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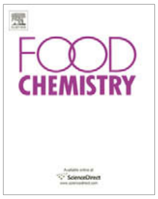

A model for predicting salt equilibria in milk and mineral-enriched milks

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ABSTRACT

The aim of this work was to present an original model predicting salt equilibria between the micellar and aqueous phases in milk and mineral-enriched milks. Calculations were based on interactions between cations (calcium, magnesium, sodium, potassium) and anions (citrate, chloride, inorganic phosphate and total caseins) according to their reciprocal affinity and considering pH, ionic strength, and solubility of calcium phosphate. In order to validate the model, the theoretical salt equilibria in milk and milks enriched with different salts ($CaCl_2$, NaCl, phosphate and citrate) at pH 6.75 were compared with experimental results published in the literature. Comparison of simulated results to known values reported in the literature showed a good agreement. In conclusion, this model constitutes an interesting tool for simulating salt equilibria in milk in order to prepare various dairy formulations with different concentrations of minerals and caseins.

1. Introduction

The salt fraction of milk contains mainly cations (calcium, magnesium, sodium, and potassium) and anions (inorganic phosphate, citrate (Cit), and chloride), and all of these macro-elements are differently distributed between the aqueous and micellar phases. Potassium, sodium and chloride ions are essentially diffusible (95%) whereas calcium (70%), inorganic phosphate (50%), magnesium (40%) and citrate (10%) are partially bound to the casein micelles (Davies & White, 1960; Holt, 1997). The nature of the colloidal calcium in casein micelles can be defined as a mixture of calcium caseinate (containing organic phosphate) and calcium inorganic phosphate salt (Holt, 1997). According to the physicochemical conditions (pH, ionic strength, addition or removal of minerals), the mineral distribution, or salt equilibria, can be considerably altered with changes in the physicochemical properties of the casein micelles (Gaucheron, Le Graët, & Schuck, 2004; Holt, 1997; Walstra, Wouters, & Geurts, 2006).

Different theoretical models have been described to calculate the salt equilibria in simple solution without proteins (Holt, Dalgleish, & Jenness, 1981; Lyster, 1981; Wood, Reid, & Elvin, 1981). For example, Wood et al. (1981) and Holt et al. (1981) developed models for calculating salt equilibria in a simulated milk salt solution and milk diffusate, respectively. Lyster (1981) described a similar model with the possibility of simulating the precipitation of calcium and magnesium phosphate salts. All of these models were useful for improving the understanding of salt equilibria. However, their dairy applications have been limited because the interactions between minerals and caseins were not considered. More recently, Holt (2004) proposed an equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. The author assumed that the micellar calcium phosphate and the calcium phosphate nanoclusters were the same. Calculations were performed from a generalised empirical formula for the nanoclusters containing calcium, magnesium, inorganic phosphate (P_i) and citrate, in association with a casein phosphorylated sequence, or phosphate centre (Little & Holt, 2004). Moreover, in this model the mole fractions of the individual caseins not complexed to the calcium phosphate through one or more of their phosphate centres are computed. Application of the thermodynamic model to the individual cow milks allowed the comparison of calculated results to those obtained experimentally by White and Davies (1958) and Davies and White (1960). The author does not specify whether his thermodynamic model can predict the salt equilibria during the addition of minerals to milk.

The aim of this work was to propose a model for calculating the salt equilibria between the aqueous and micellar phases of milks with different mineral composition. For this purpose, we extended the previous model of Holt et al. (1981) by integrating interactions of cations with casein molecules in the micellar phase and taking into account the solubility of calcium phosphate. In order to validate the model, the theoretical salt equilibria of milk and enriched milks with different salts ($CaCl_2$, NaCl, phosphate and citrate) at pH

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6.75, were compared with experimental results published in the literature.

2. Materials and methods

2.1. Description of model

The model proposed here is an extension of that described by Holt et al. (1981), calculating the mineral distribution in the milk diffusate. We have added the major constituents of the micellar phase of milk (total caseins, citrate, inorganic calcium phosphate and cations directly bound to caseins). Caseins were represented by the principal binding sites of calcium and magnesium which are the phosphoseryl residues (CN-HPser^{-}, CN-PSer^{2-}) and carboxylic groups (CN–COO^{-}) of glutamic and aspartic acids and C-terminal groups (Byler & Farrell, 1989). The differences of phosphorylation levels, association constants and the tertiary structure that may exist between the four caseins were not considered. Only the amounts of these residues and their affinity for cations were taken into account. The association constants are shown in Table 1.

