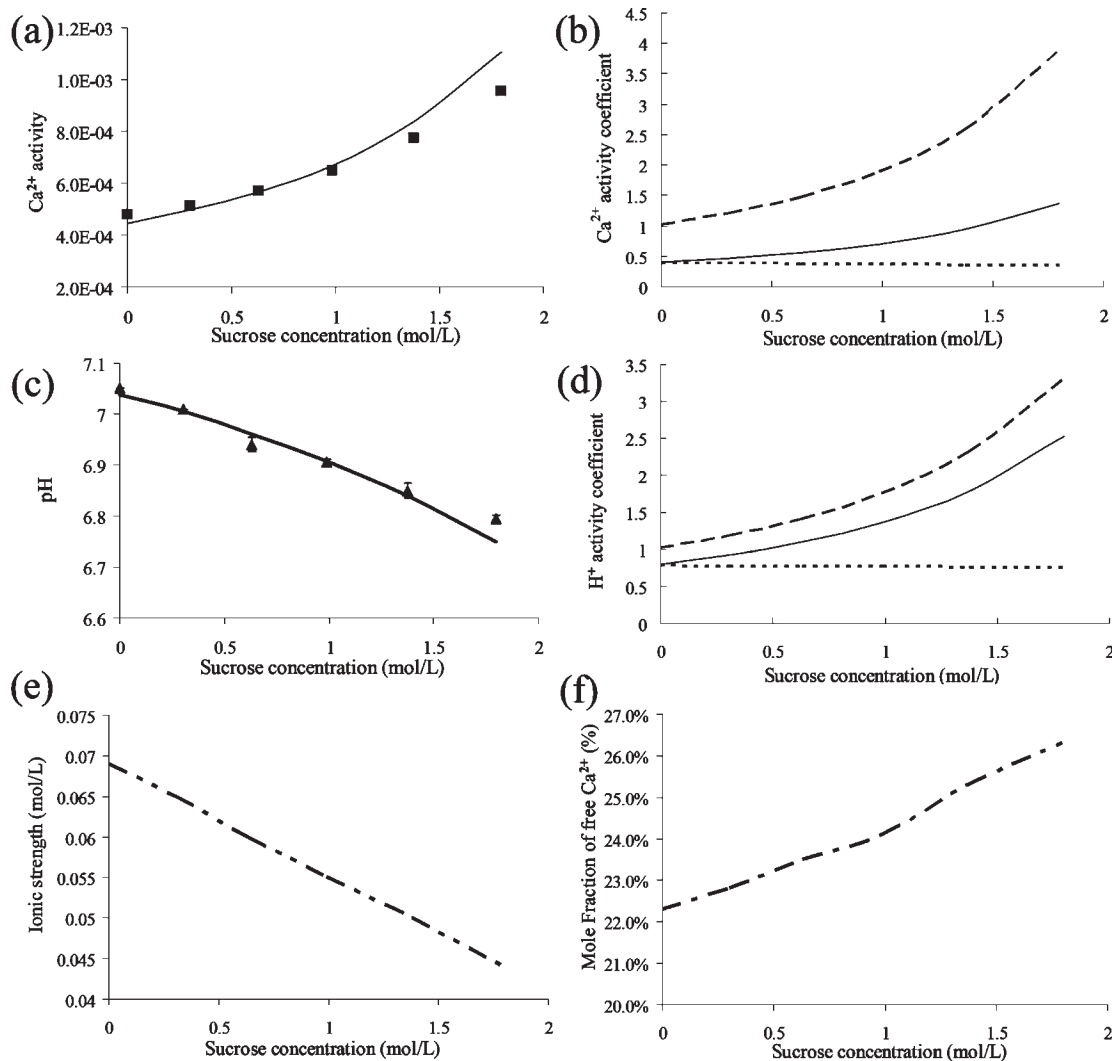
Figure 1. Influence of sucrose on ionic properties in the $CaCl_2-KCl-K_3citrate$ system. (a) and (c) represent Ca^{2+} activity and pH, respectively, as a function of sucrose concentration: (■) measured Ca^{2+} activity by Ca-ISE; (—) calculated Ca^{2+} activity by MSA model; (▲) measured pH; (—) calculated pH by MSA model. (b) and (d) represent the calculated Ca^{2+} and H^+ activity coefficient by MSA model, respectively: (---) activity coefficient of the electrostatic part; (− −) activity coefficient of the hard sphere part; (—) overall activity coefficient. (e) represents the calculated ionic strength (mol/L). (f) represents the calculated mole fraction of free Ca^{2+} as a function of sucrose concentration by the MSA model.

