Title	Enhancement of separation rate and recovery efficiency of milk whey proteins by addition of calcium and magnesium ions in batch foam separation
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Citation	Process Safety and Environmental Protection https://doi.org/10.1016/j.psep.2022.05.015
Issue Date	2022-05-07
Doc URL	http://hdl.handle.net/2115/92404
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Type	article (author version)
File Information	manuscript_20220509.pdf

Enhancement of separation rate and recovery efficiency of milk whey proteins by addition of calcium and magnesium ions in batch foam separation

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Abstract

The influence of Ca^{2+} and Mg^{2+} to milk whey solution at pH 6 on the enhancement of proteins recovery in batch foam separation was investigated. The pH of whey solution produced in cheese production was about pH 6. The separation rate was evaluated by the separation rate constant, k, by fitting to first-order kinetic equation, and the recovery efficiency was evaluated by the fraction of residual concentration of whey proteins. In the comparison of the case without and with the addition at pH 6, in the case with the addition of Ca^{2+} and Mg^{2+} in the range over 0.06 mol/L, the k became 4.4-fold and 2.0-fold larger, and the recovery efficiency became 5.3-fold and 1.9-fold larger than those in the case without the addition. The overall equilibrium adsorption constant, K, and the overall saturated adsorption density, X_s, were also affected by the addition of Ca^{2+} and Mg^{2+}. The variation tendency of K and X_s was quite different. As increasing the concentration of both Ca^{2+} and Mg^{2+}, mostly K decreased, on the other hand, X_s increased. Judging from these results, the formation of aggregates/complexes of whey proteins by the addition of Ca^{2+} and Mg^{2+} was suggested. The addition of Ca^{2+} and Mg^{2+} could enhance the separation rate and recovery efficiency.

Keywords: Foam separation, Milk whey protein, Calcium, Magnesium, Separation

Highlights

1. Addition of Ca^{2+} and Mg^{2+} enhanced recovery efficieny of milk whey proteins.

2. Addition of Ca^{2+} and Mg^{2+} also increased the separation rate of milk whey proteins.

3. Addition of Ca^{2+} is more effective than Mg^{2+} for recovery of whey proteins at pH 6.

4. Adsorption parameters can be estimated from time course data with our proposed model.

1. Introduction

In cheese production processes, milk whey is produced as a by-product. About 90 % of cow milk as a raw material became milk whey [1]. In Japan, the amount of domestic cheese production was 47,000 tons in 2019, which is reported that the amount produced increased every year. With increasing in the amount produced of cheeses, milk whey has been produced over ca. 400,000 tons in 2014. Milk whey has been reported to contain a lot of useful substances, for example, lactose (40-50 g/L), some proteins; α-lactalbumin; β-lαctogloblin (6-8 g/L), and minerals (80-100 g/L). Some effective utilization of milk whey have been attempted, for example, food additive for dietary supplements, confectionery, ice cream, livestock foods, and so on.

Foam separation technique has been recognized as a convenient and useful method to separate or enrich mainly dissolved organic substances and suspended matters which have or be added hydrophobic properties. From the viewpoint of effective usage of natural resources and industrial byproducts, recovery of useful substances, such as proteins from milk whey is very important. However, there are only a few reports about the recovery of proteins from milk whey by foam separation [2]. In the present study, foam separation experiments were conducted to recover proteins from milk whey solution. The adsorption behavior of water-soluble proteins onto the liquid-atmosphere interface was affected by the pH values of the solution [3]. In the present study, influences of the addition of Ca^{2+} and Mg^{2+} on the separation efficiency of proteins from milk whey solution by foam separation were investigated. Therefore, the addition of common divalent metal ions, such as Ca^{2+} and Mg^{2+}, can bind with protein molecules, resulting in the formation of larger aggregates or complexes. Moreover, it was expected that the electrostatic repulsive force between the aggregates/complexes was reduced by the addition of Ca^{2+} and Mg^{2+} due to the charge neutralization effect. Thus, it could be considered that the amount adsorbed and the separation rate could be improved.

The interactions between Ca^{2+} and whey proteins have been investigated by several researchers over the last 30 years [4]. In these reports, two main types of interactions have been observed between whey proteins and soluble minerals: (1) attractive intermolecular forces and (2) intramolecular binding of minerals by proteins. These attractive intermolecular forces between proteins are driven by Ca^{2+} bridge formation at the ionizable level of the protein [5]. Simons et al. (2002) reported that calcium ions have been suggested to be involved in intermolecular protein-Ca-protein cross-linking, intramolecular electrostatic shielding, or ion-induced protein conformational changes [6]. Permiakov et al. (1982) reported that magnesium ions in millimolar concentrations have little effect on the association of calcium ions with a-lactalbumin, which led them to suggest that calcium and magnesium ions bind to different sites on the protein. It appears now that one of the magnesium binding sites is the same as the calcium binding site but that the binding of magnesium is much weaker than that of calcium [7]. The addition of certain appropriate concentrations of Ca^{2+} or Mg^{2+} may improve the separation rate and recovery efficiency due to the effect of the formation of complexes/aggregates by adding Ca^{2+} or Mg^{2+} to the whey protein solution.