Micellar calcium-inorganic phosphate was integrated in the model by considering the solubility of calcium phosphate in the aqueous phase and the molar Ca/P$_i$ ratio of this salt in the micellar phase. The major forms of orthophosphates mainly present in the aqueous phase of milk, and which can interact with calcium, are $H_2PO_4^-$ and HPO_4^{2-}. The $H_2PO_4^-$ form has a low affinity for calcium (Table 1) and remains essentially as the ionic form. In contrast, the HPO_4^{2-} form has a relatively high affinity for calcium, but the formation of the $CaHPO_4$ complex in the aqueous phase is limited by its low solubility. Holt et al. (1981) calculated a concentration of $CaHPO_4$ complex close to 0.60 mM. As the milk diffusate (or aqueous phase of milk) is considered as supersaturated with respect to calcium phosphate, we assumed that 0.60 mM is the maximum concentration of the complex $CaHPO_4$. When this theoretical limit was reached, the calcium and inorganic phosphate precipitated together. With regard to the nature of micellar calcium phosphate, different views have been expressed in the literature. They are based mainly on the non-diffusible calcium/inorganic phosphate (Ca/P$_i$) ratio. For example, McGann, Buchheim, Kearney, and Richardson (1983a), McGann et al. (1983b) determined a Ca/P$_i$ of about 2 and Chaplin (1984) and Holt (1982) indicated molar Ca/P$_i$ ratios of 1.58 ± 0.03 and 1.11, respectively. In our model, we assumed an optimal molar ratio of 1.4 which gives simulated results agreeing with experimental results. For micellar citrate, its exact location in the micellar phase is not yet established (Holt, 2004). We considered, in the calculation, that 10% of the total citrate of milk was associated with the micellar phase at pH 6.75 (Davies & White, 1960; Gaucheron, Le Graët, Piot, & Boyaval, 1996).

Table 1
Intrinsic association constants (M^{-1}) used in the mode of calculation.

	H^+	Ca^{2+}	Mg^{2+}	Na^+	K^+
H_2Cit^-	1069[b]	28[a]	15[a]	1[a]	1[a]
$HCit^{2-}$	6.0×10^{4}[a]	876[a]	402[a]	10[a]	10[a]
Cit^{3-}	3.16×10^{6}[b]	2.2×10^{5}[b]	1.11×10^{5}[a]	20[a]	15.6[b]
$H_2PO_4^-$	200[b]	21[b]	12[a]	1[a]	1[a]
HPO_4^{2-}	1.12×10^{7}[b]	442[b]	838[a]	16.9[b]	13.1[b]
PO_4^{3-}	2.47×10^{12}[a]	2.88×10^{6}[a]	8.35×10^{4}[a]	100[a]	100[a]
Cl^-	–[a]	9.4[a]	5.6[a]	0.92[a]	0.92[b]
$RCOO^-$	5.71×10^{4} [a]	15[a]	19[a]	0.7[a]	0.7[a]
Casein-HPSer$^-$	200[c]	11[c]	10[c]	1[c]	1[c]
Casein-Pser^{2-}	2.50×10^{6}[c]	2.2×10^{4}[c]	8000[c]	16.9[c]	13.1[c]
Casein-COO$^-$	1.13×10^{4}[c]	15[c]	19[c]	0.7[c]	0.7[c]

[a] Data reported by Holt et al. (1981).
[b] Modified values compared to values in Holt et al. (1981)
[c] Assumed values and the symbol "–" refers to very low value (close to zero).

2.2. The computer programme work

The simplified flowchart of the computer program is indicated in Fig. 1. The formulae used to calculate the ionic strength and ionic activity coefficients, the mass balance equations constructed for all components, and the algorithm used for solving systems of equations, were as detailed by Holt et al. (1981). From the total mineral and casein concentrations, pH and ionic strength, the software successively calculated the micellar citrate concentration and the ionic activity coefficients as a function of ionic strength, and recalculated the association constants and the concentrations of individual ions and complexes in the aqueous and micellar phases of the milks, taking into account calcium phosphate solubility. The method of calculation was based on successive approximations, using a series of nested iterative loops (Fig. 1).

It is noteworthy that the calculations were valid only for 20–25 °C because the constants used in this model were determined in this temperature range.

