Aqueous Solubility of Calcium L-Lactate, Calcium D-Gluconate, and Calcium D-Lactobionate: Importance of Complex Formation for Solubility Increase by Hydroxycarboxylate Mixtures

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ABSTRACT: Among the calcium hydroxycarboxylates important for cheese quality, D-lactobionate $[K_{sp} = (7.0 \pm 0.3) \times 10^{-3}$ $mol^3 \ L^{-3}]$ and L-lactate $[K_{sp} = (5.8 \pm 0.2) \times 10^{-3} \ mol^3 \ L^{-3}]$ were found more soluble than D-gluconate $[K_{sp} = (7.1 \pm 0.2) \times 10^{-4}$ $mol^3 \ L^{-3}]$, as indicated by the solubility products determined electrochemically for aqueous 1.0 M NaCl at 25.0 °C. Still, solubility of calcium L-lactate increases by 45% in the presence of 0.50 M sodium D-gluconate and by 37% in the presence of 0.50 M sodium D-lactobionate, while solubility of calcium D-gluconate increases by 66 and 85% in the presence of 0.50 M sodium L-lactate and 0.50 M sodium D-lactobionate, respectively, as determined by complexometric titration. Sodium L-lactate and sodium D-gluconate have only little influence on solubility of calcium D-lactobionate. The increased solubility is described quantitatively by calcium binding to D-gluconate $(K_1 = 14 \pm 3 \ mol^{-1} \ L)$ in 1.0 M NaCl at 25 °C, D-lactobionate $(K_1 = 11 \pm 2 \ mol^{-1} \ L)$, and L-lactate $(K_1 = 8 \pm 2 \ mol^{-1} \ L)$, as indicated by the association constants determined electrochemically. In mixed hydroxycarboxylate solutions, calcium binding is quantitatively described by the geometric mean of the individual association constants for both aqueous 1.0 and 0.20 M NaCl, indicating a 1:1 stoichiometry for complex formation.

KEYWORDS: *Calcium lactate, calcium gluconate, calcium lactobionate, calcium salt solubility, calcium complexes*

INTRODUCTION

The calcium salts of L-lactic acid, D-gluconic acid, and lactobionic acid are receiving increasing attention as food ingredients.[1,2] Calcium L-lactate and calcium D-gluconate find use in functional food and fortified beverages for optimal bone and teeth health, while calcium D-lactobionate is important as an antioxidant with special application and in controlling water activity.[3−5] All three hydroxycarboxylates have a natural image in relation to dairy production because they, like lactate (Lact⁻), are formed during milk fermentation or, like gluconate (Gl⁻), are a glucose oxidation product appearing during chemical acidification of milk, while lactobionate (Lacto⁻) may be formed enzymatically from lactose in cheese milk and has the potential of increasing the cheese yield.[6,7] The three calcium hydroxycarboxylates are all well-defined, shelf-stable hydrates from moderate to good aqueous solubility.[8,9] Calcium lactate, moreover, has a neutral taste and is accordingly used in oral calcium supplementation to humans.[10] Calcium gluconate has traditionally found use in veterinary practice as injections to calcium-deficient dairy cattle because of the less necrotic character of even supersaturated calcium gluconate solutions compared to other calcium salt solutions.[11]

The limited solubility of many calcium salts at neutral pH may result in precipitation in calcium-fortified foods and beverages during storage, resulting in reduced calcium bioavailability.[12−14] Calcium phosphates and calcium lactate have likewise caused problems as precipitates in dairy products, such as Cheddar cheese, during aging, resulting in undesirable textures.[15,16] The solubility of calcium lactate has, in particular, been studied under various conditions of relevance for dairy production, including the presence of an excess of other calcium and lactate salts.[17,18] The addition of sodium gluconate, as a molecule with a very good sequestering power, to Cheddar cheese during production has in dairy practice been found to prevent precipitation of calcium lactate and other calcium salts in the product during aging, despite calcium gluconate being far less soluble than calcium lactate, inviting speculations of possible mechanisms for this inhibition.[19,20]

Accordingly, we have determined the solubility of calcium L-lactate, calcium D-gluconate, and calcium L-lactobionate and, for each of these salts, also the solubility in the presence of each of the two other sodium hydroxycarboxylates. For the six binary combinations of salts, solubility measurements were combined with electrochemical determination of the free calcium ion concentration, a method further used for determination of association constants of calcium to each of the three hydroxycarboxylates. Such quantification of complex binding of calcium to hydroxycarboxylate anions combined with accurate determination of solubilities of each of the three calcium hydroxycarboxylates should help the formulation/design of food and beverage products with improved calcium bioavailability and further help to prevent problematic precipitation of calcium salts in cheese and other dairy products.

