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Rheology of caseinate fractions in relation to their water holding capacity

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ABSTRACT

In this paper we relate rheological properties of whole caseinate and various caseinate fractions to the improved water holding capacity of caseinate gels on which we reported previously. In that separate paper it was found that α_s-casein (ASCN) enriched sodium caseinate (NaCN) fractions gave a substantially better water holding capacity. We hypothesized that this was due to the 'telechelic' character of ASCN. Here we systematically investigated the low shear rheology and viscoelastic properties as a function of concentration, salt content, pH and temperature. It was found that the ASCN enriched fraction had a considerably lower overlap concentration. The overlap concentration depends on the 'bulkiness' of a polymer but also on the interaction. By considering ASCN as a telechelic (two hydrophobic parts connected by a middle hydrophilic part) poly-electrolyte molecule it is understandable that ASCN has a considerably lower overlap concentration (0.058 w/w) than NaCN or β-casein, which both have an overlap concentration greater than 0.1 w/w. We tested the telechelic vis a vis the overlap concentration hypothesis by mildly cross linking NaCN and ASCN depleted NaCN using transglutaminase. Viscosity was increased by a factor 1.5 to 2 and found a lower overlap concentration by almost a factor of two. In a separate paper we also found that the water holding capacity of a caseinate gel depends directly on the cross linking of the gel, which may be physical e.g. by a telechelic ASCN, or chemical, though enzymatic cross linking.

1. Introduction

The casein protein matrix in a cheese product is responsible for its structure, texture and cooking properties. Although fat plays a role as well, it is mainly in the flavour of cheese. In a previous paper it was found that water holding capacity of casein(ate) fractions depends on the interactions and cross linking of the caseins (de Kruif, Anema, Zhu, Havea, & Coker, 2015). The working hypothesis of this paper is that the rheology of the different casein(ate) fractions is related to their previously reported water holding capacity.

The rheology of a polymer dispersion depends on the end to end distance of a polymer and the interaction between the polymers. For this investigation we used an α_{S1}-casein enriched (ASCN-RICH), β-casein (BCN) and the α_{S1}-casein-depleted caseinate (ASCN-DEPLETE). The rationale behind this is that α_{S1}-casein (ASCN) is different from BCN in that ASCN can be considered as a tri-block poly-electrolyte polymer with a hydrophilic block in between two more hydrophobic blocks (Alaimo, Wickham, & Farrell, 1999; Creamer & Harris, 1997; Holt & Sawyer, 1993; Payens & Schmidt, 1966; Swaisgood, 1992). Such telechelic molecules are responsible for a very specific rheological behaviour and are used extensively in (paint)industry (Annable, Buscall, Ettelaie, & Whittlestone, 1993).

In polymer science, a molecule like ASCN is called a telechelic molecule meaning that the two end capping hydrophobic parts may bind to different objects or may associate with neighbouring micelles. Telechelic molecules are used in stabilizing dispersions and notably paint. BCN is a di-block polymer with a hydrophilic and hydrophobic section. As a result BCN forms (soap like) micelles with a hydrophobic core and a hydrophilic corona. BCN forms 15 nm diameter micelles at temperatures above 20 °C that can be considered as a hard sphere dispersion (Kegeles, 1979; de Kruif & May, 1991; O'Connell, Grinberg, & de Kruif, 2003; Panouille,

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Durand, & Nicolai, 2005). Mikheeva, Grinberg, Grinberg, Khokhlov, and de Kruif (2003) review the micellisation of BCN and present extensive thermodynamic data. The difference in character between ASCN and BCN will be noticeable in low shear viscosity experiments and may be observed in light scattering and dynamic rheology experiments. Also studies and scaling theory indicate a difference in behaviour, notably in the scaling of rheological data by C^*: that is the overlap concentration. The overlap concentration is related to the concentration where polymers with an unperturbed radius of gyration (Rg) start to overlap. Therefore for homopolymers C^* is proportional to $1/[\eta]$, where $[\eta]$ is the intrinsic viscosity (Graessley, 1980). In θ-solvents $C^* \approx 2/[\eta]$ and for poor solvents $C^* \approx 4/[\eta]$ (Takahashi, Isono, Noda, & Nagasawa, 1985). The intrinsic viscosity is defined by $[\eta] = \eta_r/C$, thus $\eta_r = 1 + C[\eta]$ where η_r is the relative viscosity (at concentrations approaching zero).