2.3. Input data required for calculations

To calculate the salt equilibria, the data required are pH and concentrations of cations and anions (Fig. 1). These values can be chosen, depending on the mineral and casein compositions of the milks. In this study, the input data used for the calculations of salt equilibria in standard milk comprised the following total concentrations : Ca (30.0 mM), Mg (5.00 mM), Na (22.0 mM), K (38.0 mM), citrate (9.00 mM), inorganic phosphate (21.0 mM), chloride (29.0 mM), phosphoseryl residues (7.50 mM), and carboxylic groups of casein molecules (15.0 mM). To simulate enrichment of milk by salts, concentrations of $CaCl_2$ (0–20.0 mM), NaCl (0–300 mM), trisodium citrate (0–18.0 mM) or disodium hydrogen phosphate (0–20.0 mM) were added (Table 4). The pH variations induced by these salt additions were compensated by calculating relevant amounts of NaOH or HCl to maintain the pH at the constant value of 6.75.

2.4. Output data

The calculations provide the concentrations of all ionic species (free and complexed) and their distribution between micellar and aqueous phases. Some key values calculated in the aqueous phase are the ionic strength and the concentrations of ionic calcium and magnesium, the complex $CaCit^-$, $CaHPO_4$ and the monovalent ions (Na^+, K^+, Cl^-). In the micellar phase, calculations give the concentrations of cations directly bound to phosphoseryl residues and carboxylic groups, the amounts of non-diffusible calcium inorganic phosphate and micellar citrate.

3. Results and discussion

3.1. Distribution and forms of salts in milk

3.1.1. Salt distribution between aqueous and micellar phases

The mineral distribution between the micellar and aqueous phases was calculated at pH 6.75 and is listed in Table 2. These calculations were in good agreement with the salt distribution levels reported in the literature (Holt, 1997). Indeed, it is generally accepted that, for total concentrations of about 30 and 20 mM calcium and inorganic phosphate, about 10 mM concentrations of each ion are found in the micellar phase and 20 and 10 mM in the aqueous phase, respectively. As provided in the method of calculation