MATERIALS AND METHODS

Chemicals. CaCl₂·2H₂O, NaCl, NaOH, and P₄O₁₀ were all from Merck (Darmstadt, Germany). Calcium L-lactate pentahydrate, calcium D-gluconate monohydrate, calcium lactobionate monohydrate, calcium iodate, sodium L-lactate (∼98%), sodium D-lactate (≥99.0%), D-gluconic acid sodium salt (≥99%), ammonium purpurate 5,5-

Table 1. Solubility, Measured Free Calcium Ion Concentration, Calculated Free Hydroxycarboxylate Ion Concentration, $[L^-]$, and Solubility Product of Calcium Hydroxycarboxylates in Aqueous 1.0 mol L^{-1} NaCl and 25.0 °C, Taking Complex Formation into Account[a]

	solubility (g/100 mL)	$c_{Ca^{2+}}$ (mol L^{-1})	$[Ca^{2+}]$ (mol L^{-1})	total ligand concentration (mol L^{-1})	$[L^-]$ (mol L^{-1})	K_{sp} (mol^3 L^{-3})
calcium L-lactate	6.7 ± 0.1	0.307 ± 0.004	$(46.2 ± 0.5) × 10^{-3}$	0.62 ± 0.01	0.354 ± 0.004	$(5.8 ± 0.2) × 10^{-3}$
calcium D-gluconate	4.33 ± 0.04	0.1010 ± 0.0002[b]	$(38 ± 1) × 10^{-3}$	0.201 ± 0.001	0.1380 ± 0.0003	$(7.1 ± 0.2) × 10^{-4\,c}$
calcium D-lactobionate	47.3 ± 0.2	0.626 ± 0.002[d]	$(16.8 ± 0.5) × 10^{-3}$	1.253 ± 0.004	0.643 ± 0.003	$(7.0 ± 0.3) × 10^{-3}$

[a]Solubilities are presented for anhydrous calcium salts. [b]For water, a value of 0.080 mol L^{-1} was determined. [c]K_{sp} = 4.7 × 10^{-4} mol^3 L^{-3} has been reported.[30] [d]For water, a value of 0.62 mol L^{-1} was determined.

nitrilodibarbituric acid, and potassium iodate were all from Sigma-Aldrich (Steinheim, Germany). Ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate was from Sigma (St. Louis, MO). Sodium thiosulfate, 0.1 M Titrisol, was from Merck (Darmstadt, Germany). Sodium lactobionate was from Novozymes A/S (Bagsvaerd, Denmark). All aqueous solutions were made from purified water Milli-Q Plus, from Millipore Corporation (Bedford, MA).

Dissolution and Solubility Determination. Aqueous samples of calcium L-lactate pentahydrate, calcium D-gluconate monohydrate, and calcium lactobionate monohydrate were prepared by dissolving 15.0, 10.0, and 40.0 g in 100 or 50 mL, respectively. Ionic strength was adjusted with 1.0 mol L^{-1} NaCl. Samples were under constant stirring at 25.0 °C. For the dissolution study, samples were analyzed after 2 h of stirring and then in intervals of 30, 60, 90, 120, 150, 300, and 1440 min for total and free calcium ion concentrations. Samples were filtered (589/3, Whatman, Dassel, Germany) prior to each analysis. The total calcium concentration was determined by EDTA titration and free calcium ion concentration by a calcium-ion-selective electrode. Solubility, which corresponds to the total calcium concentration, was determined after 120 min of sample equilibration for each of calcium salts. All samples were prepared in duplicates.

EDTA Titration. Titrant solution of EDTA with the concentration of 0.0500 M was standardized against 0.0200 mol L^{-1} CaCl$_2$. A total of 1.000 mL of sample was transferred to a titration flask and diluted with 40 mL of Milli-Q water. A total of 2 mL of 2.0 mol L^{-1} solution of NaOH was added to a sample to maintain basic pH, and 0.3 mL of 0.50% murexid solution was used as an indicator. Samples were titrated until an initial pink color changed to dark purple, indicating the end point.

Electrochemical Determination of the Calcium Ion Concentration. The calcium ion concentration $[Ca^{2+}]$ was measured in the filtrate with a calcium-ion-selective electrode ISE25Ca with a reference REF 251 electrode from Radiometer, Copenhagen, Denmark. For electrode calibration, solutions of 0.000 100, 0.001 00, and 0.0100 mol L^{-1} Ca^{2+} were prepared from a stock solution of 1.00 mol L^{-1} CaCl$_2$. All calibration solutions were adjusted to ionic strengths of 1.0 or 0.20 with 1.0 mol L^{-1} NaCl. Calcium concentrations in the sample were determined from the linear relationships between the electrode potential measured in standard solutions and the $-\log[Ca^{2+}]$ of the standard solution according to the Nernst equation.[21] All measurements were performed at 25.0 ± 0.1 °C.

Solubility in Mixed Solutions. Solutions of sodium salts of hydroxycarboxylate ions were added to calcium salt samples at the beginning of their preparation, as described in the Dissolution and Solubility Determination section. Each sodium salt was prepared in two different concentrations, which resulted in final concentrations of 0.10 and 0.50 mol L^{-1}, after the addition to a sample of the respective calcium salt. Salts of sodium D-gluconate and sodium lactobionate were added to the calcium L-lactate sample. Sodium L-lactate and sodium lactobionate were added to the calcium D-gluconate sample. Sodium L-lactate and sodium D-gluconate were added to the calcium lactobionate sample. Samples were prepared in duplicates. Samples were under constant stirring at 25.0 °C. The total calcium concentration and free calcium ion concentration were determined after 2 h of stirring.