In this study, we investigated the influence of the addition of calcium and magnesium ions to milk whey solution on the enhancement of separation kinetics and recovery efficiency in batch foam separation. Foam separation experiments were conducted with varying the concentration of Ca^{2+} and Mg^{2+} at pH 6. We discussed the effect of the addition of Ca^{2+} and Mg^{2+} on the separation kinetics and the recovery efficiency.

2. Materials and methods

2.1. Materials

Cow milk used in this study was a marketed product containing over 8.4 wt.% nonfat milk and 3.7 wt.% milk fat. Standard substances of bovine serum albumin, α-lactalbumin, and β-lactogloblin were purchased from Sigma-Aldrich Co. Ltd. Acetonitrile and trifluoroacetic acid (TFA) were obtained from Kanto Chemical Co. Inc, Japan, as mobile phase for HPLC. $CaCl_2$ and $MgCl_2$ were purchased from Wako Pure Chemical Co., Japan. All the other reagents used in this study were also obtained from Kanto Chemical Co. Inc., Japan. All reagents were used without further purification.

2.2. Preparation of milk whey solution

A certain volume of cow milk was taken into a beaker, then, 1 M HCl aqueous solution was added to adjust the pH of the milk at pH 4.6 (the i.e.p. of casein). After adjusting the pH, the suspension was centrifuged at 3,000 rpm for 15 min. The supernatant (whey solution) was filtered with filter paper (No.2, ADVANTEC, Japan). Moreover, 1 M HCl or 1 M NaOH aqueous solutions were added to the whey solution for adjusting pH 6. After this adjusting pH value, the whey solution was again centrifuged at 3,000 rpm for 15 min. The whey solution was filtered again with filter paper (5C, ADVANTEC, Japan). The obtained whey solution was diluted to 5-fold (w/w) with distilled water in most experiments in this study. A certain concentration of Ca^{2+} or Mg^{2+} solution was added to the whey solution and it was used in the foam separation experiment. The added volume of Ca^{2+} and Mg^{2+} solution was restricted within 5% v/v of the final volume of the whey solution.

2.3. Experimental setup

Fig. 1 shows a schematic drawing of the experimental setup. The column was made of a transparent acrylic resin tube with 4.4 cm in inside diameter. The main part of the column was 70 cm in height. A sintered glass filter having an average pore size of 10-15 μm was installed at the bottom of the column as a gas distributor. Air was supplied to the column through the distributor. Pressure taps were installed along the wall at intervals of 25 cm for measuring gas holdup.

2.4. Experimental procedure

Experimental procedures for batch and continuous modes in this study were mostly the same as those in the previous studies [3,8,9]. The prepared whey solution was taken into the column (liquid height 60 cm). After this charging of the solution, the air was supplied and dispersed as bubbles through the distributor. The volumetric flow rate of air was employed at 50 cm^3/s in most experiments. The solution in the column was sampled from a sampling tap installed at the bottom of the column. The overall concentrations of the whey proteins were measured by the Lowry method using bovine serum albumin as a standard protein [10]. The pH of the whey solution in the column was measured with a pH meter (520A or 920A, ORION, U.S.). The gas holdup was estimated from the difference in static pressure between the clear and aerated liquids using a differential pressure transducer (Tem-Tech, Japan). Voltage signals were recorded by a personal computer via a digital multi-meter (PC5000a, Sanwa, Japan). Each experiment was repeated at least twice. When the variation was large, the data range is indicated by a bar in the graph. The average and the standard deviation were calculated as necessary.

3. Results and Discussion

3.1 Influence of addition of calcium and magnesium ions on proteins separation and recovery

In the previous study, we revealed that the separation rate and the recovery efficiency of milk whey proteins in foam separation were effective and highest at pH 7 among those in the range of pH 4-8. In the actual industrial process of cheese production, milk whey solution was often obtained at about pH 6.2. Bovine serum albumin (BSA), α-lactalbumin (LA), and β-lactoglobulin (LG) molecules should have a mainly negative charge at pH 6 judging from their isoelectric points. Therefore, we assumed that by adding some cations to the whey solution at pH 6, such as Ca^{2+} and Mg^{2+}, aggregates or complexes of proteins would be formed, which improves the separation rate and recovery efficiency.

Fig. 2 shows the typical time course of whey protein concentration in the column. The ordinate, C_b and C_i are the concentration of whey proteins at t and the initial concentration of whey proteins, respectively. The concentration of Ca^{2+} and Mg^{2+}added in the whey solution within the column was 0.1 M. In case of adding Ca^{2+} and Mg^{2+}, C_b/C_i became lower than that without adding, and C_b reached the residual equilibrium concentration within the column at about 120 min. Especially, the addition of Ca^{2+} made C_b/C_i much lower than that of adding Mg^{2+}. Each C_b/C_i values for without adding, Ca^{2+}, and Mg^{2+} were 0.88, 0.37, and 0.76, respectively. In the case setting 0.1 M as the initial concentration, the addition of Ca^{2+} affected greatly on recovery efficiency. To evaluate recovery kinetics, the following first-order equation was used.

$$\ln\left(\frac{C_\mathrm{b}}{C_\mathrm{i}}\right) = -kt \qquad (1)$$

Where k represents the separation rate constant. By fitting of the data to Eq. (1), a proportional relationship with a negative slope value should be obtained by a least squares regression, and k could be determined from the slope value. Fig. 2b shows the result of the fitting of the data shown in Fig. 2a to Eq. (1). Within 40 min a good linear proportional relationship was obtained, thus, k could be evaluated by the fitting of the data to Eq. (1). The values of k without and with the addition of Ca^{2+} and Mg^{2+} were 3.09×10^{-3}, 1.23×10^{-2}, and 4.65×10^{-3} min^{-1}, respectively. By the addition of Ca^{2+}, the separation rate also became much faster than that in the case of adding Mg^{2+}.In the previous study [9], the k value at pH 6 corresponded to about 0.6-fold that at pH 7.

