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On the cohesive properties of casein micelles in dense systems

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ABSTRACT

Milk casein micelles are natural colloids that behave as microgel particles. When concentrated, those particles form cohesive gels, the properties of which are still largely unknown. With this work, the objective is to bring new information about the origin of gel cohesion in such a packing of soft colloidal objects. The experimental approach is based on the following of the reswelling/redispersion behavior of concentrated gels prepared through osmotic stress under variable controlled conditions (compression degree, compression route, and duration) and subsequently immersed in a native solvent. The essential result is that gel cohesion strongly depends on the initial deformation of the casein micelles within the gels. The optimum of gel cohesion if found for intermediate deformations, *i.e.*, when the micelles have lost about half of their original volume. Below that deformation, the contact between neighboring micelles is probably too weak, so that the repulsion/attraction balance is still in favor of inter-micellar brush repulsion forces. Above that deformation, the gel also loses its cohesiveness, either because some hydrophobic inter-micellar connections are lost during the first stage of redispersion (individual micellar reswelling), or because there are simply less cohesive bonds at such high compression levels.

1. Introduction

Operations that lead to highly concentrated dispersions of a (bio-)colloidal object are now commonly encountered in chemical and food industries. It is typically the case in the dairy industry where milk is most of the time used in its concentrated form: the large-scale manufacture of cheese often requires concentration using filtration operations, while the manufacture of spray dried products such as skimmed milk powder requires evaporative concentration of milk up to 40–50% total solids prior to spray drying. In milk, the colloidal part is composed of so-called casein "micelles", sort of microgels of 50–300 nm in diameter. When milk is concentrated, the presence of those micelles has a crucial impact on the macroscopic and functional properties of the obtained products (Liu, Dunstan, & Martin, 2012). However, information is still scarce about how the casein micelles behave and interact in such concentrated regimes (Bouchoux, Cayemitte, Jardin, Gésan-Guiziou, & Cabane, 2009; Bouchoux et al., 2012, 2009; Dahbi, Alexander, Trappe, Dhont, & Schurtenberger, 2010; Dalgleish & Corredig, 2012; Nair, Alexander, Dalgleish, & Corredig, 2014; Qu, Gésan-Guiziou, & Bouchoux, 2012).

Casein micelles make up to 80% of the protein content of cow milk. They are polydisperse, irregular and roughly spherical macromolecular assemblies made of four distinct caseins (α_{s1}, α_{s2}, β, and κ) and 8% in mass of phosphate and calcium ions (Dalgleish, 2011). They also contain 76% of water in volume, which makes them some kind of natural sponge-like microgels (Bouchoux, Gésan-Guiziou, Pérez, & Cabane, 2010). The casein micelles can be considered as a matrix of proteins in which the ionic nanoclusters of calcium and phosphate act as connecting points for casein assembly (Farrell, Malin, Brown, & Qi, 2006; Horne, 2006). The κ-caseins are located on the surface of casein micelles, their hydrophilic C-terminal regions extend into the aqueous phase as a polyelectrolyte brush which stabilizes the casein micelles through electrostatic and steric repulsions (Dalgleish, 1998; De Kruif & Zhulina, 1996).

Casein micelles are cohesive particles. They can stick together and form a "gel" through various means. In the yoghurt and cheese manufacture the casein micelles are gelled by acidification or addition of rennet, respectively (Lucey, 2002). In milk filtration, there is no change in the physico-chemical characteristics of the micelles environment, and gelation is induced by the high

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concentrations attained at the membrane surface (Bouchoux, Qu, Bacchin, & Gésan-Guiziou, 2014, Gésan-Guiziou, Boyaval, & Daufin, 1999; Jimenez-Lopez et al., 2008; Le Berre & Daufin, 1996; Qu et al., 2012). Regardless of the means that results in gelation, the repulsive barrier provided by the κ-casein polyelectrolyte brush needs to be overcome to initiate the process (Dalgleish, 1998). When this repulsive barrier is somehow altered, the casein micelles experience attractive interactions and gelation occurs. At low pH, *i.e.*, during acid gelation, the polyelectrolyte brush is less charged and smaller in size through partial collapsing, thus reducing the electrostatic and steric repulsive forces (Tuinier & De Kruif, 2002). In rennet gelation, the rennet cleaves the polyelectrolyte brush, thus also reducing inter-micellar repulsions. In filtration, and similarly in osmotic stress experiments, the casein micelles gel at high concentration without altering chemically or enzymatically the κ-casein brush (Bouchoux et al., 2009). In that case, the distance between the casein micelles is small enough to "defeat" the κ-casein repulsive barrier, so that attractive forces are favored and gelation is provoked. The authors suppose that the gelation is due to bridging/binding forces between certain cohesive regions located on the surfaces of the micelles (Bouchoux et al., 2010).