The solubility of calcium L-lactate in aqueous solution in the presence of additional calcium and lactate ions was investigated using

CaCl$_2$ to adjust the calcium ion concentration and sodium L- or D-lactate to adjust the lactate ion concentration. Solutions of CaCl$_2$ and sodium L- or D-lactate were prepared with 10, 20, 100, and 200% excess concentrations of calcium or lactate ions at saturation. Solutions were equilibrating for 2 h at constant stirring at 25.0 °C. The solubility of calcium L-lactate after the addition of lactate and calcium ions was determined by EDTA titration (see the EDTA Titration section). The solubility of calcium L-lactate after the addition of calcium ions was determined by subtracting the initial calcium level.

Potentiometric Determination of the Association Constant. Sodium salts of L-lactate and D-gluconate were prepared in two different concentrations of 0.100 and 0.200 mol L^{-1}, and samples of sodium lactobionate were prepared in concentrations of 0.0100 and 0.0500 mol L^{-1} for an ionic strength of 0.20 mol L^{-1}. For an ionic strength of 1.0 mol L^{-1}, concentrations of 0.0100 and 0.100 mol L^{-1} were prepared for all sodium salts. Stability constants for mixed calcium ligand complexes were determined in samples containing sodium L-lactate and sodium D-gluconate with the total concentration of 0.100 and 0.200 mol L^{-1} for an ionic strength of 0.20 mol L^{-1} and total concentrations of 0.0200 and 0.200 mol L^{-1} for an ionic strength of 1.0 mol L^{-1}. Mixed calcium ligand complexes of lactobionate with L-lactate or D-gluconate were studied for concentrations of 0.0100 and 0.100 mol L^{-1} for the sodium salts for an ionic strength of 0.20 and 1.0 mol L^{-1}, respectively. The solution of CaCl$_2$ was added to each sample with the final concentration of 0.001 00 mol L^{-1} in the samples. All samples were equilibrating for 1 h at 25.0 °C. Free calcium ion was determined as described above. Before analysis of samples with an ionic strength of 0.20 mol L^{-1}, the calcium-ion-selective electrode was calibrated with standards of the same ionic strength and the concentration used was the same as for standards of an ionic strength of 1.0 mol L^{-1}. The free calcium ion concentration was used for calculation of the stability constant.

Stability Constant Determined by the Solubility Method. Saturated calcium iodate solutions were made from 1.00 g of Ca(IO$_3$)$_2$ added to 100 mL of water with an ionic strength adjusted by NaCl to 0.20 or 1.0 M. Solutions without any sodium hydroxycarboxylate added were used to determine the solubility product of calcium iodate used to calculate the free calcium ion concentration in solutions with added ligand. The concentration of iodate was determined in the presence of 0.040 and 0.080 M hydroxycarboxylate by iodometric titration for conditions identical to those used for determination of the solubility product of calcium iodate at 25.0 °C after 4 days of equilibration. Mixed ligands of sodium L-lactate and sodium D-gluconate, sodium lactobionate and sodium L-lactate, sodium lactobionate and sodium D-gluconate were prepared with a total final concentration of 0.040 and 0.080 mol L^{-1} for an ionic strength of 0.20 and 1.0 mol L^{-1}, respectively. A total of 5.00 mL of equilibrated and filtered solution was titrated with 0.100 mol L^{-1} sodium thiosulfate after the addition of 2.5 g of potassium iodide and 150 mL of water and 5.00 mL of 2.0 mol L^{-1} hydrochloric acid using 5.00 mL of 0.5% starch solution as an indicator toward the end of titration.[22]

■ RESULTS AND DISCUSSION

The solubility of the three calcium hydroxycarboxylates under investigation increased from calcium D-gluconate to calcium L-lactate to calcium D-lactobionate. The solubilities at 25.0 °C in

1.0 NaCl, as presented in Table 1, agree for calcium gluconate and calcium lactate with literature values.[9,23] Equilibrium is established after a few hours for all three salts, as evident from Figure 1. The free calcium ion concentrations as measured by a
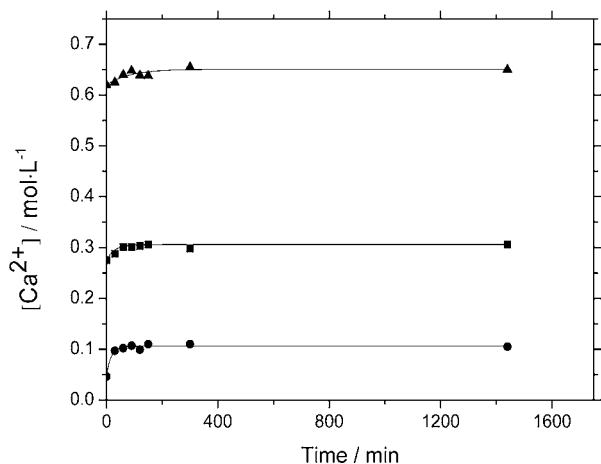
Figure 1. Dissolution of calcium hydroxycarboxylates in aqueous 1.0 mol L^{-1} NaCl at 25.0 °C: (■) calcium L-lactate, (●) calcium D-gluconate, and (▲) calcium D-lactobionate.

