

# Effect of SDS on Casein Micelles: SDS-Induced Milk Gel Formation

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**ABSTRACT:** The addition of sodium dodecyl sulfate (SDS) during skim-milk reconstitution contributed to a modification of hydrophobic interactions and, consequently, to change in the micellar structure. SDS-induced modifications in casein micelles were investigated by biochemical measurements (soluble mineral and protein analyses, granulometric and electrokinetic potential measurements, and casein micelle solvation). SDS induced micellar  $\kappa$ -casein dissociation and caused a decrease in steric, hydration, and electrostatic repulsive forces between casein micelles and as a result altered micellar stability. Consequently, SDS-modified micelle aggregation occurred. Mineral analysis indicated that Ca,  $\text{PO}_4^{3-}$  and Mg partitioning between aqueous phase and curd is similar, suggesting a possible bridging mechanism via minerals and SDS molecules.

**Key Words:** sodium dodecyl sulfate,  $\kappa$ -casein, casein micelles, milk gel formation

## Introduction

THE MAIN INTERACTIONS THAT ARE INVOLVED IN CASEIN MILK gel formation depend on attractive forces, such as hydrogen bonds (reinforced during cooling), hydrophobic interactions (reinforced at high temperature), electrostatic attractions, van der Waals attractions. An evaluation of the importance of the different interactions involved in casein gel formation is useful when determining the properties of acid or rennet milk gels.

Modification of hydrophobic interactions at the micellar structural level constitutes one approach to probe the involvement of these kinds of interactions in the mechanism of micellar casein gel formation. The addition of an anionic amphiphilic molecule, such as sodium dodecyl sulfate (SDS), which mainly interacts with the nonpolar groups of protein side chains, induces competitive breaking of hydrophobic interactions (Markus and Karush 1957; Cheeseman 1968; Cheeseman and Jeffcoat 1970; Kato and others 1984). Accordingly, hydrophobic interactions can be reduced at the micellar structural level by adding SDS during skim-milk reconstitution. This approach could permit the establishment of a relationship between the structure of SDS-modified casein micelles and their functional properties of network formation. Studies dealing with the effect of SDS on the physico-chemical properties of milk proteins have previously been reported, but these have only been conducted on isolated caseins (Cheeseman 1968; Cheeseman and Jeffcoat 1970; Cheeseman and Knight 1970).

Previously, Lefebvre-Cases and others (1998) have shown that under appropriate conditions of temperature and SDS molarities, the SDS-modified casein micelles are able to interact to form a milk gel at the natural pH of milk without adding coagulating agents. Results have suggested that SDS-induced milk gel formation requires a defined level of casein dissociation, which could also be related to a particular micellar state. However, to understand how casein micelles are able to aggregate in the presence of SDS to form a gel requires more information concerning the compositional and structural SDS-induced changes occurring at the micellar level. Our objective was to propose an explanation of the role of the presence of SDS in the aggregation of casein mi-

celles at 30 °C without adding coagulating agents.

## Materials and Methods

### Skim milk

Reconstituted modified skim milk is made by dissolving a commercial low-heat-processed powder (Laiterie Matines-sili, Plouvien, France) in deionized water (120 g/L) containing increasing amounts of SDS (0, 2, 4, 6, 8 g/L; Sigma-Aldrich, St Quentin Fallavier, France). To prevent bacterial growth, 0.04% (wt/vol) sodium azide was added. All mixtures were stored at 4 °C for 16 h to allow the milk to equilibrate.

### Granulometric analysis

A Malvern Zetasizer 3000 (Malvern Instruments, Orsay, France) was used to evaluate micelle size in both untreated and SDS-modified milk. Particles suspended in liquid undergo random Brownian or thermal motion depending on their size. The light scattered by the particles when they are illuminated by a He-Ne laser beam ( $\lambda = 633 \text{ nm}$ ) can be interpreted in terms of particle diameter.