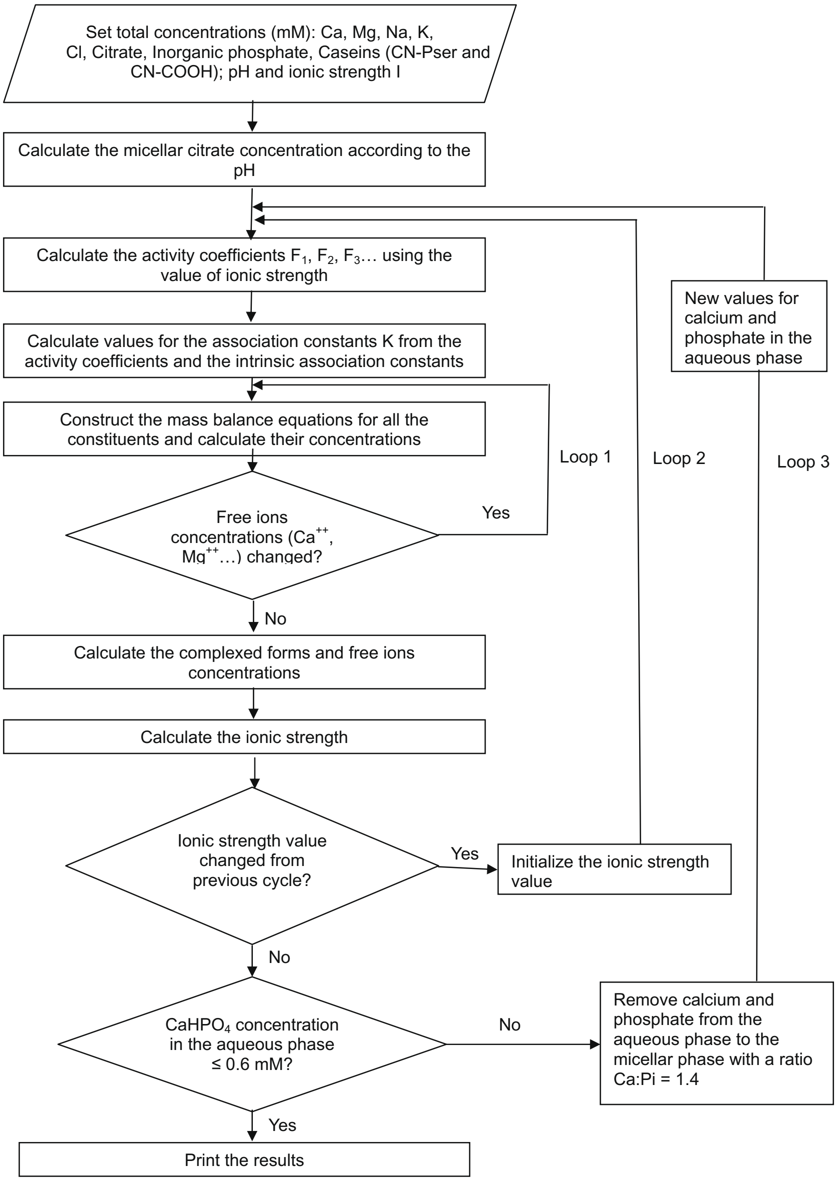

Fig. 1. The simplified flowchart of the computer programme.

Table 2
Calculation of the mineral distribution (mM) between aqueous and micellar phases of milk.

	Total[a]	Aqueous phase[b]	Micellar phase[b]
Ca	30.0	9.10	20.9
Mg	5.00	3.71	1.29
Na	22.0	21.8	0.22
K	38.0	37.7	0.35
Pi	21.0	10.0	11.0
Citrate	9.00	8.10	0.90
Cl	29.0	29.0	0.00
Phosphoseryl residues	7.50	0.00	7.5
Carboxylic groups	15.0	0.00	15.0

[a] Concentrations corresponding to input data.
[b] Theoretical concentrations in the aqueous and micellar phases.

(Fig. 1), 10% of citrate and the totality of the phosphoseryl residues and carboxylic groups were in the micellar phase. However, less than 1% of sodium and potassium ions were determined in the micellar phase, whereas it has been shown experimentally that the values were about 5% (Le Graët & Brulé, 1993). The proportion of micellar magnesium calculated (26% of the total) (Table 2) was also slightly different from that determined experimentally (about 40%). These slight differences could be due to the presence of mixed salts probably existing in milk but not considered in the calculations. In addition, the small differences observed between our results and those reported in the literature may be partly related to variations in the total concentrations of minerals and caseins.

3.1.2. Salt equilibria in the aqueous phase

In the aqueous phase, the cations and anions were free or associated among themselves according to their affinities (Table 3). Soluble calcium existed in different forms: (i) mainly associated with trivalent citrate (6.04 mM for a total of 9.30 mM of calcium), (ii) free ions (2.12 mM) and (iii) associated, to a lesser degree, with chloride and inorganic phosphate (mixture of $H_2PO_4^-$ and HPO_4^{2-}). The low concentration of calcium phosphate in the aqueous phase was due to its low solubility. At pH 6.75, the H_3Cit, H_2Cit^- and H_3PO_4 forms did not exist. The sodium, potassium and chloride

Table 3
Theoretical concentrations of minerals (mM) in the aqueous phase of milk at pH 6.75.

	Free	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
H_3Cit	0.00	0.00	0.00	0.00	0.00
H_2Cit-	0.00	0.00	0.00	0.00	0.00
$HCit^{2-}$	0.03	0.01	0.00	0.00	0.01
Cit^{3-}	0.17	6.04	1.79	0.02	0.03
H_3PO_4	0.00	0.00	0.00	0.00	0.00
$H_2PO_4^-$	3.65	0.07	0.02	0.05	0.08
HPO_4^{2-}	3.67	0.60	0.67	0.51	0.69
PO_4^{3-}	0.00	0.01	0.00	0.00	0.00
Cl^-	27.8	0.24	0.09	0.34	0.58
Ionic form		2.12	1.14	20.9	36.26

ions existed mainly as free ions. Taking into account these different associations and the concentrations of these different free ions, the ionic strength of the aqueous phase was estimated at 73 mM. As it was impossible to measure the different associated forms experimentally, our results were directly compared with other theoretical results obtained by Holt et al. (1981). For example, the calculated concentration of $CaCit^-$ was 6.96 mM according to Holt et al. (1981) compared to 6.04 mM in this work. In addition, they found a theoretical concentration for ionic calcium of 2.00 mM compared with 2.12 mM in our results. The slight differences observed were probably due to the differences in association constants and concentration values used in the two models. For the specific case of the ionic calcium concentrations in milk, wide variations, depending on the method of determination and the milk composition, have been reported in the literature. An experimental value of ionic calcium, determined by selective electrode (Lin, Lewis, & Grandison, 2006), was about 2 mM, in agreement with our calculated value.