calcium-ion-selective electrode and also presented in Table 1 became constant during this period of time. The calcium ion concentration as determined electrochemically is lower than the total calcium concentration determined by complexometric titration, especially for the most soluble calcium lactobionate and less for the least soluble calcium gluconate. Upon dissolution of calcium hydroxycarboxylates, the following two-step equilibrium is established:

$$CaL_2(s) \rightleftharpoons CaL^+ + L^- \tag{1}$$

$$CaL^+ \rightleftharpoons Ca^{2+} + L^- \tag{2}$$

where L$^-$ is lactate, gluconate, or lactobionate. The solubility is determined by the thermodynamic solubility product and will increase with ionic strength according to

$$K_{sp} = a_{Ca^{2+}} a_{L^-}^2 = [Ca^{2+}][L^-]^2 \gamma_{Ca^{2+}} \gamma_{L^-}^2 \tag{3}$$

in which $\gamma_{Ca^{2+}}$ and γ_{L^-} are the activity coefficients, which are expected to decrease for up to at least an ionic strength of 1.0. The solubilities of Table 1 as determined at the high ionic strengths resulting from 1.0 M NaCl are accordingly higher than reported for pure water.[9,23] However, it was shown by others that salt addition has only a small effect on a salt solubility.[8,18] The use of a high and fairly constant ionic strength was, however, prompted by the calculation of ionic equilibrium involved in complex formation.

The solubilities of the calcium hydroxycarboxylates are further influenced by the association of calcium ion with the hydroxycarboxylate ion L$^-$, as described by eq 2, forming a binary calcium hydroxycarboxylate complex.[17] The addition of fully dissociated calcium salts, such as calcium chloride, will influence both equilibriums of eqs 1 and 2 but in opposite direction, in effect increasing the concentration of CaL$^+$. This increase appears to be higher than the relative decrease in the concentration of L$^-$, as seen from the results presented in Figure 2, from which a slight increase in the calcium lactate concentration becomes evident with an increasing calcium chloride addition, confirming previous findings.[17,18] Lactate
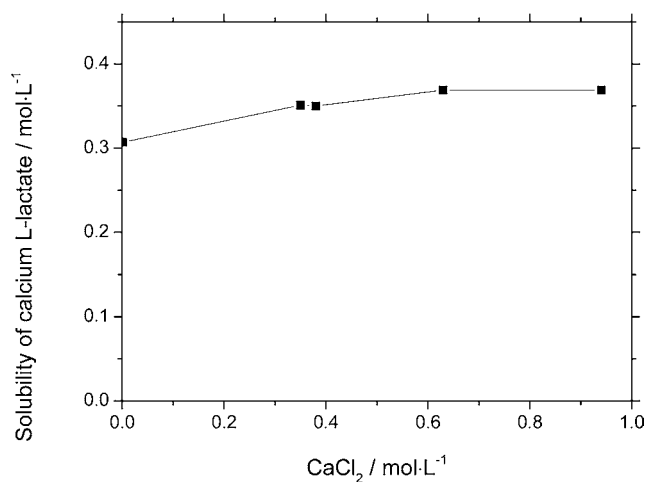
Figure 2. Effect of added calcium chloride on the solubility of calcium L-lactate in aqueous 1.0 mol L^{-1} NaCl at 25.0 °C.
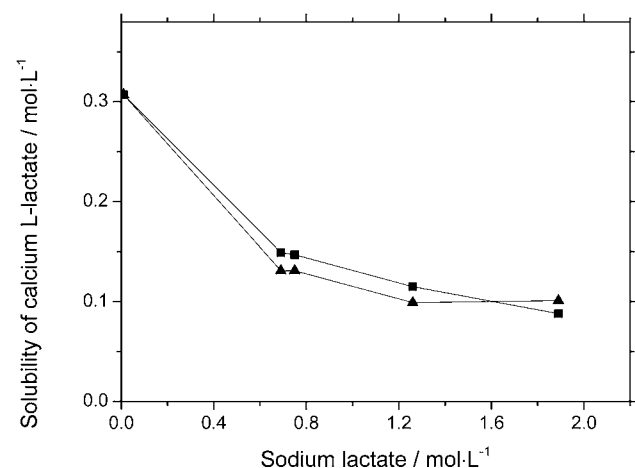
Figure 3. Effect of the addition of (■) sodium L-lactate or (▲) sodium D-lactate on the solubility of calcium L-lactate in aqueous 1.0 mol L^{-1} NaCl at 25.0 °C.

addition had the opposite effect, as may be seen from Figure 3. An increasing lactate concentration decreased the solubility of calcium lactate mainly because [L$^-$] appears squared in the solubility product defined in eq 3. This important effect of increasing the lactate concentration in cheese on calcium lactate precipitation was studied in more detail by others.[17] The solubility data of Figure 3 confirm that the presence of the enantiomeric D-lactate, which may also be formed in cheese, further decreases the solubility because of the formation of less soluble racemic calcium D,L-lactate, as also reported by others.[23]