After 2 h of incubation at 30 °C, both native and SDS-treated casein micelles were examined in their natural ionic environment by suspending them in corresponding permeate obtained by filtration through Amicon YM 10 membranes at the temperature of measurement. The milk dilution used was 1:15 (v/v). Viscosity values of permeate were determined for each SDS concentration using a Searle Rheolab viscometer (Physica Messtechnik, Stuttgart, Germany) ( $\eta = 1.1 \text{ cp}$ , 1.2 cp, and 1.2 cp for untreated milk, 2 g SDS/L and 4 g SDS/L, respectively). The refractive index of the permeate was 1.33. All assays were performed 10 times, and curves were plotted with mean values.

### Zeta potential analysis

The zeta potential of casein micelles in both untreated and SDS-modified milk samples was measured by photon correlator spectroscopy (PCS) (Malvern Instruments, Orsay, France) with light from a He-Ne laser ( $\lambda = 633 \text{ nm}$ ) and a scattering angle of 90°. When an electric field is applied across an electrolyte, charged particles suspended in the

electrolyte are attracted towards the electrode of opposite charge. The velocity of particles in a unit electric field is referred to as its electrophoretic mobility, and the zeta potential of the particles, related to the electrophoretic mobility, can be calculated from Henry's Eq. (Henri 1931):

$$\zeta = 6 \pi \eta \mu / \varepsilon f(kr)$$

where  $\zeta$  is the electrokinetic potential or zeta potential, expressed in mV.  $\eta$  and  $\varepsilon$  are, respectively, viscosity and dielectric constant of the solution at the temperature of measurement,  $kr$  depends on particle shape, and  $\mu$  is electrophoretic mobility.

Sample preparation was analogous to the measurement of particle size by PCS, except for milk dilution 1:200 (v/v). The voltage applied across the cell was 150 V. All assays were performed 10 times, and curves are plotted with mean values.

### Compositional analysis

Both untreated and SDS-modified milk were incubated for 2 h at 30 °C before compositional analysis. After milk ultracentrifugation at 149,000 g for 50 min at 30 °C with a Beckman ultracentrifuge, rotor Ti70, the supernatant was carefully removed, and its soluble protein content was quantified using the modified Lowry method (Bensadoun and Weinstein 1976). The protein content of each pellet was determined by difference between total and soluble milk protein.

The amount of SDS bound to casein micelles was determined by difference between total and nonsedimentable SDS (under the ultracentrifugation conditions as cited above) using the modified Gregory method (Marshall and Green 1980) and was expressed as g SDS/g sedimented protein.

The solvation water of casein micelles was determined by ultracentrifugation as described by Gastaldi and others (1997) and was expressed as g H<sub>2</sub>O/g sedimented protein.

Total and soluble Ca, Mg, and phosphate (PO<sub>4</sub><sup>3-</sup>) were determined in untreated and SDS-modified milks and supernatants, respectively, using an inductively coupled plasma apparatus (Jobin Yvon ICP JY24; Division d'Instrument, Longjumeau, France).

### Statistical analysis

The data shown are the means of triplicate measurements from 5 experiments that were subjected to ANOVA (Stat View; Abacus Concepts, Inc., Berkeley, Calif., U.S.A.). Fisher's protected least significant difference (PLSD) test was used to compare paired means, and differences between means were considered to be significant at  $P < 0.05$ .

