Food Research International xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

New insights about flocculation process in sodium caseinate-stabilized emulsions

Cristián Huck-Iriart [a], Juan Montes-de-Oca-Ávalos [b,c], María Lidia Herrera [b], Roberto Jorge Candal [a,d,*], Cristiano Luis Pinto-de-Oliveira [e], Iris Linares-Torriani [f]

[a] Escuela de Ciencia y Tecnología, CONICET, Universidad Nacional de San Martín (UNSAM), Campus Miguelete, 25 de Mayo y Francia, 1650 San Martín, Provincia de Buenos Aires, Argentina
[b] Instituto de Tecnología en Polímeros y Nanotecnología (ITPN), Universidad de Buenos Aires (UBA), CONICET, Facultad de Ingeniería, Las Heras 2214, C1127AAQ Buenos Aires, Argentina
[c] Departamento de Química Inorgánica, Analítica y Química Física (DQIAQF), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, Piso 3, C1428EHA Buenos Aires, Argentina
[d] Instituto de Investigación e Ingeniería Ambiental, CONICET, Universidad Nacional de San Martín (UNSAM), Campus Miguelete, 25 de Mayo y Francia, 1650 San Martín, Provincia de Buenos Aires, Argentina
[e] Instituto de Física, Universidade de Sao Paulo, Sao Paulo, SP, Brazil
[f] Instituto de Física "Gleb Wataghin", Universidade Estadual de Campinas, Campinas, SP, Brazil

ARTICLE INFO

Article history:
Received 5 June 2016
Received in revised form 13 August 2016
Accepted 20 August 2016
Available online xxxx

Keywords:
Sodium caseinate
Disaccharides
Emulsions
Flocculation
SAXS
Turbiscan

ABSTRACT

Flocculation process was studied in emulsions formulated with 10 wt.% sunflower oil, 2, 5 or 7.5 wt.% NaCas, and with or without addition of sucrose (0, 5, 10, 15, 20 or 30 wt.%). Two different processing conditions were used to prepare emulsions: ultraturrax homogenization or further homogenization by ultrasound. Emulsions with droplets with diameters above (coarse) or below (fine) 1 μm were obtained. Emulsions were analyzed for droplet size distribution by static light scattering (SLS), stability by Turbiscan, and structure by confocal laser scanning microscopy (CLSM) and small angle X-ray scattering (SAXS). SAXS data were fitted by a theoretical model that considered a system composed of poly dispersed spheres with repulsive interaction and presence of aggregates. Flocculation behavior was caused by the self-assembly properties of NaCas, but the process was more closely related to interfacial protein content than micelles concentration in the aqueous phase. The results indicated that casein aggregation was strongly affected by disaccharide addition, hydrophobic interaction of the emulsion droplets, and interactions among interfacial protein molecules. The structural changes detected in the protein micelles in different environments allowed understanding the macroscopic physical behavior observed in concentrated NaCas emulsions.

1. Introduction

Caseins are a family of unstructured amphiphilic milk proteins known as α_{S1}, α_{S2}, β and κ, which have the tendency to naturally form complex aggregates with reported average sizes of up to 300 nm (Livney, 2010). Sodium caseinate (NaCas) results from the removal of calcium phosphate and is widely used in pharmaceutical and food industries as an emulsion stabilizer and foam formation agent. NaCas exists in aqueous solution at neutral pH as a soluble mixture of casein monomers and self-assembled protein aggregates of sizes around 10–20 nm in diameter and molecular weight of ~2.5×10^5 Da, also called nanoparticles in literature (Dickinson, 2006).

Among other factors, the extent of aggregation of NaCas is affected by the presence in the aqueous phase of compounds such as disaccharides. The effect of sucrose on emulsion physical behavior was reported to be strongly dependent on the pH. At neutral pH light scattering studies indicated a reduction in aggregation but at pH close to the isoelectric point (Ip = 4.5) an increase on the radius of gyration was reported (Belyakova et al., 2003; Dickinson, 2006; Ruis, van Gruijthuijsen, Venema, & van der Linden, 2007). These results indicated that sucrose has an important effect on this system and therefore it is also of great interest to investigate how disaccharides modify the structure of sodium caseinate stabilized emulsions.