3.2. Influence of variation of Ca^{2+} and Mg^{2+} concentration on separation rate and recovery efficiency of whey proteins

The dependence of the separation rate and the recovery efficiency on the concentration of Ca^{2+} and Mg^{2+} was investigated. The results were shown in Fig. 3. As mentioned in the previous section, the addition of Ca^{2+} and Mg^{2+} affected both the separation rate and the recovery efficiency. However, the tendency of the effect was found to be different in the case of adding Ca^{2+} and Mg^{2+}.

In the absence of Ca^{2+} and Mg^{2+}, the recovery efficiency, R (= $1 - C_e/C_i$), was only 0.12 (Fig. 3a). With the addition of Ca^{2+}, the recovery efficiency improved from 0.12 to 0.66 with the increase of Ca^{2+} concentration up to 0.06 mol/L. However, in the case of adding Ca^{2+} over 0.06 mol/L, the recovery efficiency was not improved and kept almost constant at about 0.64. On the other hand, in the case of adding Mg^{2+}, the improvement of the recovery efficiency was rather small, from 0.12 to 0.23, at the Mg^{2+} concentration up to 0.1 mol/L.

As to the separation rate, k, it increased from 0.27×10^{-2} to 1.2×10^{-2} min^{-1} with increasing Ca^{2+} concentration up to 0.10 mol/L (Fig. 3b). In the case of adding Mg^{2+}, the value of k increased from 0.27×10^{-2} to 0.6×10^{-2} min^{-1} with increasing Mg^{2+} concentration up to 0.08 mol/L. However, it was rather smaller than that in the case of adding Ca^{2+}.

3.3. Influence of Ca^{2+} and Mg^{2+} concentration on the overall equilibrium adsorption constant and the overall saturated adsorption density of whey proteins onto bubble surface.

Foam separation technique itself has been adopted to determine the adsorption parameters of surface-active substances onto bubble surfaces such as the equilibrium adsorption constant and the saturated adsorption density. Many of them have been conducted in the continuous mode because it is easy to obtain the experimental data at an equilibrium state [11-19]. However, continuous mode experiments require a much larger volume of testing solutions than batch mode experiments. So, for convenience, we have proposed a simple method for the determination of the adsorption parameters by batch mode experiments [9]. In our previous study, the adsorption of milk whey proteins onto bubble surfaces could be explained by an adsorption model based on the Langmuir equation,

$$K(C_b - C_i) + \ln(C_b/C_i) = S_d X_s K \ln(V/V_i) \qquad (2)$$

where K and X_s represent the overall equilibrium adsorption constant and the overall saturated adsorption density, respectively. V and V_i represent the liquid volume within the column at time t and the initial state, respectively. S_d represents the specific bubble surface area based on the column volume within the column. The detailed derivation is referred to in the previous study [9]. By fitting of the batch mode experimental data to Eq. (2), we could obtain the values of K and X_s of the adsorption of whey proteins onto bubble surfaces in the absence and presence of Ca^{2+} and Mg^{2+}. The comparison of the experimental data and the calculated value from Eq. (2) using the obtained adsorption parameters was shown in Fig. 4. In this calculation, the data up to 40 seconds were used where the concentration of whey solution was significantly changed.

Fig. 5 shows the influence of the initial concentration of Ca^{2+} and Mg^{2+} on K and X_s. As to K, it decreased with increasing Ca^{2+} and Mg^{2+} concentration. In the absence of Ca^{2+} and Mg^{2+}, K was 172 cm^3/g, however, in the concentration range over 0.01 mol/L for both cases of Ca^{2+} and Mg^{2+}, K decreased from 172 to about 80 cm^3/g. Moreover, K for Ca^{2+} and Mg^{2+} mostly became constant although some scatters were observed. In the concentration range from 0.02 to 0.1 mol/L, the average values of K were 91.3 cm^3/g and 68.9 cm^3/g for Mg^{2+} and Ca^{2+}, respectively. As to X_s, in the case of adding Ca^{2+} and Mg^{2+}, X_s increased with increasing Ca^{2+} and Mg^{2+} concentration. However, the tendency of the increase in X_s was different from each other in the case of Ca^{2+} and Mg^{2+}. In the case of adding Ca^{2+}, an abrupt increase was observed until 0.06 mol/L, and in the concentration range over 0.06 mol/L, X_s seemed constant (average value, 5.75×10^{-6} g/cm^2). On the other hand, in the case of adding Mg^{2+}, the increase in X_s increased linearly with the increase in the concentration of Mg^{2+}. In the case with the addition of Ca^{2+} and Mg^{2+}, X_s became maximum value, 5.91×10^{-6} and 4.65×10^{-6} g/cm^2 at 0.08 and 0.1 mol/L for Ca^{2+} and Mg^{2+}, respectively, which were 2.96-fold and 2.3-fold of X_s value (1.99×10^{-6} g/cm^2) without the addition.