Table 3. Values of the Association Constants K_{ass} and the Diameters of the Cations (d_{cation}), Anions (d_{anion}), Ion Pairs (d_{ip}), and Sucrose ($d_{sucrose}$) Employed in the MSA Model To Calculate Ca^{2+} Activity and pH in the $CaCl_2-KCl-K_3citrate$ Solution

reactants	K_{ass}[a]	d_{cation} (pm)	d_{anion} (pm)	d_{ip} (pm)	$d_{sucrose}$ (pm)
K^+, Cl^-	1	350	350	350	
Ca^{2+}, Cl^-	7	480	350	415	
Ca^{2+}, $citrate^{3-}$	1.66×10^5	480	400	440	
H^+, $citrate^{3-}$	2.51×10^6	400	400	400	
sucrose					700

[a] The association constants were taken from the literature (*34*).

Table 4. Ca^{2+} Activity Coefficients at the MM and LR Levels in $CaCl_2-KCl-K_3citrate$ Solution Enriched with Sucrose using Eqs 7−10

amt of sucrose, % (w/w)	y_{Ca}^{MM}	y_{Ca}^{LR}	γ_{Ca}^{LR}
10	0.47	0.46	0.43
20	0.56	0.55	0.48
30	0.70	0.69	0.54
40	0.94	0.91	0.64

used to qualitatively explain the ion properties in milk-based systems upon addition of sugars.

Addition of Sucrose to SMUF and Skim Milk. *Excluded Volume Effect.* As in the $CaCl_2-KCl-K_3citrate$ system, the addition of sucrose increases the Ca^{2+} and H^+ ion activities in SMUF and skim milk (**Figure 2b,e**). The Ca^{2+} activity coefficient, which was calculated from the experimental free Ca^{2+} concentration and activity, increased considerably throughout the range of

sucrose concentration (**Figure 2c**). It stands to reason that the excluded volume effect accounted for the increase in Ca^{2+} and H^+ activities. The increase in Ca^{2+} activity coefficient in skim milk was larger than it was in SMUF. This is probably because skim milk contains proteins and lactose, which account for stronger excluded volume effects.