3.1.3. Minerals in the micellar phase

The minerals in the micellar phase corresponded to calcium associated with phosphoseryl residues of caseins and inorganic phosphate to form non-diffusible calcium phosphate salt. Calculations indicated that a 6.70 mM concentration of phosphoseryl residues for a total of 7.50 mM were combined with 5.34 mM calcium and 1.14 mM magnesium. This theoretical association, corresponding to 86.4% binding of the casein molecules, was considered to be correct since it is accepted that close to 90% of phosphoseryl residues is associated with calcium and magnesium. About 50% of total calcium and inorganic phosphate concentrations was in the form of non-diffusible calcium phosphate. The molar ratio of this salt was 1.4 according to the method of calculation. The calculations also indicated the presence of 0.90 mM citrate, i.e., 10% of the total citrate concentration associated with the micellar phase. This parameter was fixed in our method of calculation and was chosen because it was reported in several studies (Davies & White, 1960; Gaucheron et al., 1996).

3.2. Changes in salt distribution in modified milks

3.2.1. Addition of calcium chloride

The calculated results for the new mineral distribution after addition of $CaCl_2$ are presented in Fig. 2. To maintain the pH value at 6.75, the model calculated a relevant amount of added NaOH. For example, when 20 mM $CaCl_2$ was added, 8 mM NaOH was calculated and also taken into account in the calculations of the salt equilibria. Increases in the concentrations of aqueous calcium, ionic calcium and calcium bound to casein were determined theoretically. At the same time, the inorganic phosphate concentration in the aqueous phase decreased. The other overall consequence of the addition of $CaCl_2$ was an increase in ionic strength (73 mM in the absence of added $CaCl_2$ compared to 103 mM in the presence of 20 mM added $CaCl_2$) (Fig. 2). To verify that these results were in good agreement, comparison was made with those obtained experimentally by various authors. The results showed that addition of calcium to milk increases the calcium concentration in the aqueous phase with a parallel decrease in inorganic phosphate concentration (Green & Marshall, 1977; Philippe, Gaucheron, Le Graët, Michel, & Garem, 2003; Philippe, Le Graët, & Gaucheron, 2005; Tessier & Rose, 1958; Udabage, McKinnon, & Augustin, 2000; Van Hooydonk, Hagedoorn, & Boerrigter, 1986; Zuraw, Smietana, Szpendowski, & Chojnowski, 1986). For example, Udabage et al. (2000) reported an increase in the diffusible calcium concentration from 9.10 mM in the absence of added calcium to 15.5 mM in the presence of 20 mM added calcium. Under the same conditions, they observed a decrease in the inorganic phosphate concentration from 10.6 to 4.20 mM in the aqueous phase. Similary, Philippe et al. (2003) reported concentrations of calcium and inorganic phosphate of 8.77 and 10.5 mM in the absence of added calcium compared to 10.8 and 6.11 mM in the presence of 9.00 mM added calcium, respectively. Moreover, part of the calcium precipitated with the inorganic phosphate because milk is saturated in calcium phosphate. Other authors (Gastaldi, Pellegrini, Lagaude, & Tarodo de la Fuente, 1994; Lin et al., 2006; Solorza-Feria, 2001; Udabage et al., 2000) have reported that the ionic calcium concentration was increased when the levels of added calcium were high. For example, Lin et al. (2006) reported an increase in the ionic calcium concentration from 1.81 mM in the absence of added calcium to 2.71 mM after addition of 10 mM Ca. All of these variations, observed experimentally, were quantitatively similar to those calculated with our model.