The influence of the addition of Ca^{2+} and Mg^{2+} on K and X_s might be considered qualitatively as follows. Whey proteins might form something to be aggregates or complexes by binding Ca^{2+} and Mg^{2+}. The whey protein-Ca^{2+}/Mg^{2+} complexes became lesser adsorption strength onto bubble surfaces than that of whey proteins without binding Ca^{2+} and Mg^{2+}. However, due to the formation of the complexes, the adsorption density of whey proteins increased with the increase in the concentration of Ca^{2+} and Mg^{2+}, suggesting that the complexes could be larger with the increase in the concentration of Ca^{2+} and Mg^{2+}. The main components of proteins in milk whey solution were estimated as LA, LG, and BSA from the result of HPLC analysis. The isoelectric points of LG, LA, and BSA are 5.2, 4.7-5.1, and 4.7-4.9, respectively. At pH 6, these proteins have negative charge, thus, whey proteins were aggregated by the divalent metal ions. Judging from the results shown in Figs. 2-5, Ca^{2+} could rather affect the formation of the complexes than Mg^{2+}.

It has been reported that LG molecule prefers to bind Ca^{2+} than Mg^{2+} in whey solution [20]. Moreover, in addition to LG, LG has also been reported to bind more Ca^{2+} than Mg^{2+} in the pH range of 5.5-6.5 [21]. Zhu and Damodaran (1994) investigated the influence of Ca^{2+} and Mg^{2+} on aggregation, foamability, and foam stability of whey proteins solution and reported that maximum aggregation was given at 0.02-0.04 M, and best foamability was given at 0.02 M, and foam stability depended on the incubation time of aggregation [22]. The basic elementary reactions for the binding of Ca^{2+} and Mg^{2+} to whey proteins to form complexes/aggregates can be expressed as follows.

$$2P + M \rightarrow P_2M$$

Where, P and M represent whey proteins, Ca^{2+} and Mg^{2+}, respectively. It is considered that a single Ca^{2+} or Mg^{2+} ion must bridge the whey protein molecules. Because of the estimation of K and X_s of whey proteins by foam separation has not been reported except for our study, we cannot compare our results with the values in other literature. The values of K with the addition of Ca^{2+} and Mg^{2+} were lower than that without the addition. We speculate that it is due to the decrease in the collision frequency with the bubble surface by forming complexes and aggregates. On the other hand, the value of X_s became large by the addition of Ca^{2+} and Mg^{2+}, because the size of complexes and aggregates are much larger than proteins. From a practical viewpoint, it is considered that the addition of Ca^{2+} is much more effective for the recovery of whey proteins at pH 6.

4. Conclusions

We investigated the influence of the addition of Ca^{2+} and Mg^{2+} on the separation rate and the recovery efficiency. As we expected, the addition of Ca^{2+} and Mg^{2+} to whey proteins solution (pH 6) could enhance the separation rate and the recovery efficiency as compared with the case without the addition. It could be considered that the addition of Ca^{2+} or Mg^{2+} to the whey protein have enhanced the coagulation of the whey proteins, which may have affected the separation rate and recovery efficiency. In addition, in the present experimental range, the overall saturated adsorption density was estimated to be 4-5 times larger than in the case without the addition of Ca^{2+} and Mg^{2+}. This fact could also support our consideration described above. In a further study, we would improve the additive enhancement and this separation process for a continuous mode.

Nomenclature

C_b = concentration of proteins in milk whey solution at time t [kg/m^3]

C_e = residual concentration of proteins in milk whey solution [kg/m^3]

C_i = initial concentration of proteins in milk whey solution [kg/m^3]

k = separation rate constant defined in Eq.(1) [s^{-1}]

K = overall equilibrium adsorption constant for milk whey proteins [m^3/kg]

S_d = specific bubble surface area based on column volume within column [m^2/m^3]

t = time [s]

V = liquid volume within column [m^3]

V_i = initial liquid volume within column [m^3]

X_s = overall saturated adsorption density of whey proteins onto bubble surface [kg/m^2]

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Figures captions

Fig. 1. Schematic diagram of the experimental setup for batch foam separation. 1. bubble column; 2. gas distributor; 3. pressure measuring tap; 4. gas flow meter; 5. flow control valve; 6. nitrogen gas cylinder; 7. pressure/voltage transducer; 8. amplifier; 9. voltmeter; 10. personal computer, 11. sampling tap, 12. feeding tap (continuous mode).

Fig. 2. (a) Typical time courses of concentration of milk whey protein recovery within the column. The concentration of Ca^{2+} and Mg^{2+} was 0.1 mol/L. (b) Fitting of the data of Fig. 1a to Eq. (1) for estimation of the separation rate constant, k.

