Lecithin-sodium caseinate self-assembled complexes as emulsifying agents in oil-in-water emulsion: Acidic medium approach

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ABSTRACT

Surfactant-polyelectrolyte complexes (SPECs) based on lecithin and sodium caseinate were produced and the effects of such binding on the physical, chemical and emulsifying properties were evaluated and compared with the two ingredients in isolation. Negative, neutral, and positive charged SPECs were obtained. Zeta potential values and size distributions of the SPECs were dependent on the mass ratio between compounds. Electrostatic association decreased the polydispersity index in comparison with pure compounds solutions. Analysis of interfacial properties showed that solutions containing SPECs promoted a greater reduction of surface tension and interfacial tension with sunflower oil when compared with pure compounds solutions. Emulsions produced with SPECs in 10:1 lecithin:sodium caseinate ratio proved to be more stable than emulsions prepared with pure compounds. Thus, the complexation improved the emulsifying properties of lecithin and sodium caseinate establishing SPECs as potential natural emulsifiers.

1. Introduction

Substitution of synthetic compounds by natural products is a growing consumer demand. In the food area, the challenge on emulsion stabilization is to obtain natural emulsifiers with the same properties as the synthetic ones, such as low cost and high performance (Dickinson, 1993; Ozturk and McClements, 2016). In this scope, electrostatic association between natural ingredients with emulsifying and/or stabilizing properties is a strategy for improving these characteristics.

The interaction between surfactant and polyelectrolytes generally are called SPECs (surfactant-polyelectrolyte complexes) and lead to micelle-like aggregates, complexes, coacervates, precipitates and gels (Guzmán et al., 2016). These new structures may promote the synergy of pure compounds enhancing desirable characteristics, such as reduction of interfacial tension, greater stability and smaller droplet size of an emulsion (Dickinson, 1993; Goldfeld et al., 2015; Mantovani et al., 2016). The properties of SPECs can be mainly associated with the phase behavior of these mixtures, both in bulk and at the interfaces. This latter aspect is especially relevant because of their use in the stabilization of dispersed systems such as foams and emulsions (Guzmán et al., 2016). In most of these applications, these mixed systems promote changes in rheological behavior, stabilization or modification of their adsorption at surfaces (Gradzielski and Hoffmann, 2018).

Lecithin is a surfactant obtained from different natural sources such as soy and eggs. It is widely used as emulsifier, but its application is limited by its solubility in several solvents, which directly interferes on its HLB (Whitehurst, 2004; Somasundaran, 2006). It is a complex mixture of phospholipids and other compounds, such as tocopherol and glycolipids. Lecithin presents amphoteric character and neutral net charge at physiologic conditions. In acidic pH, the carboxylic and phosphate groups are ionized promoting a negative zeta potential to lecithin solutions (Whitehurst, 2004).

Sodium caseinate is a salt of caseins, a group of amphiphilic proteins with flexible structures (αs1, αs2, β, and κ-caseins) (Dalgleish, 1998; De Kruif and Holt, 2003). High heat stability and the ability to melt are properties of caseinates. In addition, when in solution, caseinates presents excellent surfactant property due to its hydrophilic and hydrophobic segments (Sarode et al., 2016) However, the emulsifying properties of sodium caseinate decrease at acid pH. In such pH values, the net attractive forces between the casein molecules increase, resulting in a self-association of the adsorbed and non-adsorbed protein components (Allen et al., 2006), which could lead to emulsion destabilization.

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Recent literature has presented few studies among these natural compounds aiming at improving their emulsifying properties. However, the use of sodium caseinate at acidic pH and the previous electrostatic complexation of compounds with subsequent application as emulsifier has not been reported in the literature. Therefore, the objective of this work was to produce, characterize and evaluate the application of SPECs based on lecithin and sodium caseinate as emulsifying agents.

2. Material and methods

2.1. Materials

Sodium Caseinate (NaCas) (protein 87 g/100 g) was obtained from Alibra (Brazil). Soy lecithin (Lip) (LIPOID S45, phosphatidylcholine 51.8%) was obtained from Lipoid (Germany). Sunflower oil (SFO) (80.1% C18:1 cis oleic) was obtained from Cargill (Brazil). All other reagents used were of analytical grade. Ultrapure water, used to prepare the compounds dispersions, was obtained with a Milli-Q Ultrapure water purification system (Millipore, USA).