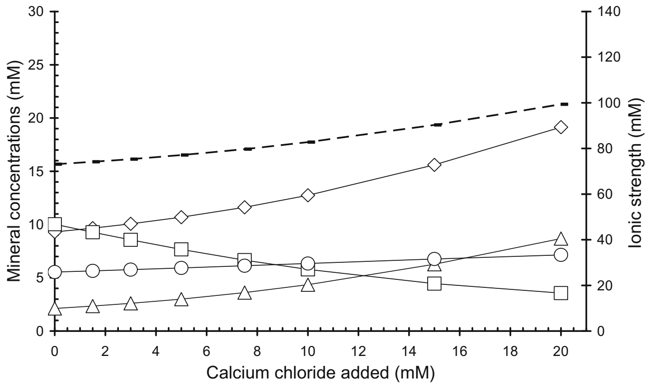

Fig. 2. Influence of $CaCl_2$ addition on the salt equilibria of milk. The symbols correspond to the contents of (◇) calcium in the aqueous phase, (□) phosphate in the aqueous phase, (△) ionic calcium, (○) calcium bound to caseins and (--) ionic strength.

3.2.2. Addition of sodium chloride

Calculations of the new theoretical distribution of minerals after addition of NaCl to milk are presented in Fig. 3. When 300 mM NaCl was added to milk, 2.85 mM NaOH was calculated as necessary to maintain the pH at 6.75. The addition of NaCl resulted in increases in the concentrations of aqueous and free calcium and a decrease in the concentration of calcium bound to casein molecules. The concentration of inorganic phosphate in the aqueous phase increased at the same time. Another theoretical result was an increase in ionic strength (73 mM in the absence of NaCl to about 340 mM in the presence of 300 mM added NaCl) (Fig. 3). Our theoretical results were slightly different from those obtained experimentally by some authors (Gaucheron, Le Graët, & Briard, 2000; Grufferty & Fox, 1985; Huppertz & Fox, 2006; Le Graët & Brulé, 1993; Zoon, Van Vliet, & Walstra, 1989). They re-

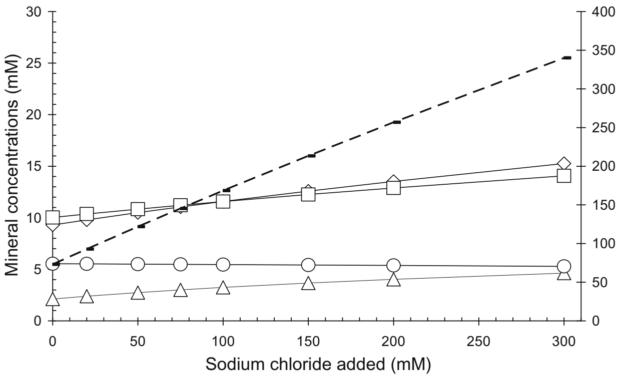

Fig. 3. Influence of NaCl addition on the salt equilibria of milk. The symbols correspond to the contents of (◇) calcium in the aqueous phase, (□) phosphate in the aqueous phase, (△) ionic calcium, (○) calcium bound to caseins and (--) ionic strength.

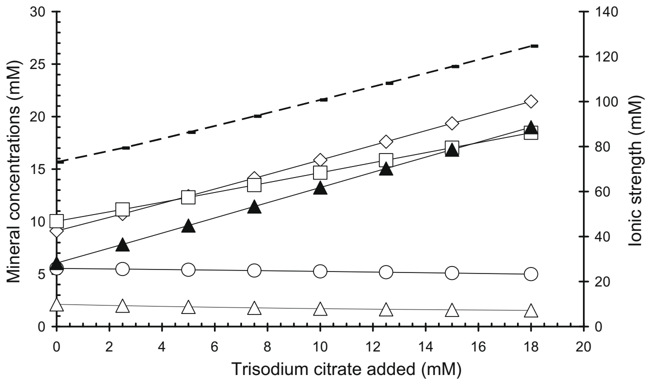

Fig. 4. Influence of citrate addition on the salt equilibria of milk. The symbols correspond to the contents of (◇) calcium in the aqueous phase, (□) phosphate in the aqueous phase, (△) ionic calcium, (○) calcium bound to caseins, (▲) calcium bound to citrate in diffusible phase and (--) ionic strength.

ported that addition of NaCl resulted in solubilisation of micellar calcium but not inorganic phosphate. They suggested exchanges of calcium linked directly to the phosphoseryl residues of the casein molecules with the added sodium. In this case, the nanoclusters composed of calcium phosphate were not changed and, consequently, inorganic phosphate was not solubilised. This preferential solubilisation of calcium in the presence of NaCl was not envisaged in the method of calculation because the low association constants of monovalent cations for caseins used in the model (Table 1) do not lead to their exchange against calcium. In contrast, our results were in accordance with those obtained by other authors (Aoki, Umeda, & Nakano, 1999; Famelart, Le Graët, & Raulot, 1999) that indicated that the addition of NaCl increased the concentrations of calcium and inorganic phosphate in the aqueous phase. In our model of calculation, the addition of NaCl to milk induced an increase in ionic strength with a resulting reduction in the ion activity coefficients and increase in the dissociation of calcium phosphate. The concentration of ionic calcium was also increased (Table 4).