Fig. 3. Influence of the concentration of Ca^{2+} and Mg^{2+} in whey solution on (a) the recovery efficiency, C/C_i, and (b) the separation rate constant, k. R for Ca^{2+} (0.06-0.1 mol/L), average = 0.636 and standard deviation = 0.023. R for Mg^{2+} (0.02-0.1 mol/L), average = 0.228 and standard deviation = 0.087.

Fig. 4. Typical results of fitting of data to Eq. (2) for determination of the overall equilibrium adsorption constant, K, and the overall saturated adsorption density, X_s, of whey proteins.

Fig. 5. Influence of the concentration of Ca^{2+} and Mg^{2+} in whey solution on (a) the overall equilibrium adsorption constant, K, and (b) the overall saturated adsorption density, X_s. K for Ca^{2+} (0.04-0.1 mol/L), average = 68.9 and standard deviation = 12.3. K for Mg^{2+} (0.02-0.1 mol/L), average = 91.4 and standard deviation = 16.5. X_s for Ca^{2+} (0.06-0.1 mol/L), average = 5.74×10^{-6} and standard deviation = 1.58×10^{-7}.

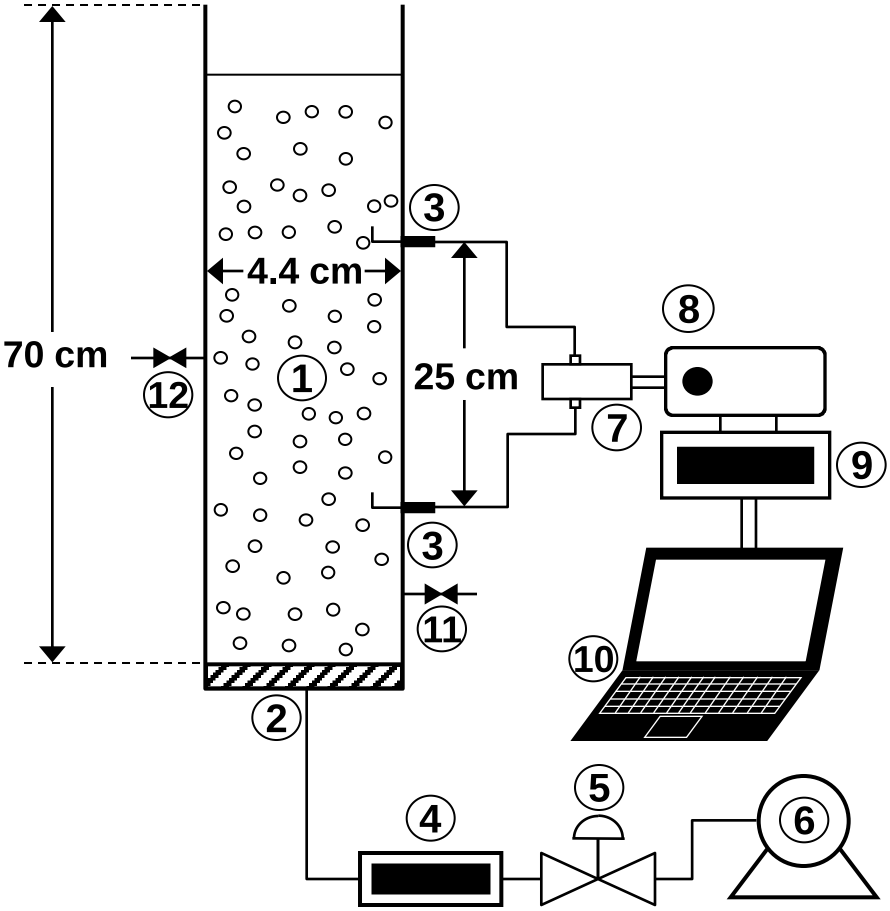

Fig. 1. Schematic diagram of the experimental setup for batch foam separation. 1. bubble column; 2. gas distributor; 3. pressure measuring tap; 4. gas flow meter; 5. flow control valve; 6. nitrogen gas cylinder; 7. pressure/voltage transducer; 8. amplifier; 9. voltmeter; 10. personal computer, 11. sampling tap, 12. feeding tap (continuous mode).