The solubility products of Table 1 were calculated using the concentration of calcium and hydroxycarboxylate as determined from the total calcium $c_{Ca^{2+}}$ and free calcium [Ca^{2+}] concentrations according to

$$K_{sp} = [Ca^{2+}][L^-]^2 = [Ca^{2+}](c_{Ca^{2+}} + [Ca^{2+}])^2 \tag{4}$$

Independent determination of K_1 for lactate, gluconate, and lactobionate was based on two methods for further use in the mixed ligand solutions. From electrochemically determined free calcium ion concentrations, the complex concentrations were calculated according to

$$[CaL^+] = c_{Ca^{2+}} - [Ca^{2+}] \tag{5}$$

Table 2. Association Constants for Calcium Hydroxycarboxylate Complexes with a 1:1 Stoichiometry, K_1, in 0.20 or 1.0 mol L^{-1} NaCl at 25.0 °C[a]

ligand	$K_1{}^{\dagger}$ (mol^{-1} L)		$K_1{}^{\ddagger}$ (mol^{-1} L)	
	0.20 mol L^{-1} NaCl	1.0 mol L^{-1} NaCl	0.20 mol L^{-1} NaCl	1.0 mol L^{-1} NaCl
lactate	12 ± 3	8 ± 2	7 ± 1	6.2 ± 0.2
gluconate	31 ± 3	14 ± 3	14 ± 2	14 ± 8
lactobionate	25 ± 5	11 ± 2	19 ± 2	16 ± 4
lactate + gluconate	19 ± 3 (19)	15 ± 4 (11)	10 ± 3 (10)	6 ± 3 (9)
lactobionate + lactate	16 ± 4 (17)	11.5 ± 0.6 (9)	15.1 ± 0.1 (12)	8 ± 3 (10)
lactobionate + gluconate	19 ± 7 (28)	13 ± 2 (12)	19 ± 8 (16)	15 ± 3 (15)

[a]$K_1{}^{\dagger}$ is the association constant determined electrochemically using a calcium-ion-selective electrode, and $K_1{}^{\ddagger}$ is the association constant determined from solubility of calcium iodate by iodometric titration. Numbers in parentheses are predicted constants for equimolar hydroxycarboxylate mixtures.

and the association constant from

$$K_1 = \frac{[\text{CaL}^+]}{[\text{Ca}^{2+}][\text{L}^-]} \quad\quad (6)$$

using

$$[\text{L}^-] = c_{\text{L}^-} - [\text{CaL}^+] \quad\quad (7)$$

to calculate the free hydroxycarboxylate concentration, $[\text{L}^-]$, from total hydroxycarboxylate, c_{L^-}, and the concentration of the 1:1 calcium hydroxycarboxylate complex, $[\text{CaL}^+]$. For the other method, the increase in solubility of the sparingly soluble calcium iodate because of complex formation likewise provided the equilibrium concentration of $[\text{Ca}^{2+}]$ and $[\text{CaL}^+]$. In the absence of complex-forming hydroxycarboxylate, the concentration of calcium ion relates to the iodate concentration, as determined by iodometric titration, according to

$$\text{Ca(IO}_3\text{)}_2(s) \rightleftharpoons \text{Ca}^{2+} + 2\text{IO}_3^- \quad\quad (8)$$
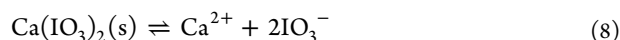
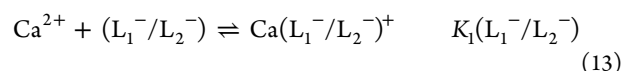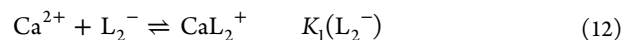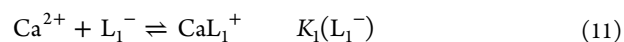$$[\text{Ca}^{2+}] = \frac{1}{2}[\text{IO}_3^-] \quad\quad (9)$$

from which the solubility product was calculated

$$K_{\text{sp}} = [\text{Ca}^{2+}][\text{IO}_3^-]^2 = \frac{1}{2}[\text{IO}_3^-]^3 \quad\quad (10)$$

For 25.0 °C, K_{sp} for calcium iodate was found to have the value of 8.7×10^{-6} mol^3 L^{-3} in 0.20 mol L^{-1} NaCl and the value of 3.2×10^{-5} mol^3 L^{-3} in 1.0 mol L^{-1} NaCl, in fair agreement with the literature value for comparable conditions, i.e., 2.2×10^{-5} mol^3 L^{-3}.[24] In the presence of a calcium ligand, the solubility of calcium iodate will increase (eq 2) and the complex concentration was calculated from eq 5, where $c_{\text{Ca}^{2+}}$ is the total calcium concentration and $[\text{Ca}^{2+}]$ is the free calcium concentration, as determined from eq 9.

Generally, carbohydrate complexes with metal ions have low stability in aqueous solutions, because of the poor electron densities on hydroxylic oxygen as a donor atom. Thereby, introduction of an anchoring group into a carbohydrate molecule, such as a carboxylate, may promote the binding between metal and ligand.[25] Calcium was found to bind most strongly to gluconate according to the value of K_1, as determined electrochemically but with comparable strength to lactobionate, as seen from the results presented in Table 2. Comparable binding of lactobionate to calcium seems to be reasonable, because within the lactobionate molecule, the gluconate moiety is responsible for binding to metal ions.[2] Lactate binds calcium with less affinity. The complex constant as defined in eq 6 is expected to decrease with an increasing