3.2.3. Addition of trisodium citrate

The addition of trisodium citrate to milk also resulted in a new theoretical distribution of minerals (Fig. 4). To maintain the pH at 6.75, the model calculated an added concentration of 15.2 mM HCl for an addition of 18 mM trisodium citrate. Increases in concentrations of calcium and inorganic phosphate were determined in the aqueous phase. The molar solubilised calcium/solubilised inorganic phosphate ratio was 1.4 (this parameter is fixed in the method of calculation) and an increase in the contents of calcium bound to citrate in the aqueous phase was determined. Slight decreases in the concentrations of ionic calcium (Table 4) and calcium bound to casein were also calculated. The ionic strength was consequently increased from 73 mM in the absence of citrate to about 124 mM in the presence of 18 mM added citrate. Our theoretical results were in agreement with many experimental results which also showed increases in the concentrations of calcium and inorganic phosphate in the aqueous phase after addition of citrate (Casiraghi & Lucisano, 1991; Gatti, Alvrez, & Suarez Sala, 1999; Le Ray et al., 1998; Odagiri & Nickerson, 1965; Udabage et al., 2000). For example, Udabage et al. (2000) reported that the addition of 10 mM citrate induced an increase in calcium concentration in the aqueous phase from 9.10 mM in the absence of added citrate to 16.2 mM in the presence of added 10 mM citrate. In the same sample, the concentrations of inorganic phosphate in the aqueous phase went from 10.6 to 14.6 mM. These authors also reported that the calcium activities were decreased from 0.76 in the absence of citrate to 0.52 mM in the presence of 10 mM added citrate. Our calculations showed values of calcium activity from 0.93 in the absence of citrate to 0.69 mM in the presence of 10 mM citrate. All the variations observed experimentally by these different authors were quantitatively similar to those obtained by our calculation method, suggesting an optimal choice of parameters in the model. This demineralisation of casein micelles was related to the high affinity of the citrate molecule for calcium ions (Table 1). Because of the calcium chelation by citrate, a displacement of calcium from the micellar phase towards the aqueous phase was thus induced. Solubilisation of calcium and inorganic phosphate occurred at the same time because calcium and inorganic phosphate are strongly bound in the micellar phase.

3.2.4. Addition of disodium hydrogen phosphate

The calculations indicated that addition of disodium hydrogen phosphate induced changes in the salt distribution (Fig. 5). To maintain the pH at 6.75, the model calculated an added concentration of 3.46 mM HCl for an addition of 20 mM disodium hydrogen phosphate. Decreases in the concentrations of soluble calcium and ionic calcium were calculated. At the same time, the concentration of inorganic phosphate in the aqueous phase and the amount of non-diffusible inorganic calcium phosphate were increased. Fol-

Table 4
Calculated concentrations of ionic calcium (Ca^{2+}) in milks modified by salt additions.

$CaCl_2$ added	Ca^{2+}	NaCl added	Ca^{2+}	Na_3Cit added	Ca^{2+}	Na_2HPO_4 added	Ca^{2+}
0.00	2.12	0.00	2.12	0.00	2.12	0.00	2.12
1.50	2.34	20.0	2.39	2.50	1.98	2.00	1.84
3.00	2.60	50.0	2.74	5.00	1.87	4.00	1.64
5.00	3.00	75.0	3.01	7.50	1.78	5.00	1.56
7.50	3.61	100	3.25	10.0	1.71	7.50	1.39
10.00	4.35	150	3.67	12.5	1.64	10.0	1.25
15.00	6.27	200	4.03	15.0	1.59	15.0	1.07
20.00	8.68	300	4.63	18.0	1.54	20.0	0.94