However, the experimentally derived values of Ca^{2+} activity coefficients of SMUF and skim milk in the absence of sucrose were far too low compared to the value commonly calculated by the Davies equation, which is 0.40 for both SMUF and skim milk (**Table 5**). On the one hand, we speculate that a systematic deviation is generated by the Ca-ISE methodology at lower
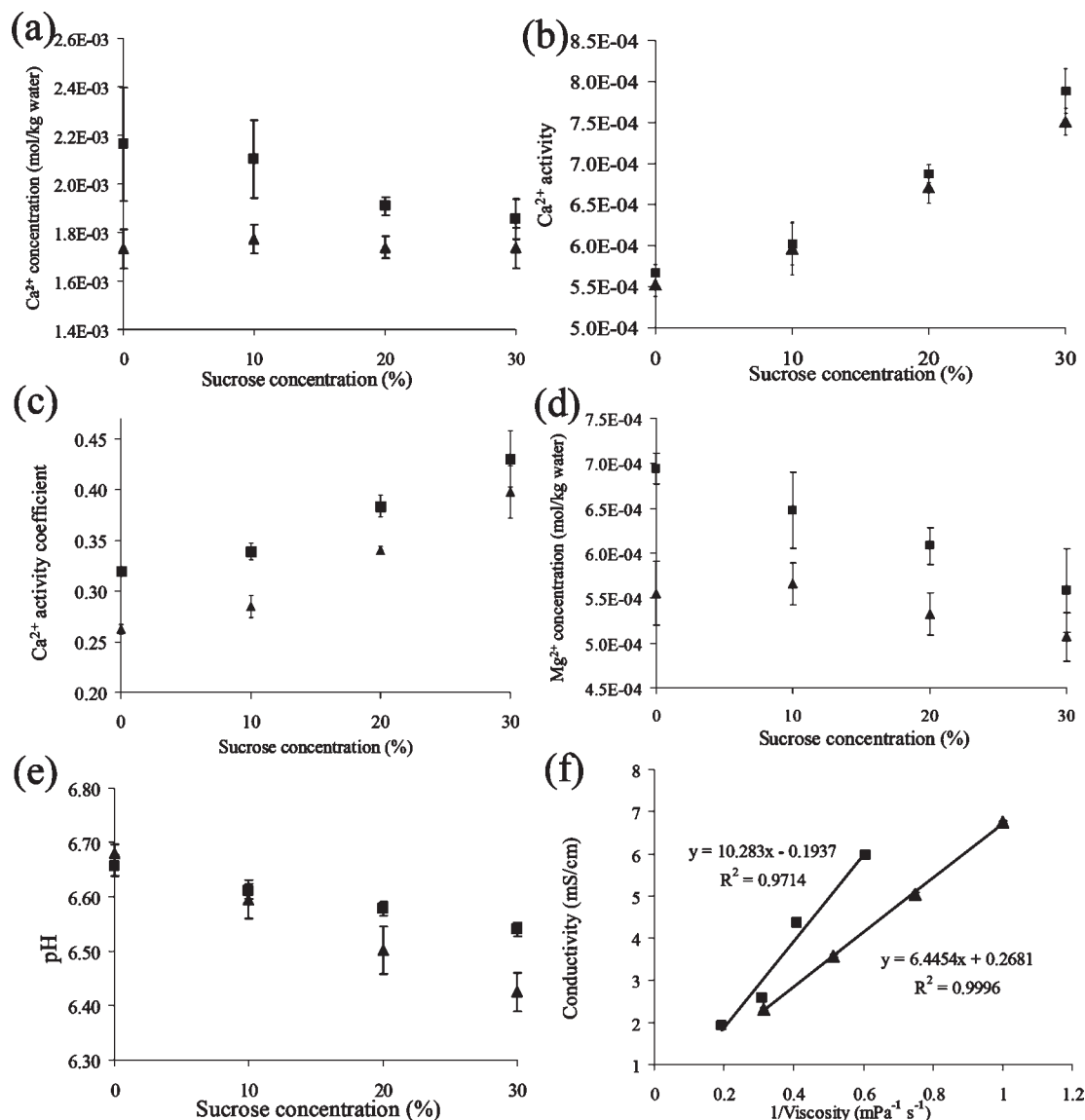
Figure 2. Influence of sucrose on ion properties ((▲) SMUF; (■) skim milk): (a) Ca^{2+} concentration measured by DMT; (b) Ca^{2+} activity measured by Ca-ISE; (c) Ca^{2+} activity coefficient calculated from experimental data; (d) Mg^{2+} concentration measured by DMT; (e) pH in SMUF and skim milk measured by pH electrode; (f) relation between conductivity and viscosity at different sucrose concentrations.

ionic strength ($I < 0.09$ mol/kg of water) for the Ca^{2+} activity measurement, since these results are consistent with earlier observations (*24*, *30*). On the other hand, the calculated Ca activity coefficient by the Davies equation may overestimate the actual value in a multicomponent solution (*31*). We are in the process of analyzing the causes of the large differences in individual Ca ion activity coefficient between experimental and calculated results and will discuss the issue in a separate publication.