The present paper focuses on the interaction forces that are involved in the gelation of dispersions of casein micelles at high concentrations. The objective here is not to understand the exact "nature" of these forces (hydrophobic or electrostatics for instance), like it was done in previous works dedicated to rennet or acid gelation (De Kruif, 1998; De Kruif & Zhulina, 1996; Mellema, Leermakers, & De Kruif, 1999; Niki, Kohyama, Sano, & Nishinari, 1994; Tuinier & De Kruif, 2002). Instead, this paper centers on the *conditions of formation* of these bonds, as well as on their *resistance* or *reversibility* when the concentration process is stopped. Former osmotic pressure or filtration studies only led to indirect and partial indications about these two issues, and those particular problems have never been explored in a systematic way until now. The approach consists first in making gels of casein micelles through osmotic compression in controlled conditions, *i.e.*, compression degree (=osmotic pressure), compression route (=direct or gradual compression), and compression duration. The gel is then placed in an adequate solvent and the extent of gel re-swelling and/or gel re-dispersion is examined. Such experiments aim at addressing the following important questions:

(1) *Redispersion process.* How do the cohesive bonds in the gels of casein micelles evolve when the external force of compression is removed? Do they break? If yes, what is the nature of the redispersed objects? Are they still "casein micelles"?
(2) *Gel cohesion vs. gel formation.* Is there any relations between the way the gels are formed (compression route, degree, and duration) and their auto-cohesion properties? What does it tell about the general properties of the cohesive interactions that "make" the gels?

2. Materials & methods

2.1. Casein micelle dispersions

All dispersions were prepared from Native PhosphoCaseinate powder (NPC) dispersed in a solvent made from ultrafiltration of skimmed milk (UF permeate). In such dispersions, it is commonly admitted that the caseins are organized into casein micelles that are close to their "native" state (Famelart, Lepesant, Gaucheron, Le Graet, & Schuck, 1996). The NPC powder was prepared according to a protocol developed by (Pierre, Fauquant, Le Graët, Piot, & Maubois, 1992) and (Schuck et al., 1994). Its average composition is given in Table 1.

The UF permeate was obtained through membrane ultrafiltration (5000 Da cut-off) of a fresh skimmed milk. The totality of the milk protein fraction, i.e. caseins and whey proteins, is eliminated through this operation. The UF permeate contains the milk minerals, lactose, and a few other low molar mass molecules (Jenness & Koops, 1962), and thus corresponds to the "native" solvent of the casein micelle. Both thiomersal and sodium azide, purchased from Sigma–Aldrich (St. Louis, MO, USA), were added to the UF permeate as preservatives at 0.02% and 0.05% (w/w) respectively.

The casein micelle dispersions (herein called NPC dispersions) were prepared by thoroughly mixing the NPC powder with UF permeate for 15 h at 35 °C. This protocol was shown to be efficient for solubilizing the powder in totality (Gaiani et al., 2006).

2.2. Making the gel

The gels were prepared using osmotic stress, a concentration technique that is based on water exchange between the sample, placed in a dialysis bag, and a reservoir of known osmotic pressure (Bouchoux, Cayemitte, et al., 2009; Parsegian, Rand, Fuller, & Rau, 1986). Standard regenerated cellulose Spectra/Por 2 dialysis bags with a molecular mass cutoff of 12,000–14,000 Da were used (Spectrum Laboratories, Rancho Dominguez, CA). The stressing solutions were prepared by diluting poly(ethylene glycol) (PEG) in UF permeate. Two PEG were used, both purchased from Fluka (Buchs, Switzerland). A polymer with a molar mass of 35,000 Da was used for the osmotic stress experiments at pressures under 5 bar; which corresponds to a casein concentration of the sample <500 g/L (Bouchoux, Cayemitte, et al., 2009). This size of the PEG was chosen for avoiding the permeation of the polymer molecules through the dialysis bags. For pressures higher than 5 bar, PEG 35,000 could not be used for a question of solubility (5 bar corresponds to 20% (w/w) of PEG 35,000, which is almost the saturation concentration). In that case, PEG 20,000 was chosen as its solubility goes up to 50% (w/w), which corresponds to a maximum osmotic pressure of ~100 bar. It is theoretically possible that some of this smaller PEG passes through the 12,000–14,000 Da dialysis bags through reptation and migrates from the stressing solution to the stressed casein dispersions during the compression process. However, such a phenomenon can be neglected due to the rapid gel formation at such high compression levels, and to the consecutive low diffusion of PEG into the gel. The relationship between osmotic pressure Π (bar) and PEG concentration [PEG] (%, w/w) are:

$$\mathrm{Log}(\Pi \times 10^5) = a + b\,[\mathrm{PEG}]^c \quad (1)$$

with $a = 0.49$, $b = 2.5$ and $c = 0.24$ for PEG 35,000 (Bouchoux, Cayemitte, et al., 2009), and $a = 0.57$, $b = 2.75$ and $c = 0.21$ for PEG 20,000 (lpsb.nichd.nih.gov, 2013).

Liquid casein micelles dispersions of moderate concentration (120 g/L of caseins) were placed in dialysis bags and immersed in the PEG stressing solutions kept at 20 °C. In order to obtain a

Table 1
Composition of NPC powder.

	TS (%, w/w)	Minerals (% TS)	Caseins (% TS)	Noncasein nitrogen matter (% TS)	Nonprotein nitrogen matter (% TS)
NPC	91.0	8.5	85.6	4.6	0.6

All values are given as averages ± 0.2%. NPC: Native PhosphoCaseinate, TS: total solid. Nitrogen content was measured through the Kjeldahl method and was converted into equivalent nitrogen matter through multiplication by a conversion factor of 6.38 (FIL-IDF, 1993).