ionic strength, in agreement with the experimental results, as was also found for binding of calcium to amino acids.[26] The solubility method gave comparable results for these association constants for the two ionic strengths, providing further evidence for the validity of the methods. Literature values are available for the association constants for lactate and gluconate under slightly different conditions and, again, in fair agreement with the present results. For calcium lactate, a value of 11 mol^{-1} L and, for calcium gluconate, a value of 16 mol^{-1} L were thus reported for an ionic strength of 0.20.[27] Stronger binding of calcium to gluconate than to lactate was also reported by others with comparable binding strength.[28] The strength of calcium binding to sugar acids, as similar systems, was increased when an increasing number of coordination sites.[29] The association constants were also determined for equimolar concentrations of the three hydroxycarboxylate combinations. Assuming that 1:1 stoichiometry is dominating, the apparent constants determined for these mixtures are related to individual constants according to
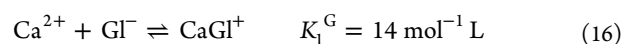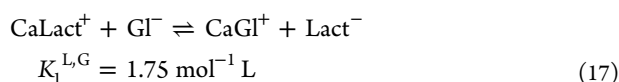
$$\text{Ca}^{2+} + \text{L}_1^- \rightleftharpoons \text{CaL}_1^+ \quad\quad K_1(\text{L}_1^-) \quad\quad (11)$$

$$\text{Ca}^{2+} + \text{L}_2^- \rightleftharpoons \text{CaL}_2^+ \quad\quad K_1(\text{L}_2^-) \quad\quad (12)$$

$$\text{Ca}^{2+} + (\text{L}_1^-/\text{L}_2^-) \rightleftharpoons \text{Ca}(\text{L}_1^-/\text{L}_2^-)^+ \quad\quad K_1(\text{L}_1^-/\text{L}_2^-) \quad\quad (13)$$

through

$$K_1(\text{L}_1^-/\text{L}_2^-) = \sqrt{K_1(\text{L}_1^-)(K_2(\text{L}_2^-)} \quad\quad (14)$$

introducing an average ligand $(\text{L}_1^- \times \text{L}_2^-)$. As may be seen from Table 2, the K_1 "predicted" from the relationship of eq 14 as the geometric mean based on the additivity of the reaction free energy is in fair agreement with the experimental findings. This agreement further adds support to the validity of a 1:1 stoichiometry for calcium complex formation with mixtures of hydroxycarboxylates at the high ionic strength.

The solubility of calcium L-lactate was found to increase in the presence of sodium D-gluconate and to a lesser degree in the presence of sodium D-lactobionate, as may be seen from Table 2. On the basis of dissolution rates estimated from Figure 1, 2 h of equilibration was allowed in these experiments without any further change in solubility over a longer period of time, as tested for several of the solutions. The distribution among the relevant species was calculated using the following equations for the combination of calcium lactate and sodium gluconate:

$$\text{Ca}^{2+} + \text{Lact}^- \rightleftharpoons \text{CaLact}^+ \quad\quad K_1^{\text{L}} = 8 \text{ mol}^{-1} \text{ L} \quad\quad (15)$$

$$\text{Ca}^{2+} + \text{Gl}^- \rightleftharpoons \text{CaGl}^+ \quad\quad K_1^{\text{G}} = 14 \text{ mol}^{-1} \text{ L} \quad\quad (16)$$

$$CaLact^+ + Gl^- \rightleftharpoons CaGl^+ + Lact^-$$
$$K_1^{L,G} = 1.75 \text{ mol}^{-1} \text{ L} \tag{17}$$

$$\frac{[Lact^-][CaGl^+]}{[Gl^-][CaLact^+]} = 1.75 \tag{18}$$

$$[CaLact^+] + [CaGl^+] = c_{Ca^{2+}} - [Ca^{2+}] \tag{19}$$

$$[Lact^-] = 2c_{Ca^{2+}} - [CaLact^+] \tag{20}$$

$$[Gl^-] = c^o_{NaGl} - [CaGl^+] \tag{21}$$

where c^o_{NaGl} is the initial sodium gluconate concentration, $c_{Ca^{2+}}$ is the solubility of calcium lactate as determined by titration, and $[Ca^{2+}]$ is the measured free calcium ion concentration. Equations 18−21 now hold the information necessary to calculate the free concentrations of lactate, gluconate, the lactate complex, $[CaLact^+]$, and the gluconate complex, $[CaGl^+]$, as the four unknowns of these equations. The calculations were performed by iteration to yield the results shown in Table 3 for $c^o_{NaGl} = 0.10$ and 0.50 mol L^{-1}.

Similar calculations were performed for the solubility determination of calcium lactate in the presence of 0.10 and 0.50 mol L^{-1} sodium lactobionate (Table 3), the combinations of calcium gluconate and sodium lactate (Table 4), the combinations of calcium gluconate and sodium lactobionate (Table 4), the combinations of calcium lactobionate and sodium lactate (Table 5), and the combinations of calcium lactobionate and sodium gluconate (Table 5), each for the two initial concentrations of the sodium hydroxycarboxylate of 0.10 and 0.50 mol L^{-1}.

The actual distribution between free calcium, free hydroxycarboxylates, and calcium hydroxycarboxylate complexes is presented in Tables 3−5. Clearly the increase in solubility of calcium lactate by 0.10 and 0.50 mol L^{-1} sodium gluconate by 12 and 45%, respectively, is due to complex binding of calcium to gluconate. Sodium lactobionate increases the solubility of calcium lactate similarly by 10 and 36%, respectively. The effect on the more sparingly soluble calcium gluconate is even more significant, with 39 and 66% increase in the presence of 0.10 and 0.50 mol L^{-1} sodium lactate, respectively, and with 41 and 85% in the presence of sodium lactobionate, respectively. In contrast, the solubility of the most soluble calcium lactobionate is hardly affected, with a solubility increase of only 8% seen by the presence of 0.50 mol L^{-1} sodium gluconate. Notably, for all 12 solutions with an increased solubility of the calcium hydroxycarboxylates, even for calcium lactobionate, a significant decrease in the free calcium ion concentration is seen. The most significant decrease was observed for calcium gluconate in the presence of 0.50 mol L^{-1} sodium lactobionate, which decreases the free calcium concentration from 0.038 to 0.0060 mol L^{-1}.