A correction was made to adjust the experimental Ca^{2+} activity coefficients in SMUF according to the method described by Geerts et al. (*11*). Briefly, the experimental Ca^{2+} activities were plotted as a function of the added sugar concentration. The Ca^{2+} activities were found to correlate linearly with the amount of sugar added (**Table 6**). Since the free Ca^{2+} concentration of SMUF is not influenced by the additional sugar (**Figure 2a** and **Table 8**), this indicates that the increase in Ca^{2+} activity coefficient is the same as the increase in Ca^{2+} activity after addition of sugar. That is, the ratios of Ca^{2+} activity coefficients in the presence and absence of sugar

Table 5. Ca^{2+} Activity Coefficients (γ_{Ca}^{2+}) (SD)[a] of SMUF and Skim Milk in the Absence of Sugar

	SMUF[b]	skim milk[b]	theor[c]
γ_{Ca}^{2+}	0.32 (0.00)	0.26 (0.01)	0.40

[a] $n = 2$. [b] Activity coefficients were calculated from the Ca^{2+} activity (Ca-ISE method) (**Table 6**) and Ca^{2+} concentration (DMT method) (**Table 8**). [c] Value was calculated by the Davies equation.

should be equal. **Table 6** gives the adjusted Ca^{2+} activity coefficients in the presence of sugars in SMUF. Interestingly, the adjusted Ca^{2+} activity coefficients in SMUF are nearly same as the calculated values (γ_{Ca}^{LR}) of the $CaCl_2-KCl-K_3$citrate solution enriched with sucrose by the MSA model (**Table 4**). This implies that the approach used by Geerts et al. (*11*) gives good prediction of Ca^{2+} activity coefficients in electrolyte solutions. For skim milk, the Ca^{2+} activity also increased linearly with the content of sugar (**Table 6**). However, the free Ca^{2+} concentration decreased with the added sugar: e.g., sucrose (**Figure 2a**). Thus, the Ca^{2+} activity coefficients

Table 6. Effect of Added Sugar on the Ca^{2+} Activity and the Ca^{2+} Activity Coefficient ($\gamma_{Ca^{2+},S}$) (in Terms of Molality) of SMUF and Skim Milk

| | | Ca^{2+} activity (SD) [a] | | | | | | | | |
| | | SMUF | | | | skim milk | | | | fit eq [f] $a_{Ca^{2+}} = bs + c$ |
sugar	amt, % (w/w)	measd [b]	calcd [c]	ratio [d]	$\gamma_{Ca^{2+},S}$ [e]	measd [b]	calcd [c]	ratio [d]	$\gamma_{Ca^{2+},S}$ [e]	
none	0	0.55 (0.01)	0.55	1.000	0.40	0.57 (0.01)	0.57	1.000	0.40	
sucrose	5	0.57 (0.02)				0.58 (0.02)				$a_{Ca^{2+}} = 0.0007s + 0.00055$ (SMUF)
	10	0.60 (0.03)	0.62	1.122	0.45	0.60 (0.03)	0.64	1.129	0.46	$R^2 = 0.984$
	20	0.67 (0.02)	0.69	1.249	0.50	0.69 (0.02)	0.71	1.252	0.57	$a_{Ca^{2+}} = 0.0007s + 0.00057$ (skim milk)
	30	0.75 (0.02)	0.76	1.375	0.55	0.79 (0.03)	0.78	1.376	0.64	$R^2 = 0.961$
	40	0.92 (0.01)				0.94 (0.05)				
lactose	5	0.57 (0.03)	0.59	1.059	0.42	0.58 (0.00)		1.059	0.42	$a_{Ca^{2+}} = 0.0007s + 0.00055$ (SMUF)
	10	0.61 (0.03)	0.62	1.122	0.45	0.60 (0.02)		1.122	0.44	$R^2 = 0.970$
	15	0.65 (0.02)	0.66	1.185	0.47					
maltose	5	0.56 (0.00)	0.58	1.041	0.42	0.58 (0.02)	0.59	1.041	0.42	$a_{Ca^{2+}} = 0.0005s + 0.00055$ (SMUF)
	10	0.59 (0.03)	0.60	1.086	0.43	0.60 (0.05)	0.61	1.076	0.44	$R^2 = 0.953$
	15	0.61 (0.03)	0.63	1.131	0.45	0.62 (0.03)	0.63	1.111	0.44	$a_{Ca^{2+}} = 0.0004s + 0.00057$ (skim milk)
	20	0.66 (0.03)	0.65	1.176	0.47	0.65 (0.02)	0.65	1.146	0.46	$R^2 = 0.974$
trehalose	5	0.58 (0.02)	0.59	1.059	0.42					
	10	0.62 (0.01)	0.62	1.122	0.45					$a_{Ca^{2+}} = 0.0005s + 0.00055$ (SMUF)
	15	0.66 (0.01)	0.66	1.185	0.47					$R^2 = 0.987$