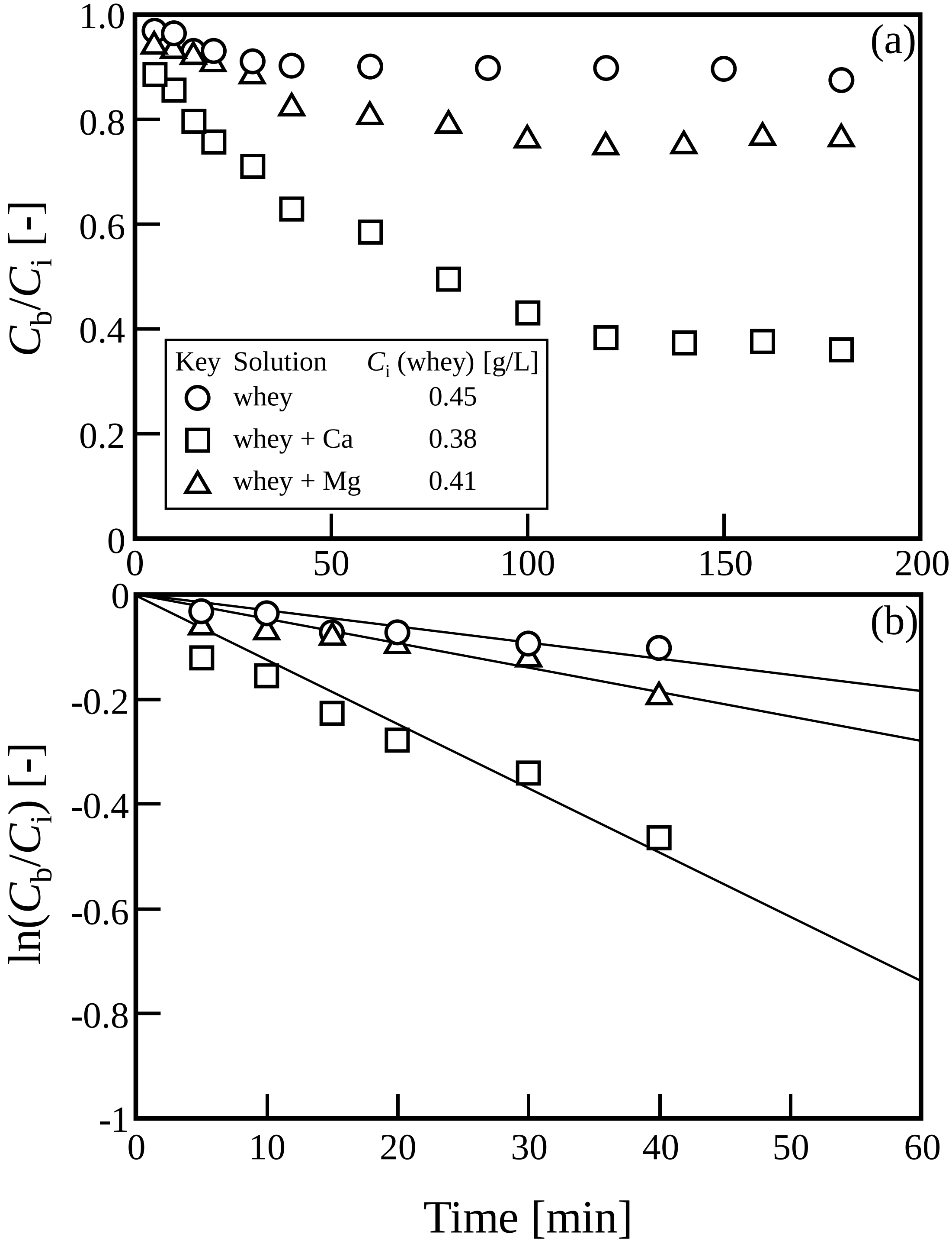

Fig. 2. (a) Typical time courses of concentration of milk whey protein recovery within the column. The concentration of Ca^{2+} and Mg^{2+} was 0.1 mol/L. (b) Fitting of the data of Fig. 1a to Eq. (1) for estimation of the separation rate constant, *k*.