2.2. Stock solutions preparation

Lecithin (Lip) 2% (w/w) was solubilized in ethyl alcohol and, then, an adequate volume was added in Milli-Q water to obtain 0.2% aqueous lecithin (w/w). Sodium caseinate (NaCas) 0.2% (w/w) was solubilized in Milli-Q water. The pH of both solutions was adjusted to 3.5. At this pH value, the compounds presented the highest difference of charges, which is suitable for electrostatic association (based on previous tests and not shown).

2.3. Surfactant-polyelectrolyte complexes (SPECs) preparation

SPECs were prepared by mixing the stock solutions (0.2% w/w) in a rotor-stator device (Ultraturrax model T-10, Ika, Germany) at 2000 rpm for 2 min, at room temperature. Previous tests (not shown) varying the mass ratios between compounds were carried out in order to obtain cationic, neutral e anionic SPECs. The formulations which presented these characteristics were 10:1, 5:1 and 1:1 Lip:NaCas ratios (Table 1). The total final concentration in the formulations was kept constant at 0.2% (w/v). After preparation, the formulations were stored at 10 °C for 24 h before analysis.

2.4. Physical-chemical characterization: size distribution and zeta potential analysis

Size distributions of pure compounds and SPECs were determined by Dynamic Light Scattering (DLS) in a Zetasizer Nano ZS (Malvern, UK), with backscatter detection at 173°. The Zeta potential values were determined by microelectrophoresis in the same equipment. All measurements were carried out at 25 ± 0.5 °C.

2.5. Surface and interfacial measurements

Solutions containing SPECs or pure compounds at 0.2% (w/v) were used to evaluate the surface tension (γs) (air/water interface) and interfacial tension (γml) with sunflower oil (~80% C18:1). These parameters were measured at 25 ± 1 °C by pendant drop method in a_pal

Table 1

| Formulation     | Lecithin (mg/mL) | Sodium Caseinate (mg/mL) |
|-----------------|------------------|--------------------------|
| LipNaCas 10:1   | 1.81             | 0.19                     |
| LipNaCas 5:1    | 1.66             | 0.34                     |
| LipNaCas 1:1    | 1.00             | 1.00                     |

Table 2

| SPECs and pure compounds | Zeta potential (mV) | Diameter (nm) | PDI Peak 1 | Peak 2 |
|--------------------------|---------------------|---------------|------------|--------|
| Pure Lip                 | -45.2 ± 1.5a        | 672.6 ± 125.6 | 0.8 ± 0.1  |
| Lip:NaCas 10:1           | -34.9 ± 0.6b        | 290.9 ± 12.5  | 0.2 ± 0.1  |
| Lip:NaCas 5:1            | -0.3 ± 0.2c         | 1518.5 ± 125.6| 1 ± 0.0    |
| Lip:NaCas 1:1            | +25.4 ± 0.2d        | 249.9 ± 0.3b  | 0.4 ± 0.1  |
| Pure NaCas               | +29.9 ± 0.7e        | 301.3 ± 33.1b | 0.6 ± 0.1  |

"* Different lowercase letters on the same column indicate a significant difference between values (p-value <0.05).

Tracker S tensiometer (Teclis, France). Tests were carried out in triplicate, each with an individual duration of 2000 s. Data of initial and equilibrium tensions were analyzed. The equilibrium values of surface tension (γeq) were also studied as a function of lecithin concentration.

2.6. Production of oil in water emulsions

2.6.1. Emulsion preparation

30/70 (w/w) O/W model emulsions were prepared using SPECs dispersions (0.2% (w/v)) and sunflower oil (SFO). Emulsions composed only by lecithin or sodium caseinate (0.2% (w/v)) as emulsifying agents were prepared as control. The emulsions were homogenized in a rotor-stator device (Ultraturrax model T-10, Ika, Germany) at 30,000 rpm for 2 min at room temperature (25 ± 1 °C). The pH of aqueous phase was kept constant in 3.5.