[a] All the values of Ca^{2+} activities and standard deviations were obtained by using their original values multiplied by a factor of 1000. The experiment was conducted in triplicate. [b] The Ca^{2+} activities were measured by the Ca-ISE method. [c] The Ca^{2+} activities were calculated by equations, which were obtained by linearly fitting the measured Ca^{2+} activities as a function of sugar concentrations (see the column "fit eq"). [d] Ca^{2+} activity ratio between the calculated Ca^{2+} activity and the measured Ca^{2+} activity in the absence of sugars. [e] The adjusted Ca^{2+} activity coefficient, calculated by using eq 15 [f] Based on 3 decimal Ca^{2+} activity data; s is the concentration of sugar (%) (w/w).

Table 7. Concentration (mmol/kg of Water) of Major Ions in Milk Serum[a]

| | | concn (mmol/kg of water) | | | | |
sugar	amt, % (w/w)	[Ca] (SD)[b]	[K] (SD)	[Mg] (SD)	[Na] (SD)	[P][c] (SD)
none	0	11.1 (0.2)	45.8 (1.1)	3.9 (0.1)	20.7 (0.3)	15.8 (0.5)
sucrose	10	10.7 (0.7)	46.4 (1.0)	3.8 (0.3)	20.4 (0.8)	15.9 (0.2)
	20	10.9 (0.1)	45.4 (1.0)	3.7 (0.1)	19.9 (0.4)	15.4 (1.2)
	30	11.1 (0.4)	44.3 (1.4)	3.6 (0.1)	19.9 (0.8)	16.0 (0.6)
lactose	10	11.4 (0.2)	48.8 (0.9)	3.9 (0.1)	21.4 (0.4)	16.6 (0.2)
maltose	10	11.5 (0.3)	48.8 (0.4)	4.0 (0.2)	21.5 (0.2)	16.2 (0.4)

[a] Concentrations (mmol/kg of water) of the major ions in skim milk: [Ca] = 36.4, [Mg] = 5.8, [Na] = 22.1, [K] = 50.2, [P_i] = 22.6, [Cl] = 34.0, [citrate] = 11.2, [SO_4] = 1.1. [b] $n = 2$. [c] [P] represents the sum of the concentration of inorganic phosphate and organic phosphate in milk serum, which is measured by ICP-AES.

(**Table 6**) need to take into account the change in the Ca^{2+} concentration and can be calculated as

$$\frac{a_{Ca^{2+},S}}{a_{Ca^{2+},B}} = \frac{m_{Ca^{2+},S}}{m_{Ca^{2+},B}} \frac{\gamma_{Ca^{2+},S}}{\gamma_{Ca^{2+},B}} \quad (14)$$

Equation 14 can be rearranged as

$$\gamma_{Ca^{2+},S} = \gamma_{Ca^{2+},B} \frac{a_{Ca^{2+},S}}{a_{Ca^{2+},B}} \frac{m_{Ca^{2+},B}}{m_{Ca^{2+},S}} \quad (15)$$