## Results and Discussion

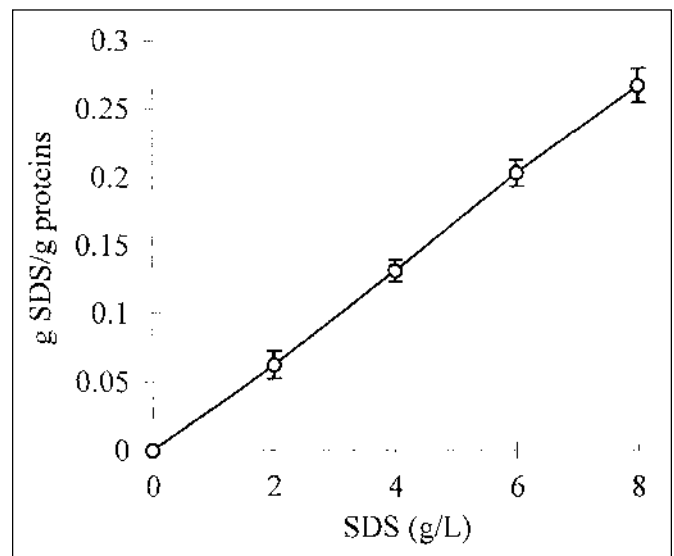
**I**N A PREVIOUS PAPER, LEFEBVRE-CASES AND OTHERS (1998), combining turbidimetry, rheology, and SEM analysis, pointed out that under appropriate temperature conditions, SDS could cause casein micelles to interact to form a micellar association resulting in a gel. This phenomenon occurs at a specific SDS concentration (4 g/L SDS) and could be related to a particular micellar state.

Figure 1 shows that the amount of SDS bound to casein micelles increases with increasing SDS concentration. This observation agrees with that of Marshall and Green (1980) who suggested that casein micelles contain many equivalent SDS binding sites, such as hydrophobic binding sites. Nevertheless, because of their amphipolar nature, SDS molecules

can interact with casein micelles either via the anionic part or the hydrophobic part.

As to the anionic interaction, at the pH of milk, an SDS molecule could associate electrostatically through its negatively charged oxygen from the sulfate group (SO<sub>3</sub><sup>-</sup>), either with quaternary nitrogen of casein (such as N<sup>+</sup> in His, Arg, Lys) or with the calcium located in the colloidal calcium phosphate (CCP), leading to an increase in the micellar hydrophobicity (Green 1982a, 1982b). Hydrophobic interactions would further increase with increasing temperature, up to the point at which the attractive force between the SDS-modified micelles would be sufficient to overcome the repulsive forces so that aggregation would occur. Nevertheless, according to this explanation, NH<sub>3</sub><sup>+</sup>—SO<sub>3</sub><sup>-</sup> binding should have released casein carboxyl residues initially involved in electrostatic interactions (NH<sub>3</sub><sup>+</sup>—COO<sup>-</sup>), resulting in milk acidification. However, pH measurement does not confirm this hypothesis since the pH value remained constant during the entire SDS-induced milk gel formation process (data not shown).

As to the hydrophobic interaction, SDS could interact with the casein micelle through its hydrophobic moiety (Markus and Karush 1957; Cheeseman 1968; Cheeseman and Jeffcoat 1970; Cheeseman and Knight 1970; Kato and others 1984). Observations seem to support this hypothesis. Indeed, SDS could compete with the  $\kappa$ -casein molecule for hydrophobic sites and in so doing progressively cause  $\kappa$ -casein dissociation from the micelle at increasing SDS concentration. The effect of SDS on the soluble protein content (Figure 2) shows that the extent of micellar casein dissociation is proportional to SDS concentration. Previous work (Lefebvre-Cases and others 1998) has shown that  $\kappa$ -casein was the only protein that is entirely dissociated from the micelle at 4 g SDS/L, whereas the maximum dissociation of  $\alpha_{s1}$ - and  $\beta$ -casein was reached at 8 g SDS/L. According to Cheeseman and Jeffcoat (1970), the SDS-binding properties of casein depend on the nature of the casein. The casein:SDS ratio (w/w) required for maximum binding is 1:1.1, 1:1.2, and 1:3.4 for  $\kappa$ ,  $\alpha_{s1}$ , and  $\beta$ -casein, respectively. In our study, the  $\kappa$ -casein:SDS ratio (w/w) obtained for 4 g SDS/L was 1:1.18, suggesting that



**Figure 1—Adsorption of SDS by casein micelles as a function of SDS concentration**

maximum SDS-binding to  $\kappa$ -casein was attained, thus leading to maximum micellar  $\kappa$ -casein dissociation.