2.6.2. Droplet size distribution

The droplet size distribution was determined by a laser diffraction method using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK). The emulsions were dispersed in water at the rotational velocity of 2000 rpm. The droplet size was expressed as the volume mean diameter (D[4,3]) calculated according to Eq. (1). The emulsions were analyzed immediately and after 48 h of their preparation.

$$D_{4,3} = \frac{\sum d_i n_i}{\sum n_i}$$

where n_i is the droplet number with diameter d_i.

2.7. Statistical analysis

All measurements were performed in triplicate and shown as mean ± standard deviation. The data were analyzed by one-way ANOVA and Tukey HSD test (p < 0.05), using the software PAST - Paleontological statistics software package for education and data analysis (Hammer et al., 2001).

3. Results and discussion

3.1. SPECs characterization: size distribution and zeta potential

Table 2 shows the size distribution and zeta potential values of SPECs and pure compounds. The analyses were carried out at the pH of SPECs formation (3.5). Pure lecithin and sodium caseinate showed opposite charges at pH 3.5, therefore they are prone to electrostatic complexation (Table 2). The positive net charge of sodium caseinate is related to the presence of carboxylic and phosphate groups on its hydrophilic head.

Both pure compounds showed high polydispersity index and bimodal
distribution (Table 2), which is related to the presence of distinct structures in both solutions. Sodium caseinate showed bimodal distribution with peaks ranging from 54.3 to 301.3 nm. These results were attributed to the different sizes of casein fragments. The lecithin solution also showed a bimodal distribution, with peaks centred at 125 and 670 nm (Table 2). Such result may be attributed to the formation of aggregates, due to the low solubility of lecithin in aqueous medium (Berg, 2002; Whitehurst, 2004).

Depending on the ratio between compounds, different complexes were obtained (Table 2). When a small amount of caseinate is complexed with lecithin (LipNaCas 10:1 dispersion), a significant decrease in size and polydispersity (p-value < 0.05) was observed in relation to pure lecithin (Table 2). It was believed that the addition of sodium caseinate reduce the aggregation of the phospholipids. In addition, the net charge of LipNaCas 10:1 dispersion was negative and smaller than zeta potential value of pure Lip, suggesting that complexation between sodium caseinate and lecithin occurred.

Complexation between compounds in the Lip: NaCas ratio 5:1 resulted in insoluble aggregates with micrometric size. This result was related to the neutralization of the positive and negative charges of pure compounds, observed by the Zeta potential value close to neutrality. In an equal ratio of compounds (LipNaCas 1:1), sodium caseinate is in excess due to its higher charge density in relation to charge density of lecithin. The size and polydispersity were decreased in comparison with pure NaCas (Table 2). The zeta potential value of LipNaCas 1:1 decreased in relation to zeta potential of pure NaCas suggesting that phospholipids were bonded at caseinate fragments.

### 3.2. Surface properties

Solutions of pure compounds and dispersions of SPECs showed distinct behaviors at the air-water interface. Regarding pure compounds, the lecithin solution presented greater activity (Fig. 1) in comparison with the sodium caseinate solution. Although both solutions present amphiphilic properties lecithin resulted in a better performance once it presents smaller molecule size (section 3.1) when compared to the amphiphilic fractions of caseinate (αs1- and β-caseins) (Whitehurst, 2004; Woodward et al., 2009). Such factor favors a faster interface saturation.

The behavior of the SPECs dispersions at the air-water interface depended on the ratio between compounds. Lip:NaCas 10:1 showed greater interfacial decay rate on the 100 first seconds, evidenced by its steeper slope (Fig. 1) when compared with pure lecithin, pure caseinate solution, and other SPECs formulations. It was believed that in this formulation the new arrangement between compounds promoted synergy and then, showed better activity. On the other hand, SPECs produced by Lip:NaCas 5:1 and Lip:NaCas 1:1 showed a slightly higher surface activity when compared to pure NaCas. The increase of surface activity for Lip:NaCas 5:1 and Lip:NaCas 1:1 in comparison to pure NaCas was attributed to the surfactant bonded to its sites (Dickinson and Golding, 1997; Karbowiak et al., 2006), improving the surface activity of NaCas.