The ionic product of the free concentrations of calcium and the actual hydroxycarboxylate, $[Ca^{2+}][L^-]^2$, is calculated for L = Lact$^-$ in Table 3, for L = Gl$^-$ in Table 4, and for L = Lacto$^-$ in Table 5. For Table 3, $[Ca^{2+}][Lact^-]^2$ is rather constant and the average value of $6.1 \pm 0.4 \times 10^{-3}$ mol^3 L^{-3} is very similar to the solubility product of calcium lactate $[K_{sp} = (5.8 \pm 0.2) \times 10^{-3}$ mol^3 L^{-3}; Table 1] for the same conditions. For Table 4, $[Ca^{2+}][Gl^-]^2$ has an average value of $(7.4 \pm 2.7) \times 10^{-4}$ mol^3 L^{-3}, very similar to the solubility product of calcium gluconate $[K_{sp} = (7.1 \pm 0.2) \times 10^{-4}$ mol^3 L^{-3}; Table 1]. For Table 5, $[Ca^{2+}][Lacto^-]^2$ has an average value of $(8.0 \pm 0.7) \times 10^{-3}$

Table 3. Solubility of Calcium L-Lactate in Aqueous 1.0 mol L^{-1} NaCl at 25.0 °C Determined by Complexometric Calcium Titration after the Addition of 0.10 or 0.50 mol L^{-1} Sodium D-Gluconate or Sodium D-Lactobionate[a]

hydroxy carboxylate (mol L^{-1})	calcium L-lactate solubility (g/100 mL)	$c_{Ca^{2+}}$ (mol L^{-1})	$[Ca^{2+}]$ (mol L^{-1})	$[Lact^-]$ (mol L^{-1})	$[Gl^-]$ or $[Lacto^-]$ (mol L^{-1})	$[CaLact^+]$ (mol L^{-1})	$[CaGl^+]$ or $[CaLacto^+]$	$[Ca^{2+}][Lact^-]^2$	$[Ca^{2+}][Gl^-]^2$ or $[Ca^{2+}][Lacto^-]^2$
Sodium Gluconate									
0.10	7.63 ± 0.04	0.350 ± 0.002	(35.8 ± 0.8) × 10^{-3}	0.425	0.059	0.270	0.044	6.5 × 10^{-3}	1.2 × 10^{-4}
0.50	9.74 ± 0.04	0.446 ± 0.002	(14.1 ± 0.1) × 10^{-3}	0.653	0.308	0.237	0.195	6.0 × 10^{-3}	1.3 × 10^{-3}
Sodium Lactobionate									
0.10	7.3 ± 0.2	0.338 ± 0.005	(32.2 ± 0.1) × 10^{-3}	0.412	0.058	0.255	0.051	5.5 × 10^{-3}	2.4 × 10^{-3}
0.50	9.2 ± 0.1	0.419 ± 0.005	(13.7 ± 0.2) × 10^{-3}	0.678	0.377	0.159	0.123	6.3 × 10^{-3}	1.9 × 10^{-3}

[a]The free calcium ion concentration, $[Ca^{2+}]$, was determined electrochemically. Free lactate, $[Lact^-]$, free gluconate, $[Gl^-]$, and free lactobionate, $[Lacto^-]$, concentrations were calculated from total and free calcium concentrations, as described in the text by an iterative procedure, together with the concentration of the calcium complexes of lactate, $[CaLact^+]$, gluconate, $[CaGl^+]$, and lactobionate, $[CaLacto^+]$, using the stability constants of Table 1.

Table 4. Solubility of Calcium D-Gluconate in Aqueous 1.0 mol L⁻¹ NaCl at 25.0 °C Determined by Complexometric Calcium Titration after the Addition of 0.10 or 0.50 mol L⁻¹ Sodium L-Lactate or Sodium D-Lactobionate[a]

hydroxycarboxylate (mol L⁻¹)	calcium D-gluconate solubility (g/100 mL)	$c_{Ca^{2+}}$ (mol L⁻¹)	[Ca²⁺] (mol L⁻¹)	[Gl⁻] (mol L⁻¹)	[Lact⁻] or [Lacto⁻] (mol L⁻¹)	[CaGl⁺] (mol L⁻¹)	[CaLact⁺] or [CaLacto⁻]	$[Ca^{2+}][Gl^-]^2$	$[Ca^{2+}][Lact^-]^2$ or $[Ca^{2+}][Lacto^-]^2$
				Sodium Lactate					
0.10	5.8 ± 0.3	0.14 ± 0.01	(30.9 ± 0.3) × 10⁻³	0.190	0.080	0.081	0.019	1.1 × 10⁻³	2.0 × 10⁻⁴
0.50	7.2 ± 0.1	0.168 ± 0.004	(9.5 ± 0.3) × 10⁻³	0.250	0.428	0.080	0.078	5.9 × 10⁻⁴	1.7 × 10⁻³
				Sodium Lactobionate					
0.10	6.1 ± 0.1	0.142 ± 0.002	(23.5 ± 0.6) × 10⁻³	0.177	0.066	0.108	0.032	7.6 × 10⁻⁴	2.4 × 10⁻⁵
0.50	8.0 ± 0.3	0.185 ± 0.002	(5.95 ± 0.01) × 10⁻³	0.287	0.404	0.085	0.094	4.9 × 10⁻⁴	5.2 × 10⁻⁵