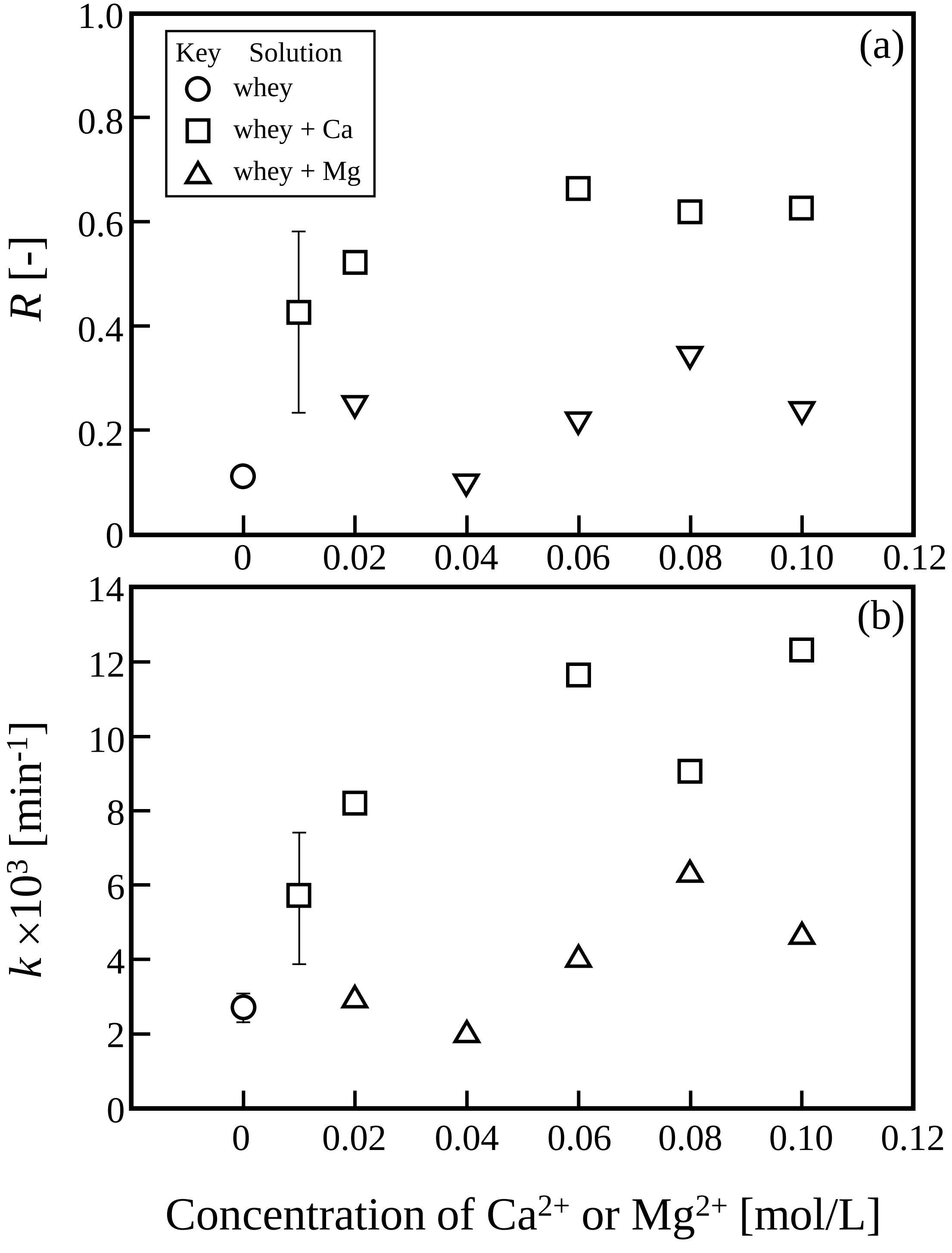

Fig. 3. Influence of the concentration of Ca^{2+} and Mg^{2+} in whey solution on (a) the recovery efficiency, *R*, and (b) the separation rate constant, *k*. *R* for Ca^{2+} (0.06-0.1 mol/L), average = 0.636 and standard deviation = 0.023. *R* for Mg^{2+} (0.02-0.1 mol/L), average = 0.228 and standard deviation = 0.087.

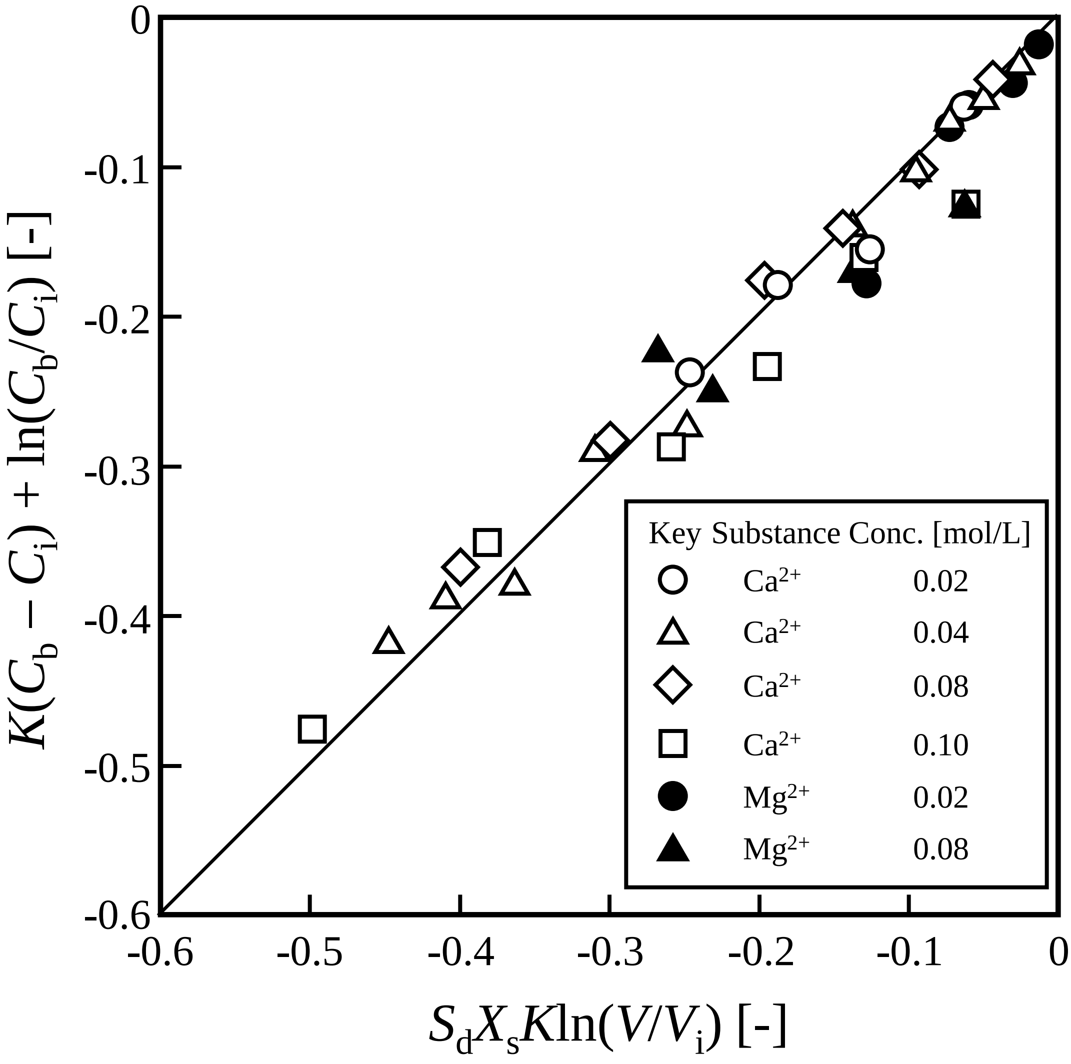

Fig. 4. Typical results of fitting of data to Eq. (2) for determination of the overall equilibrium adsorption constant, *K*, and the overall saturated adsorption density, X_s, of whey proteins.