$\gamma_{Ca^{2+},S}$ represents the adjusted molal Ca^{2+} activity coefficient at a certain sugar concentration. $\gamma_{Ca^{2+},B}$ is the molal Ca^{2+} activity coefficient of milk in the absence of sugar (**Table 5**). $m_{Ca^{2+},S}$ and $a_{Ca^{2+},S}$ are the molal Ca^{2+} concentration and the calculated Ca^{2+} activity of milk in the presence of sugar, respectively (**Tables 6** and **8**). $m_{Ca^{2+},B}$ and $a_{Ca^{2+},B}$ are the Ca^{2+} concentration and the calculated Ca^{2+} activity of milk in the absence of sugar, respectively (**Tables 6** and **8**). For the milk samples without measurement of the Ca^{2+} concentrations, the same correction method was used as described for SMUF.

Hydration Effect. **Figure 2a,d** shows that the concentrations of free Ca^{2+} and Mg^{2+} ions in SMUF do not change significantly throughout the range of sucrose concentrations, whereas in skim milk they appear to decrease as the sucrose concentration increases. It seems that ion pairs are formed in skim milk, but not in SMUF in the presence of sucrose. **Figure 2f** shows that the conductivity has a linear relationship with the inverse of viscosity in SMUF; thus, there is no evidence for ion pair formation in this case. However, this situation is less clear for milk. These results indicate that the effect of sucrose on the ionic properties of skim milk differ from those for SMUF, which apparently cannot be explained solely by excluded volume effects.

Sucrose can bind free water molecules and decrease water activity. This leads to a change in hydration properties of solutes. The differences in solute−water interactions and changes to the hydration sphere around solute species (*10*) may account for the difference in ion pair formation between SMUF and skim milk. The major compositional differences between SMUF and skim milk are the proteins: namely, casein micelles and whey proteins and lactose. Studies suggest that the preferential exclusion of sugar molecules from the casein domain results in preferential hydration of the caseins (*6*, *7*, *32*) and of the globular proteins (*3*, *32*). The increase in hydration of milk proteins, and the ability of sucrose to bind water, can cause considerable dehydration of ions, resulting in ion pair formation in milk. Although MSA theory does not directly consider the solute−water interaction, it is possible to integrate the hydration properties of ions into the MSA model, for instance, by taking into account the dependence of the size of hydrated ions on sucrose concentration. This would require experimentally determining the change in diameters of hydrated ions as an increase in sucrose concentration. Thus, the MSA model may possibly describe the changes in free ion concentrations in case of changes in hydration.

In addition, the ion equilibria between the aqueous phase and colloidal phase are not significantly affected throughout the range of sucrose concentration, as shown by the concentrations of ions in milk serum (**Table 7**). The influence of sucrose on ion speciation in milk appears to occur only in milk serum.

Comparison among Sugars. With respect to the four disaccharides, sucrose and trehalose are nonreducing sugars, while lactose and maltose are reducing sugars. **Tables 6−8** summarize the influence of the four disaccharides on the thermodynamic

Table 8. Comparison of the Influence of Disaccharides on Electrolyte Properties of SMUF and Skim Milk