The behavior of surfactant-polyelectrolyte complexes can be better understood analyzing the graph of the equilibrium surface tension versus lecithin concentration (Fig. 2). In presence of sodium caseinate, low concentrations of lecithin (Lip:NaCas 1:1 and Lip:NaCas 5:1) increase the equilibrium surface tension as compared to same concentrations of lecithin in absence of NaCas. When lecithin concentration is further increased (Lip:NaCas 10:1) the equilibrium surface tension markedly decreases. Based on these data it is believed that the critical aggregation concentration (Cac) for lecithin-sodium caseinate system is approximately the lecithin concentration of formulation Lip: NaCas NaCas 5.1 It is generally admitted that, in presence of polyelectrolyte, such as NaCas, surfactant start to form structures above Cac, thus promoting greater surface activity as seen in Lip: NaCas 10:1 (Dickinson and Golding, 1997; Jain et al., 2004).

### 3.3. Interfacial properties

The oil-water interface was more sensitive to the changes in Lip: NaCas ratios when compared with the air-water interface (Fig. 3). The Lip:NaCas 10:1 and Lip:NaCas 5:1 formulations showed a greater velocity in the interface stabilization, which is evidenced by their higher slopes (Fig. 3) in comparison with pure lecithin solution, pure sodium caseinate, and the Lip:NaCas 1:1 formulation.

These results were attributed to the interactions between SPECs, the amount of lecithin present in each formulation and to the chemical composition of the sunflower oil used in this study (80.1% cis oleic acid).
Oleic acid is a long-chain unsaturated fatty acid (18 carbons). Its length and the double bond decrease its structural flexibility and, therefore, plays a role in the anchoring of a given emulsifier at the oil-water interface (Dickinson and Golding, 1997; Karbowiak et al., 2006; Gomes et al., 2018).

Regarding pure compounds, lecithin showed lower equilibrium tension values than pure NaCas (Lip = 1.25 ± 0.4 mN/m; NaCas = 12.37 ± 0.3 mN/m), evidencing its greater ability to stabilize the interface. This was attributed to the hydrophobic interactions of carbon chains between phospholipids and sunflower oil when compared to interactions of hydrophobic sites of the NaCas and the long chains of the SPO (Dickinson and Golding 1997; Gomes et al., 2018). The small molecular size of phospholipids also had influence on this activity. SPECs (Lip:NaCas 10:1 and 5:1) promoted increased hydrophobic interactions between the sunflower oil and lecithin phospholipid chains in comparison with pure compounds and formulation Lip:NaCas 1:1. This fact increased the velocity of stabilization. Neutral charged SPECs are known in literature by their ability to decrease the interfacial tension (Dickinson and Golding, 1997; Karbowiak et al., 2006; Mirtallo et al., 2010).

Lip: NaCas 1:1 did not reach the equilibrium in the evaluated time. This fact was attributed to a different coverage of the interface in comparison with other SPECs formulations. In formulation Lip:NaCas 1:1 there is excess of sodium caseinate, thus, the interface stabilization is
resulted from the conformational changes in sodium caseinate structure. Sodium caseinate form films from aqueous solutions because of its random coil nature and its ability to form extensive intermolecular hydrogen, electrostatic and hydrophobic bonds, resulting in an increase of the interchain cohesion (Dickinson and Golding 1997; Dickinson, 1999; Mirtallo et al., 2010). Additionally, the adsorption of sodium caseinate at the interface is a consequence of its flexibility and the considerable numbers of hydrophobic residues present in these proteins. When a protein adsorbs at the oil-water interface, the hydrophobic regions of their structures (created by clusters of appropriate amino acid side chains) lie on, or possibly partially dissolve in the oil phase. This kind of change in protein structure is known as surface denaturation (Dickinson and Golding 1997; Dickinson, 1999).

3.4. Production of O/W emulsions with SPECs dispersions

Different behaviors were observed when sunflower oil was emulsified with SPECs dispersions or pure compounds solutions (Fig. 4). The particles measured in pure dispersions (Table 2) reached the oil-water interface promoting stabilization and thus, droplets were formed. Immediately after production, emulsions produced with pure compounds showed particles with smaller sizes in relation to emulsions produced with SPECs dispersions (Lip = 19.3 ± 3.0 μm; NaCas = 37.4 ± 19.0 μm) (Fig. 4). This fact can be attributed to the presence of smaller structures (vesicles, bilayers, and micelles) in pure compounds solutions (section 3.1, Table 2, peak 2). These small structures rapidly adsorb to the droplets interface, prevents flocculation and creaming. Despite the structural differences of pure compounds, both adsorb to the oil-water interface. Phospholipids provide stability to emulsions by both mechanical and electrostatic mechanisms (Dickinson, 1999). In addition, the sodium caseinate has functional properties, which include emulsification, water- and fat-binding, thickening, and gelation (Purtado et al., 2017).