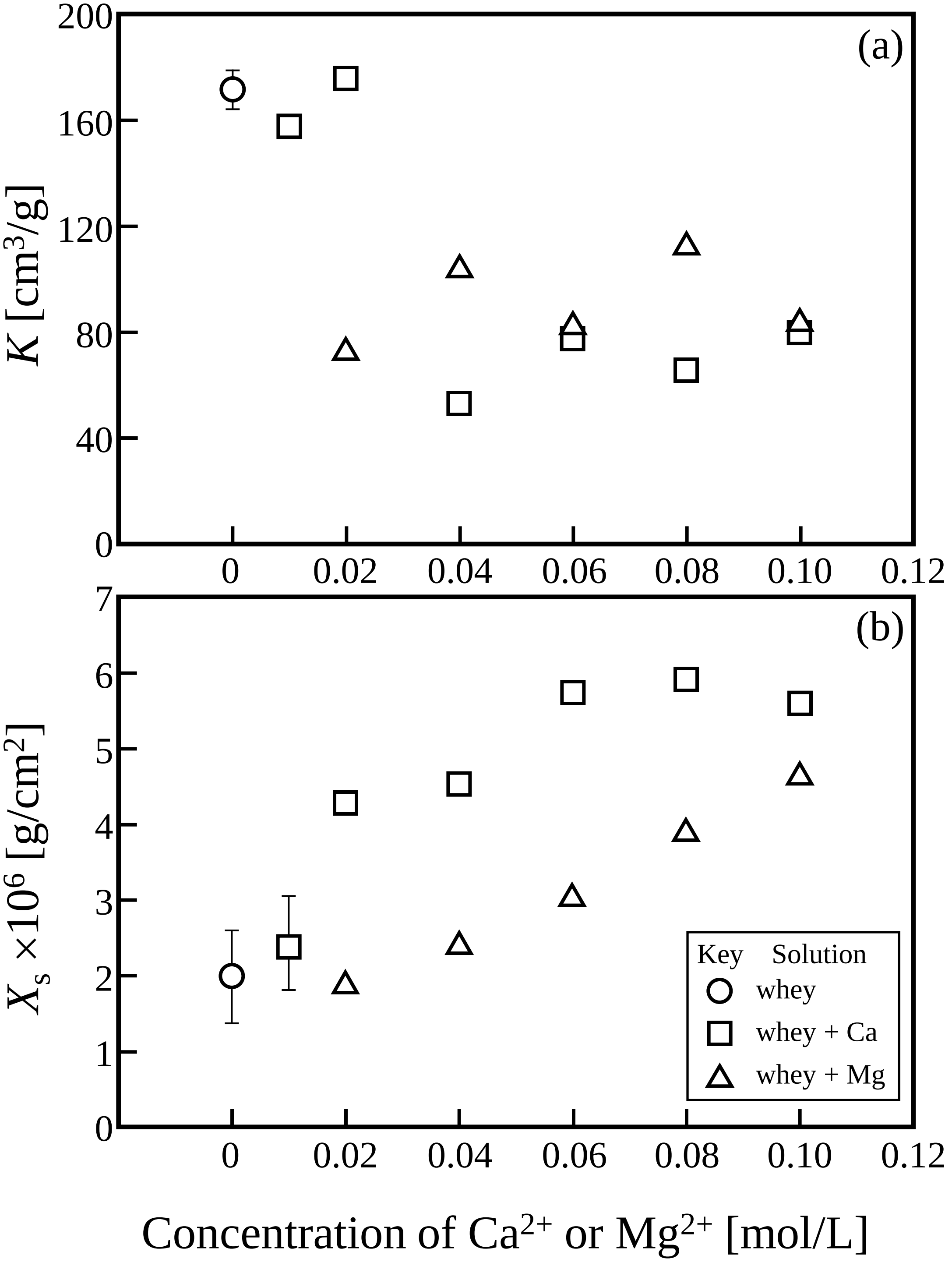

Fig. 5. Influence of the concentration of Ca^{2+} and Mg^{2+} in whey solution on (a) the overall equilibrium adsorption constant, K, and (b) the overall saturated adsorption density, X_s. K for Ca^{2+} (0.04-0.1 mol/L), average = 68.9 and standard deviation = 12.3. K for Mg^{2+} (0.02-0.1 mol/L), average = 91.4 and standard deviation = 16.5. X_s for Ca^{2+} (0.06-0.1 mol/L), average = 5.74×10^{-6} and standard deviation = 1.58×10^{-7}.