Moreover, because SDS detergent mainly reacts with the hydrophobic regions in the casein molecule, our results point out the strong possibility that hydrophobic interactions form the basis of interactions between  $\kappa$ -casein and casein micelles and that  $\kappa$ -casein must be easily accessible, that is, located predominantly at the outside of casein micelles. Other caseins, such as  $\alpha_{s1}$ -,  $\alpha_{s2}$ -, and  $\beta$ -casein, would be mainly located in the interior and be bound to the micellar structure through both hydrophobic interactions and amino-acid phosphate side chains.

In comparison with untreated milk, the soluble amounts of Ca, Mg, and phosphate remained constant as SDS concentration increased, except for the highest SDS concentration (Figure 3). So, it can be assumed that the SDS treatment applied to milk did not modify the mineral balance between micellar and aqueous phases. That result seems surprising insofar as SDS-induced casein release has previously been reported. Because colloidal calcium phosphate (CCP) acts as a binding agent between micellar caseins (Schmidt 1980,

1982), the SDS-induced micellar casein dissociation should have been accompanied by a micellar mineral solubilization. Consequently, it could be suggested that solubilization of micellar Ca, Mg, and phosphate ions could be masked by concomitant mineral bridge formation between SDS-modified micelles. According to this hypothesis, a part of these minerals would be trapped by the anionic group of SDS, and, therefore, no change in the mineral content of the soluble phase of milk would be observed in the presence of SDS. An attempt at an explanation is illustrated in Figure 4, which is a greatly oversimplified model of a mineral bridge between aggregated SDS-modified micelles. For the highest SDS concentration (8 g/L), the micellar demineralization observed might appear as a result of the SDS-induced casein micelle fragmentation into small units (Cheeseman and Jeffcoat 1970; Lefebvre-Cases and others 1998).

As can be seen in Figure 5, the average micellar size remained unchanged for 2 g/L SDS-treated milk, whereas it increased greatly for 4 g/L. At higher SDS concentrations, larger particles are formed, and the laser granulometer cannot be used effectively.

That micellar aggregation occurred from 4 g SDS/L agrees with data by Lefebvre-Cases and others (1998) whose electron microscopic observations showed that casein micelles start to come together closer from 4 g/L SDS-treated milk.

Monitoring the  $\zeta$ -potential of casein micelles provides information on micellar surface charge. For untreated milk,