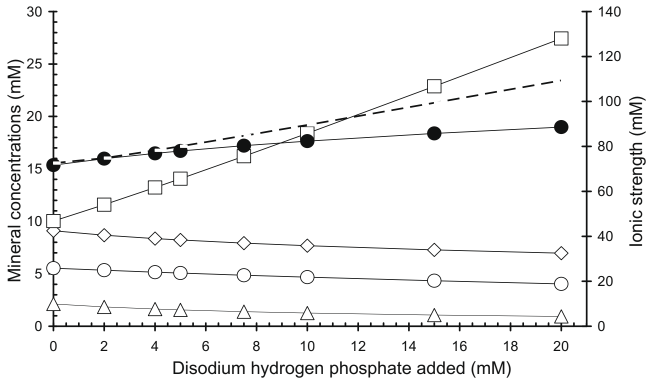

Fig. 5. Influence of phosphate addition on the salt equilibria of milk. The symbols correspond to the contents of (◇) calcium in the aqueous phase, (□) phosphate in the aqueous phase, (△) ionic calcium, (○) calcium bound to caseins, (●) non-diffusible inorganic calcium phosphate and (--) ionic strength.

lowing this addition of phosphate and the new mineral distribution, an increase in the ionic strength was determined theoretically (73 mM in the absence of disodium hydrogen phosphate increasing to about 109 mM in the presence of 20 mM phosphate). These results were in agreement with those obtained experimentally by many authors. Indeed, all the results showed that most of the added phosphate remained in the aqueous phase. Moreover, a decrease in the concentration of calcium in the aqueous phase is usually described (Augustin & Clarke, 1990; Gaucher, Piot, Beaucher, & Gaucheron, 2007; Lagaude & Tarodo de la Fuente, 1986; Udabage et al., 2000). For example, Gaucher et al. (2007) reported a decrease in calcium concentration in the aqueous phase from 9.7 mM in the absence of phosphate to 8 mM in the presence of 20 mM added phosphate. Similarly, Udabage et al. (2000) reported a decrease in calcium concentration from 9.10 mM without addition of phosphate to 7.82 mM in the presence of 20 mM phosphate. The latter authors also reported that calcium activity was decreased from 0.76 in the absence of phosphate to 0.40 mM in the presence of 20 mM added phosphate. Our calculations showed values of calcium activity from 0.93 in the absence of phosphate to 0.34 mM in the presence of 20 mM of added phosphate. Part of the added phosphate interacts with the free calcium (it is directly available in the aqueous phase) to form a precipitate of calcium phosphate. The association between calcium and phosphate is highly probable because the HPO_4^{2-} form (present at pH 6.75) has a high affinity for calcium (Table 1).

4. Strengths and limits of the model

The mineral distributions in different milks (normal or with added $CaCl_2$, NaCl, citrate or phosphate) were calculated theoretically. This model of calculation incorporates the association constants between cations (calcium, magnesium, sodium and potassium) and anions (inorganic and organic phosphates, citrate, chloride and carboxyl groups) and the solubility of inorganic calcium phosphate. The results obtained by our theoretical model in the various mineral conditions tested in this study were in agreement with those obtained experimentally. This agreement suggests that the parameters used in this model are correct. Thus, this model can be considered very satisfactory although improvements are still needed to expand its use in the dairy industry. Currently, the salt equilibria in milk were calculated by this model at 20–25 °C because the association constants taken from the literature and used in the model are determined in this temperature range. So, it should be interesting to include other temperatures used during heat treatments and processing of milk to cheese and yogurt. But this would require experimental determination of the association constants. Another limitation of the model was that only the total caseins were considered and there was no distinction made between the individual caseins. The calculated results quantitatively agree with experimental results. However, the current model cannot determine the location of non-diffusible citrate and inorganic calcium phosphate in the micellar phase. It is noteworthy that calculations are based on interactions, in the aqueous phase, between cations and anions with 1:1 stoichiometry and the mixed salts are not considered. Currently, components such as, carboxylic acids (acetate, lactate, propionate), sulphate, nitrate are also integrated in this model (not shown in this paper because not used for calculations of salt repartition in standard milk). Other components of milk, such as whey proteins, trace elements (Fe, Cu, Zn) can be added to extend to model. Some external compounds, e.g. chelating agents (EDTA, polyphosphates, hexametaphosphate) can also be integrated. Finally, as calcium can interact with a number of fatty acids to form salts, it would be interesting to develop a model by integrating the fat phase. In conclusion, this software is proposed as a new interesting tool for calculating salt distribution between aqueous and micellar phases of milk and dairy formulations. It may be useful for researchers and dairy processors wishing to test new products and to determine effects of salt addition to milk.

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