[a]The free calcium ion concentration, [Ca²⁺], was determined electrochemically. Free lactate, [Lact⁻], free gluconate, [Gl⁻], and free lactobionate, [Lacto⁻], concentrations were calculated from total and free calcium concentrations, as described in the text by an iterative procedure, together with the concentration of the calcium complexes of lactate, [CaLact⁺], gluconate, [CaGl⁺], and lactobionate, [CaLacto⁺], using the stability constants of Table 1.

Table 5. Solubility of Calcium D-Lactobionate in Aqueous 1.0 mol L⁻¹ NaCl at 25.0 °C Determined by Complexometric Calcium Titration after the Addition of 0.10 or 0.50 mol L⁻¹ Sodium L-Lactate or Sodium D-Gluconate[a]

hydroxy carboxylate (mol L⁻¹)	calcium D-lactobionate solubility (g/100 mL)	$c_{Ca^{2+}}$ (mol L⁻¹)	[Ca²⁺] (mol L⁻¹)	[Lacto⁻] (mol L⁻¹)	[Lact⁻] or [Gl⁻] (mol L⁻¹)	[CaLacto⁺] (mol L⁻¹)	[CaLact⁺] or [CaGl⁺] (mol L⁻¹)	$[Ca^{2+}][Lacto^-]^2$	$[Ca^{2+}][Lact^-]^2$ or $[Ca^{2+}][Gl^-]^2$
				Sodium Lactate					
0.10	47.9 ± 0.5	0.634 ± 0.002	(16.0 ± 0.1) × 10⁻³	0.686	0.064	0.578	0.040	7.5 × 10⁻³	6.6 × 10⁻⁵
0.50	50.9 ± 0.5	0.673 ± 0.004	(12.23 ± 0.03) × 10⁻³	0.847	0.342	0.511	0.150	8.8 × 10⁻³	1.4 × 10⁻³
				Sodium Gluconate					
0.10	47.3 ± 0.2	0.626 ± 0.002	(15.3 ± 0.1) × 10⁻³	0.694	0.046	0.564	0.047	7.4 × 10⁻³	3.2 × 10⁻⁵
0.50	51.0 ± 0.1	0.676 ± 0.001	(10.3 ± 0.2) × 10⁻³	0.890	0.294	0.469	0.197	8.2 × 10⁻³	2.8 × 10⁻³

[a]The free calcium ion concentration, [Ca²⁺], was determined electrochemically. Free lactate, [Lact⁻], free gluconate, [Gl⁻], and free lactobionate, [Lacto⁻], concentrations were calculated from total and free calcium concentrations, as described in the text by an iterative procedure, together with the concentration of the calcium complexes of lactate, [CaLact⁺], gluconate, [CaGl⁺], and lactobionate, [CaLacto⁺], using the stability constants of Table 1.

mol^3 L^{-3}, very similar to the solubility product of calcium lactobionate [K_{sp} = (7.0 ± 0.3) × 10^{-3} mol^3 L^{-3}; Table 1]. Solid calcium lactate, solid calcium gluconate, and solid calcium lactobionate are clearly in equilibrium with the solutions of the other hydroxycarboxylates present as sodium salts. In contrast, the other ionic products, [Ca^{2+}][Gl$^-$]^2 and [Ca^{2+}][Lacto$^-$]^2 for the calcium lactate experiments of Table 3, [Ca^{2+}][Lact$^-$]^2 and [Ca^{2+}][Lacto$^-$]^2 for the calcium gluconate experiments of Table 4, and [Ca^{2+}][Lact$^-$]^2 and [Ca^{2+}][Gl$^-$]^2 for the calcium lactobionate experiments of Table 5, are less constant and, in most cases, far below the respective solubility products. The solutions are accordingly not saturated with these calcium hydroxycarboxylates.

The increased solubility of calcium hydroxycarboxylates by other hydroxycarboxylates, considered relevant for cheese quality and calcium fortification of food and beverages, may be considered to be a thermodynamic rather than kinetic phenomenon, because equilibrium considerations and calculations quantitatively account for the high increase in solubility of moderately soluble calcium gluconate and calcium lactate. A combination of association constants and solubility products allowed for a speciation of calcium in such a saturated aqueous system. Notably 1:1 complexes of calcium and individual hydroxycarboxylates were found to provide a quantitative description even in the saturated solutions, with no evidence for the formation of mixed ligand complexes of calcium under these conditions. From a practical point of view, lactobionate should be tested for its capability to prevent precipitation of calcium lactate under similar conditions as sodium gluconate is currently used. More generally, the results presented in this study may find use not only in the dairy industry but should be of importance for the improvement of foods and beverages fortified with calcium. Such functional foods are of growing popularity, but precipitation of calcium salts often causes problems and decreases bioavailability.

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Notes
The authors declare no competing financial interest.

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