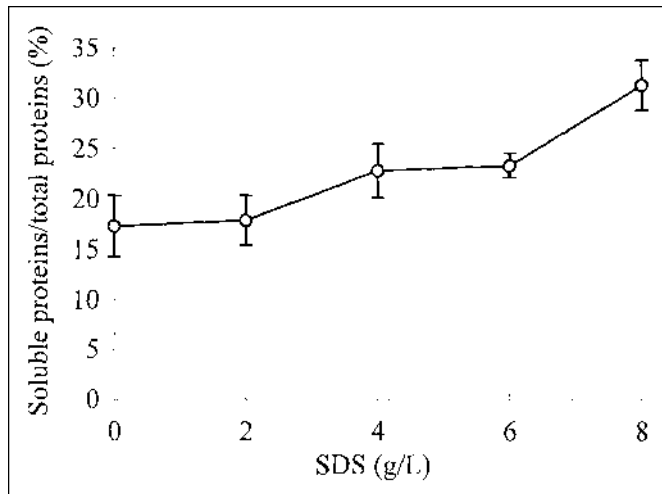


Figure 2—Total protein solubilization as a function of SDS concentration

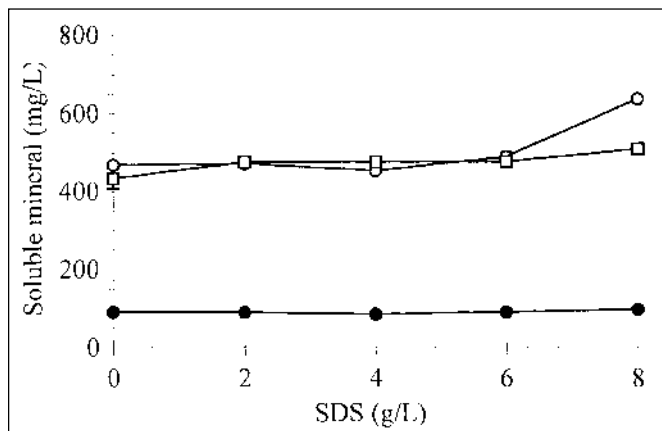


Figure 3—Changes in calcium (Ca), magnesium (Mg), and phosphate ion (P) content of milk aqueous phase as a function of SDS concentration: Ca (□); Mg (○); P (●)

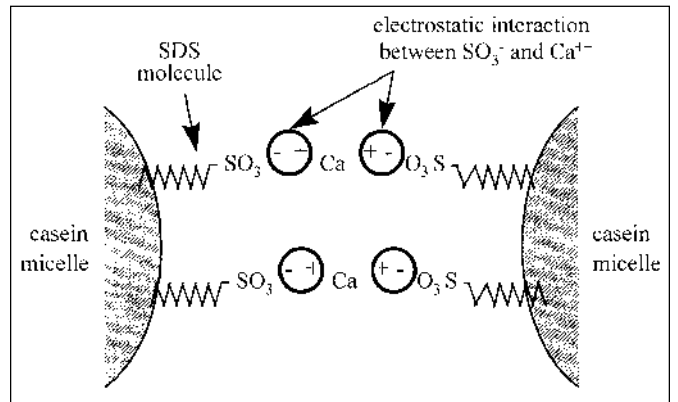


Figure 4—Hypothetical model of interaction between SDS-modified micelle and  $\text{Ca}^{2+}$

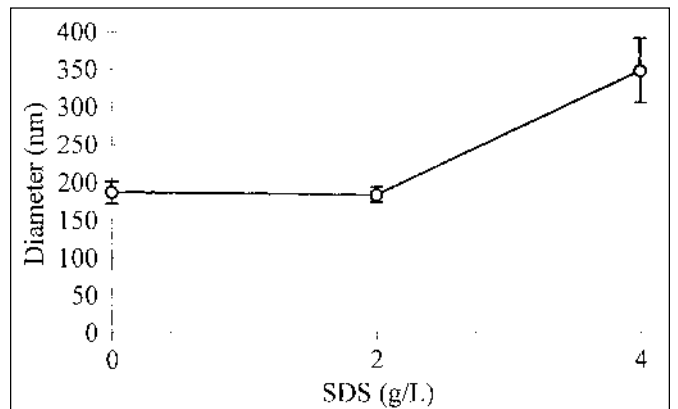


Figure 5—Micellar dia as a function of SDS concentration

the surface of casein micelles appears negatively charged, and its  $\zeta$ -potential value is  $-21.7 \text{ mV} \pm 2.0$  (Figure 6). At the lowest SDS concentration (2 g/L), this value decreased to  $-31.7 \text{ mV} \pm 2.8$  and then increases at higher SDS concentration (4 g/L) to reach the same value as untreated milk ( $-21.3 \text{ mV} \pm 1.8$ ). This result is in accordance with findings by Pearce (1976) who found that the  $\zeta$ -potential of casein micelles was altered by adding charged detergent molecules that are adsorbed by the micelles. According to that author, SDS caused an increase in the  $\zeta$ -potential of casein micelles when this detergent was used at concentrations less than required to solubilize proteins. Results lead us to conclude that SDS-induced changes in the micellar negative charge are complicated and not proportional to the SDS concentration, suggesting that SDS could act differently as a function of its concentration. Using 2 g SDS/L results in an increase in the net negative charge of casein micelles, suggesting that the negative charge carried by the SDS anionic group ( $\text{SO}_3^-$ ) is added to the negative charge of the C-terminal part of the  $\kappa$ -casein molecule. Using a higher SDS concentration (4 g/L), the reduction in the micellar negative charge could be attributed to the partial micellar  $\alpha_s$ - and  $\beta$ -casein dissociation and the total micellar  $\kappa$ -casein dissociation (Lefebvre-Cases and others 1998), which are all negatively charged at the pH value of milk (Swaisgood 1982). Therefore, although more negatively charged SDS molecules tend to bind to the micellar structure at increasing SDS concentration, the net negative charge of the micelle observed for 4 g SDS/L is similar to its initial value. Consequently, the micellar aggregation phenomenon observed from this SDS concentration is difficult to reconcile with an increase in micellar net negative charge. So, the question arises as to how highly negatively charged micelles do aggregate.