When dispersions containing SPECs were used for sunflower oil emulsification, an increase in initial droplet sizes was observed when compared with emulsions produced with pure compounds (Fig. 4). The increased droplet diameter is related to the SPECs sizes (section 3.1, Table 2). Due to their larger sizes, it is more difficult to produce fine emulsions (Dickinson, 1999).

After 48 h of production, emulsions prepared with pure compounds showed increased particles sizes suggesting coalescence processes. These results were attributed to low concentration of emulsifier (0.2% w/w), high oil fraction (30% of total weight) and low compatibility of these compounds in producing O/W emulsions. Lecithin HLB range varies from 2 to 8 being it predominantly oil-loving (Whitehurst, 2004). On the other hand, sodium caseinate, despite its amphiphilic character, presents a better role as a stabilizing ingredient increasing the emulsions viscosities (Dalgleish, 1998; De Kruijff and Holt, 2003; Goldfeld et al., 2015).

Emulsions prepared with SPECs dispersions in ratios Lip:NaCas 1:1 and 5:1 showed significant differences in particle sizes in period evaluated. Emulsion prepared with SPECs dispersion at ratio Lip:NaCas 10:1 showed no significant difference (p-value < 0.05) in droplet size immediately and after 48 h of production. These results suggests that depending on the ratio between pure compounds, electrostatic association may promote synergy and generating particles with improved emulsifying properties. These results are in accordance with results presented in section 3.3.

4. Conclusions

Lecithin-sodium caseinate complexes may be produced with different charges and sizes depending on the mass ratio of the pure compounds. These complexes have a better performance in reducing the surface tension and interfacial tension with sunflower oil in comparison with pure compounds. Emulsions produced with different SPECs showed that complexes formed with the Lip:NaCas ratio of 10:1 promoted a better O/W emulsion stability in comparison with emulsions prepared with pure compounds. Thus, it can be concluded that the prior complexation between lecithin and sodium caseinate, in certain ratios, promotes an improvement in its emulsifying properties.

CRediT authorship contribution statement

Antonio Matias Navarrete de Toledo: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Carolina Siqueira Franco Picon: Conceptualization, Methodology, Resources, Supervision, Funding acquisition. Ana Carla Kawazoe Sato: Conceptualization, Methodology, Resources, Supervision, Funding acquisition.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Allen, K.E., Dickinson, E., Murray, B., 2006. Acidified sodium caseinate emulsion foams containing liquid fat: a comparison with whipped cream. Lebensm. Wiss. Technol. 39, 225–234. https://doi.org/10.1016/j.lwt.2005.02.004.

Berg, J.M., 2002. Biochemistry. In: Lubert Stryer., 5 ed., pp. 319–335 New York.

Dalgleish, D.G., 1998. Casein micelles as colloids: surface structures and stabilities. J. Dairy Sci. 81, 3013–3018. https://doi.org/10.3168/jds.S0022-0302(98)75865-5.

De Kruijf, C., Holt, C., 2003. Casein micelle structure, functions and interactions. In: Advanced Dairy Chemistry—1 Proteins. Springer, New York, pp. 233–276. https://doi.org/10.1007/978-1-4419-8602-3_5.

Dickinson, E., 1993. Towards more natural emulsifiers. Trends Food Sci. Technol. 4, 330–333. https://doi.org/10.1016/0924-2244(93)90103-H.

Dickinson, E., 1999. Adsorbed protein layers at fluid interfaces: interactions, structure and surface rheology. Colloids Surf., B 15, 161–176. https://doi.org/10.1016/S0927-7765(99)00042-9.

Dickinson, E., Golding, M., 1997. Rheology of sodium caseinate stabilized oil-in-water emulsions. J. Colloid Interface Sci. 191 (1), 166–176. https://doi.org/10.1006/jcis.1997.4939.