In order to obtain unbiased results when studying emulsion stability as well as structural properties, the experimental techniques applied have to be as little invasive as possible. Scattering methods are a good example of such techniques and can provide important information on the structural and dynamical properties of heterogeneous fluids

* Corresponding author at: Escuela de Ciencia y Tecnología, CONICET, Universidad Nacional de San Martín (UNSAM), Campus Miguelete, 25 de Mayo y Francia, 1650 San Martín, Provincia de Buenos Aires, Argentina.
E-mail addresses: chinorgchem@gmail.com (C. Huck-Iriart), jmontesdeocaav@gmail.com (J. Montes-de-Oca-Ávalos), mlidiaherrera@gmail.com (M.L. Herrera), rjcandal@gmail.com (R.J. Candal), crislpo@if.usp.br (C.L. Pinto-de-Oliveira), torriani@ifi.unicamp.br (I. Linares-Torriani).

http://dx.doi.org/10.1016/j.foodres.2016.08.026
0963-9969/

(Alexander & Dalgleish, 2006). However, some of those techniques require a substantial dilution of the samples. This dilution disrupts emulsion structures modifying the actual system. Therefore, the ability to study the stability of food emulsions in their undiluted forms may reveal subtle correlations affecting emulsions physical changes. A relatively recently developed technique, the Turbiscan method, allows registration of the turbidity profile of an emulsion along the height of a glass tube filled with the emulsion. The analysis of the turbidity profiles with time leads to quantitative data on the stability of the studied emulsions and allows making objective comparisons between different emulsions (Mengual, Meunier, Cayre, Puech, & Snabre, 1999a). Food emulsions that can be described by the Turbiscan method have droplet sizes typically between 0.1 and 100 μm (Huck-Iriart, Álvarez-Cerimedo, Candal, & Herrera, 2011; Huck-Iriart, Pinzones Ruiz-Henestrosa, Candal, & Herrera, 2013). Small Angle X-Ray Scattering (SAXS) is a non-invasive technique generally used in materials and life science. Due to the short wavelength of X-Rays, this technique provides structural information on colloidal particles and complex fluids in the 1–100 nm size range. Combining both techniques, Turbiscan and SAXS, experimental data may be obtained in a wide range of sizes.

For the correct analysis and interpretation of the SAXS data, a possible approach is to assume a certain model for the system, based on previous knowledge, and compare it with the measured data using least square methods (Pedersen, 1997). In the past, simple models were used to describe milk casein in dilute solution (Holt, Kruif, Tuinier, & Timmis, 2003) and some important structural information was reported. However, in emulsions system, the detailed nature of the molecular interactions involved in the casein micelles formation and the arrangement of the proteins in the casein micellar aggregates is still not completely established. Notwithstanding, the existing data serves as a basis to propose a working micellar model that describes the structure of more complex systems, such as NaCas oil-in-water (O/W) emulsions.

Emulsions are thermodynamically unstable liquid-liquid dispersions. The common strategies to kinetically stabilize these systems involve modification of the liquid-liquid surface tension, the droplet size and the viscosity of the continuum phase (Ivanov & Kralchevsky, 1997). These strategies are not independent since a complex interaction between proteins, surfactants, additives and liquid phases might occur (Jourdain, Schmitt, Leser, Murray, & Dickinson, 2009; Woodward, Gunning, Mackie, Wilde, & Morris, 2009). Stability of sodium caseinate emulsions has been widely investigated (Dickinson, Golding, & Povey, 1997; Hemar et al., 2003; Belyakova et al., 2003; McClements, 2004), to name a few. Flocculation and creaming were the most frequent macroscopic manifestations reported. According to the literature, flocculation occurred above some critical concentration of stabilizing polymer and was sensitive to its concentration. Flocculation was especially related to the excess of unadsorbed protein (Dickinson et al., 1997; Dickinson & Golding, 1997). Although flocculation mechanism was widely investigated, recent studies using non disturbing techniques showed that flocculation process was more complex than reported in literature (Álvarez-Cerimedo, Huck-Iriart, & Herrera, 2010; Huck-Iriart et al., 2013). The results reported in those studies suggest that in sodium caseinate-stabilized emulsions, flocculation is a process that remains fairly poorly understood.

The aim of the present work was to further study flocculation process at the micro and nanoscales from a few nanometers up to hundreds of microns.