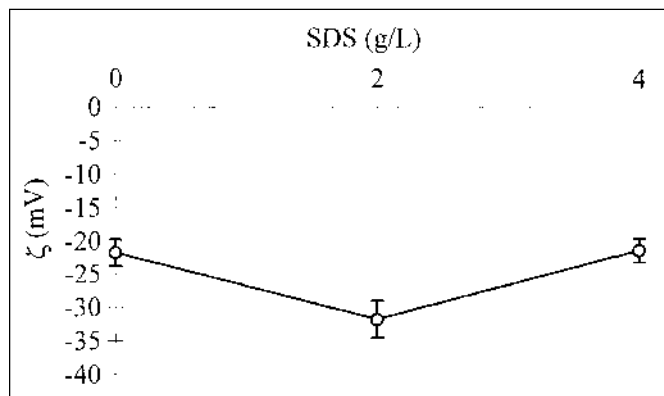
According to the literature, the importance of  $\kappa$ -casein in maintaining the stability of the casein micelle is well established (Vaugh and von Hippel 1956). Indeed, the stability against aggregation may be due to both steric, hydration, and electrostatic repulsion forces (Lenoir 1985; Walstra 1990; Bringe and Kinsella 1991) caused by the hydrophilic C-terminal part of  $\kappa$ -casein molecule. Consequently, casein micelle aggregation occurs when the  $\kappa$ -casein hairs are removed by: (1) chymosin-catalyzed hydrolysis (Walstra 1979; Darling and van Hooydonk 1981; Walstra and others 1981), (2) ethanol-induced collapse (Horne 1984; Griffin and others 1986; Horne and Davidson 1986; Griffin 1990), and (3) charge neutraliza-

tion due to pH reduction (Darling 1982; Horne and Davidson 1986).

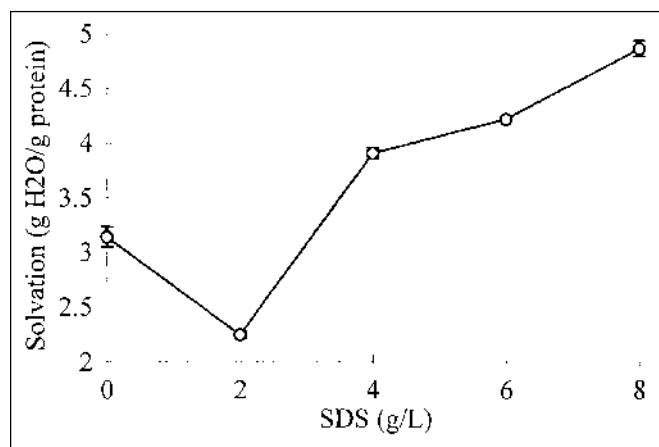
Because 4 g/L SDS-treated casein micelles, having a similar net negative charge to that of untreated milk, are able to come closer, it could be assumed that the loss in micellar stability is essentially due to the important SDS-induced micellar  $\kappa$ -casein dissociation. The resulting reduction in both steric, hydration, and electrostatic repulsion forces caused by the hairs of  $\kappa$ -casein at the micellar surface seems to be sufficient to cause micelles to aggregate.