Furtado, G.F., Mantovani, R.A., Consoli, L., Hubinger, M.D., Cunha, R.L., 2017. Structural and emulsifying properties of sodium caseinate and lactoferrin influenced by ultrasound process. Food Hydrocolloids 63, 178–188. https://doi.org/10.1016/j.foodhyd.2016.08.038.

Goldfeld, M., Malec, A., Podella, C., Rulison, C., 2015. Proteins as surfactant enhancers for environmental and industrial applications. J. Petrol Environ. Biotechnol. 6, 211. https://doi.org/10.4172/2157-7463.1000211.

Gomes, A., Costa, A.L.R., Cunha, R.L., 2018. Cunha Impact of oil type and WPI/Tween 80 ratio at the oil-water interface: adsorption, interfacial rheology and emulsion features. Colloids Surf., B 164, 272–280. https://doi.org/10.1016/j.colsurfb.2018.01.032.

Gradielski, M., Hoffmann, I., 2018. Polyelectrolyte-surfactant complexes (PESCs) composed of oppositely charged components. Curr. Opin. Colloid Interface Sci. 35, 124–141. https://doi.org/10.1016/J.COCS.2018.01.017.

Guzmán, E., Llamas, S., Maestro, A., Fernández-Pena, L., Akanno, A., Miller, R., Ortega, F., Rubio, R.G., 2016. Polymer-surfactant systems in bulk and at fluid interfaces. Adv. Colloid Interface Sci. 233, 38–64. https://doi.org/10.1016/j.cis.2015.11.001.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Paleontol. Electron. 4 art. 4. 9pp.

Jain, N., Trabelsi, S., Guilliet, S., McLoughlin, D., Langevin, D., Letellier, P., turmine, M., 2004. Critical aggregation concentration in mixed solutions of anionic polyelectrolytes and cationic surfactants. Langmuir 20 (20), 8496–8503. https://doi.org/10.1021/la0489918, 2004.

Karbowiak, T., Debeauffort, F., Voiley, A., 2006. Importance of surface tension characterization for food, pharmaceutical and packaging products: a review. Crit. Rev. Food Sci. Nutr. 46 (5), 391–407. https://doi.org/10.1080/1040839059100888.

Mantovani, R.A., Fattori, J., Michelon, M., Cunha, R.L., 2016. Formation and pH-stability of whey protein fibrils in the presence of lecithin. Food Hydrocolloids 60, 288–298. https://doi.org/10.1016/j.foodhyd.2016.03.039.

Mirallo, J.M., Dasta, J.K., Kleinschmidt, K.C., Varon, J., 2010. State of art review: intravenous fat emulsions: current applications, safety profile and clinical implications. Annu. Pharmacother. 44 (4), 688-700. https://doi.org/10.1345/aph.1M626.

Ozturk, Ö., McClements, D.J., 2016. Progress in natural emulsifiers for utilization in food emulsions. Curr. Opin. Food Sci. 7, 1–6.

Perrechil, F.A., Santana, R.C., Lima, D.B., Polastro, M.Z., Cunha, R.L., 2014. Emulsifying properties of Maillard conjugates produced from sodium caseinate and locust bean gum. Braz. J. Chem. Eng. 31, 429–438. https://doi.org/10.1590/0104-6502-20140312-0000228.

Sarode, A.R., Sawale, P.D., Khedkar, C.D., Kalyankar, S.D., Pawshe, R.D., 2016. Casein and caseinate: methods of manufacture. In: Encyclopedia of Food and Health. Academic Press, pp. 676–682. https://doi.org/10.1016/B978-0-12-384947-2.00122-7.

Somasundaran, P., 2006. Encyclopedia of Surface and Colloid Science, 2 ed. Taylor & Francis, New York.

Whitehurst, R.J., 2004. Emulsifiers in Food Technology. Blackwell Publishing Ltd. Online, ISBN 9780470995747. https://doi.org/10.1002/9780470995747.

Woodward, N.C., Gunning, A.P., Mackie, A.R., Wilde, P.J., Morris, V.J., 2009. Comparison of the orogenic displacement of sodium caseinate with the caseins from the air-water interface by nonionic surfactants. Langmuir 25 (12), 6739–6744. https://doi.org/10.1021/la902414q.