2. Materials and methods

2.1. Materials

α,α-Trehalose dihydrate and sucrose from Sigma (Sigma–Aldrich, St. Louis, Mo., USA) were used without any further purification. HPLC water was used for all experimental work. Sodium caseinate (NaCas) was obtained from ICN (ICN Biomedical, Inc., Aurora, Ohio, USA) and used without any further purification. The oil phase was commercial sunflower seed oil (SFO) which main fatty acids were identified as C16:0, C18:0, C18:1, and C18:2 with percentages of 6.7%, 3.6%, 21.9%, and 66.3%, respectively.

2.2. Emulsion preparation

Aqueous phase may contain no sucrose or 5, 10, 15, 20, or 30 wt.% sucrose added to the continuous phase. Oil phase was commercial SFO and in all emulsions represented 10 wt.%. NaCas was used as emulsifier at 2.0, 5.0, and 7.5 wt.% in the aqueous phase. Oil and aqueous phases were mixed using an Ultra-Turrax (UT) T8 high speed blender (S 8 N-5G dispersing tool, IKA Labortechnik, Janke & Kunkel, GmbH & Co., Staufen, Germany), operated at 20,000 rpm for 1 min. Coarse emulsions (UT) were obtained after repeating this process three times. The resultant coarse emulsions were further homogenized for 20 min using an ultrasonic liquid processing (US), VIBRA CELL, VCX model (Sonics & Materials, Inc., Newtown, CT, USA). The temperature of the sample-cell was controlled by means of a water bath set at 15 °C with a temperature cut down control of 40 ± 1 °C during ultrasound treatment. After ultrasound treatment fine emulsions were obtained (UT + US). Then, the samples were cooled quiescently to ambient temperature (22.5 °C). Subsequently they were analyzed for particle size distribution, stability in quiescent conditions and microstructure. The pHs of the SFO emulsions were 6.66 ± 0.05, close to 7. No buffer was added to the emulsions. Experiments were done in duplicate and results were averaged.

2.3. Particle size analysis

The particle size distribution of the emulsions was determined immediately after emulsion preparation by static light scattering (SLS) using a Mastersizer 2000 with a Hydro 2000MU as dispersion unit (Malvern Instruments Ltd., UK). The pump speed was settled at 1800 RPM. Refraction index for the oil phase was 1.4694. Determinations were conducted in duplicate and values of standard deviations were <0.2 μm. Distributions were expressed as differential volume. One of the parameters selected to characterize distributions was the volume-weighted mean diameter ($D_{4,3}$) since it has been reported that it is more sensitive to fat droplet aggregation than Sauter mean diameter ($D_{3,2}$) (Relkin & Sourdet, 2005). Other parameters selected to describe the distribution were distribution width (W) and volume percentage of particles exceeding 1 μm in diameter ($\%V_{d>1}$) (Thanasukarn, Pongsawatmanit, & McClements, 2006).

2.4. Emulsion stability

The stability of the emulsions was analyzed using a vertical scan analyzer Turbiscan MA 2000 (Formulaction, Toulouse, France). This equipment allows the optical characterization of any type of dispersion (Mengual, Meunier, Cayre, Puech, & Snabre, 1999b). A scheme of the equipment was reported in Pan, Tomás, and Añón (2002). The samples were put in a flat-bottomed cylindrical glass measurement cell and scanned from the bottom to the top. The backscattering (BS) and transmission (T) profiles as a function of the sample height (total height = 60 mm) were studied in quiescent conditions at 22.5 °C. The mechanism making the dispersion unstable was deduced from the transmission or the backscattering data. Measurements of the emulsions were performed immediately after preparation and at different time intervals: 2 min per step over the period of 30 min for course emulsions (Ultra Turrax, UT), and twice a day during a week for fine emulsions (UT + Ultrasound, US).

Creaming was detected using the Turbiscan as it induced a variation of the concentration between the top and the bottom of the cell. The curves obtained by plotting ΔBS vs. tube length (where ΔBS is calculated by subtracting the BS profile at t = 0 from the profile at t = t_i, $\Delta BS = BS_{ti} - BS_0$, in the so called reference mode), displayed a typical shape

Please cite this article as: Huck-Iriart, C., et al., New insights about flocculation process in sodium caseinate-stabilized emulsions, *Food Research International* (2016), http://dx.doi.org/10.1016/j.foodres.2016.08.026