Figure 7 shows that for the lowest SDS concentration (2 g/L), micellar solvation decreased significantly ( $P < 0.05$ ) from 3.14 g water/g prot  $\pm 0.09$  to 2.25 g water/g prot  $\pm 0.03$ , as compared to the untreated milk. On the contrary, for SDS concentrations higher than 2 g/L, casein micellar solvation increased, and the extent of this increase could be related to the SDS concentration. This change in casein micelle solvation, which reached a minimum for 2 g SDS/L, could be correlated to the aggregation state revealed by granulometric measurements (Figure 5) and by SEM, turbidimetry, and rheology studies previously reported by Lefebvre-Cases and others (1998). A decrease in micellar solvation could be attributed to an SDS-induced  $\kappa$ -casein dissociation, and this could be observed for 2 g SDS/L. For higher SDS concentrations, a decrease in micellar solvation probably occurs, but the aggregation phenomenon that takes place could mask any further decrease in casein micelle solvation. So, the increase in micellar solvation determined by ultracentrifugation measurements could be further indication of a higher water-holding capacity of the aggregated structure than a consequence of an increase in solvation of the micellar particles (Gastaldi and others 1997).

A possible explanation of SDS-induced milk gel formation without coagulating agents is that in binding to hydrophobic regions of the casein molecules, the detergent molecule induces structural changes in casein micelles. SDS competes with the casein molecule for hydrophobic sites and in so doing causes  $\kappa$ -casein dissociation from the micelle. This occurs until all available hydrophobic binding sites are occupied by SDS molecules. When the  $\kappa$ -casein-induced stabilizing power of casein micelles is destroyed, micelles begin to lose their individuality. Concurrently, this phenomenon could be enhanced by a hypothesized bridging mechanism,



**Figure 6**—Micellar zeta potential as a function of SDS concentration



**Figure 7**—Micellar solvation in milk as a function of SDS concentration

via minerals and SDS. In this way, milk could coagulate without rennet or acidification. Because 6 g SDS/L appears to be the optimum concentration to obtain a milk gel, it could be assumed that this mechanism of SDS-induced milk gel formation requires a defined level of casein dissociation, which may be related to a particular micellar state. Indeed, for lower and higher SDS concentrations, the amount of casein dissociated from the micelle is, respectively, lower or higher, but then no gel can be created. For the highest SDS concentrations (6 g/L and 8 g/L), the important micellar casein dissociation would result in a decrease in casein micelle size, which makes more difficult the internalization of hydrophobic residues. Consequently, it might be possible that structural rearrangement of casein micelles, initiated by the SDS-induced removal of  $\alpha$ s-,  $\beta$ -, and  $\kappa$ -casein, enhances micellar hydrophobicity. As a result, hydrophobic interactions between the SDS-modified micelles could be favored. This is compatible with curd syneresis, observed for 6 g/L and 8 g/L SDS concentrations (data not shown), which could be generally initiated by increasing the temperature, that is, increasing hydrophobic interactions between casein micelle particles. When hydrophobic interactions between micelle particles are numerous enough, it could lead to higher stress in the strands of the gel network. The resulting endogenous pressure would then cause the network to shrink by localized breakage of bonds, thus inducing whey expulsion (syneresis).

### Conclusions

DEPENDING ON SDS CONCENTRATION AND TEMPERATURE, casein micelles are able to interact to form a gel network without the addition of coagulating agents. Observations lead us to conclude that SDS-modified micelle aggregation would appear mainly as a consequence of a reduction in the repulsive barrier caused by the hydrophilic C-terminal region of  $\kappa$ -casein. Nevertheless, SDS-induced milk gel formation is based on complex mechanisms that involves chemical and physical changes at the micellar level. An understanding of these mechanisms is necessary for future product development involving coagulations.

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