sugar	amt, % (w/w)	Ca²⁺ concn (mmol/kg) (SD)[b]		pH (SD)[a]		conductivity (mS/cm) (SD)[a]		aW (SD)[a]	
		SMUF	skim milk	SMUF	skim milk	SMUF	skim milk	SMUF	skim milk
none	0	1.73 (0.08)	2.16 (0.23)	6.68 (0.02)	6.66 (0.02)	6.74 (0.04)	5.96 (0.09)	0.999 (0.001)	0.996 (0.001)
sucrose	5			6.65 (0.04)	6.63 (0.01)	5.85 (0.07)	5.09 (0.05)	0.997 (0.001)	0.994 (0.001)
	10	1.77 (0.06)	2.10 (0.16)	6.60 (0.04)	6.61 (0.01)	5.05 (0.05)	4.37 (0.10)	0.995 (0.001)	0.990 (0.001)
	20	1.74 (0.04)	1.91 (0.04)	6.50 (0.04)	6.58 (0.01)	3.58 (0.04)	2.59 (0.08)	0.987 (0.001)	0.982 (0.001)
	30	1.74 (0.08)	1.86 (0.09)	6.43 (0.04)	6.54 (0.01)	2.32 (0.03)	1.92 (0.06)	0.977 (0.001)	0.970 (0.001)
	40			6.36 (0.05)	6.52 (0.02)	1.34 (0.02)	1.02 (0.03)	0.962 (0.001)	0.952 (0.001)
lactose	5			6.61 (0.01)	6.63 (0.01)	5.83 (0.04)	5.07 (0.06)	0.997 (0.001)	0.994 (0.001)
	10	1.80 (0.03)	2.21 (0.12)	6.55 (0.04)	6.61 (0.01)	4.98 (0.04)	4.33 (0.08)	0.994 (0.001)	0.992 (0.001)
	15			6.49 (0.02)		4.19 (0.04)		0.993 (0.001)	
maltose	5			6.60 (0.04)	6.64 (0.01)	5.81 (0.06)	5.07 (0.06)	0.997 (0.001)	0.994 (0.001)
	10	1.73 (0.05)	2.15 (0.10)	6.55 (0.05)	6.61 (0.01)	4.99 (0.06)	4.35 (0.07)	0.995 (0.001)	0.992 (0.001)
	15			6.50 (0.02)	6.59 (0.01)	4.21 (0.05)	3.59 (0.06)	0.992 (0.001)	0.987 (0.001)
	20			6.49 (0.02)	6.56 (0.03)	3.49 (0.05)	2.54 (0.06)	0.986 (0.001)	0.984 (0.001)
trehalose	5			6.62 (0.01)		5.81 (0.01)		0.996 (0.001)	
	10	1.84 (0.03)		6.58 (0.01)		4.94 (0.01)		0.992 (0.001)	
	15			6.53 (0.01)		4.12 (0.01)		0.990 (0.001)	

[a] $n = 3$. [b] $n = 2$.

properties of SMUF and skim milk. Generally, all sugars give rise to similar effects on pH, Ca²⁺ activity and concentration, Ca²⁺ activity coefficient, conductivity, water activity, and ion equilibria. This suggests that it is the excluded volume effect which is the main factor in the impact on thermodynamic properties of ions in the solution, rather than the type of sugars. Still, slight differences in solution properties remain. First, the reducing sugars resulted in slightly lower pH than the nonreducing sugars in SMUF, which is possibly due to a release of hydrogen ions from the active hydroxyl group at their reducing end. Second, trehalose causes slightly lower water activities and higher Ca²⁺ activities than did the other sugars in SMUF. This may be due to its binding a larger number of water molecules than do maltose and sucrose, thus affecting the structure of water to a greater extent (*33*).

In conclusion, the MSA theory appears to satisfactorily explain the influence of disaccharides on the thermodynamic properties of ions in CaCl₂−KCl−K₃citrate solution and to qualitatively describe ion properties in SMUF and skim milk upon addition of disaccharides. The large increase in ion activity coefficient, which is caused by excluded volume effects, accounts for the significant increase in Ca²⁺ activity and decrease in pH in milk systems. However, the excluded volume effect is not the only explanation for the decrease in free Ca²⁺ and Mg²⁺ concentration in skim milk enriched with sucrose. The finding that sucrose addition causes preferential hydration of milk proteins may provide insights to understand the decrease in free Ca²⁺ and Mg²⁺ concentrations in milk. All the disaccharides do not appear to significantly alter the original ion equilibria between the serum and colloidal phases. The influence of disaccharides on the free ion concentrations seems to happen only in milk serum. Last, but not least, no significant difference is found among sugars, since all the sugars have similar impacts on the thermodynamic properties of ions in milk systems.

Further details of the calculation in MathCad (version 14.0) of Ca²⁺ and H⁺ ion activity coefficients in the CaCl₂−KCl−K₃citrate−sucrose solutions can be obtained from the authors upon request.

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