In practice a plot of viscosity against concentration shows a clear change in 'slope' at the transition from the dilute to semi-dilute state and this (extrapolated) transition point is considered as the overlap concentration. It is hypothesized that a 'telechelic' ASCN will have a lower C^* than BCN or NaCN. It is for that reason we present the results of a number of rheology and light scattering experiments.

2. Materials and methods

2.1. Sodium caseinate, BCN, ASCN-RICH and ASCN_DEPLETE caseinate samples

Sodium caseinate (NaCN) was obtained from the Fonterra Cooperative Group, New Zealand. BCN was produced by a previously described method (Huppertz et al., 2006; Ram, Loh, Love, & Elston, 1994). The ASCN-RICH and ASCN-DEPLETE casein samples are complementary fractions and were gifts from Satyendra Ram (Fonterra Cooperative Group). The protein content of the ASCN-RICH sample was about 70% α_{s1}-casein, 11% BCN with the remainder being predominantly α_{s2}-casein with only a trace of κ-casein. The protein content of the ASCN-DEPLETE samples was about 28% α_{s1}-casein, 54% BCN with the remainder being predominantly κ-casein with only a trace of α_{s2}-casein. The protein content of the BCN sample was >90% BCN, with the remainder being a predominantly α_{s1}-casein, and only a trace of α_{s2}-casein. The BCN, ASCN-RICH and ASCN-DEPLETE casein products were converted to caseinates by preparing dispersions in deionised water at the desired concentrations and adjusting these solutions to pH 6.5 with 3 M NaOH. Subsequent pH adjustments, if required, were done by the slow addition of 1 M NaOH or 1 M HCl.

2.2. Transglutaminase cross-linking of casein suspensions

Transglutaminase (TGASE, 1% active TGA preparation, Activa YG, Ajinomoto Group, Tokyo, Japan) was used to cross-link the casein samples. BCN and ASCN-DEPLETE solutions (10% w/w) were prepared and the pH was adjusted to 6.5. A small amount of sodium azide (0.02% w/w) was added as preservative. The samples were stirred for ~12 h. Subsamples of 100 ml were taken and TGASE (0.16 g/100 mL) was added under continuous stirring. The samples were incubated at different temperatures (ambient or 2.5 °C, 30 °C, 40 °C and 50 °C).

2.3. Rheological measurements

The rheological measurements were made using a Paar MCR301 stress-controlled rheometer equipped with a cup (diameter 28.929 mm) and bob (diameter: 26.673 mm, length: 39.997 mm) system (Anton Paar, Graz, Austria). Measurements were made in the linear regime for the oscillatory measurements, while shear data were measured between 0.1 and 100 s^{-1}.

2.4. Dynamic light scattering

Dynamic light scattering (DLS) was performed using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, Malvern, Worcestershire, U.K.) as has been described previously (Anema & Li, 2003).

3. Results and discussion

3.1. Relative viscosity of NaCN

The relative viscosity (dispersion viscosity divided by solvent viscosity) of NaCN was measured as function of concentration and the results are shown in Fig. 1. We observed that a semi-log plot gives two linear regimes. The intersection of these linear regimes was designated as C^*, the so-called overlap concentration. Takahashi et al. (1985) defined the overlap concentration as *'the critical concentration at which polymer coils begin to overlap each other'*. Experimentally it is characterized by a more or less abrupt change in the slope of the viscosity versus concentration curve. The value of C^* depends on molecular mass and the expansion factor of the polymer, which is a measure of the end to end distance of a polymer.

We also plotted data of Farrer and Lips (1999), with the ordinate values multiplied by 10 for clarity. Very extensive and detailed studies of caseinate micelles under various conditions and using rheological and light scattering methods were made by the group of Nicolai et al. in Le Mans, France (Panouille, Benyahia, Durand, & Nicolai, 2005; Pitkowski, Durand, & Nicolai, 2008; Panouille, Durand, Nicolai, Larquet, & Boisset, 2005; Panouille, Nicolai, & Durand, 2004; Pitkowski, Nicolai, & Durand, 2007; Pitkowski, Nicolai, & Durand, 2008a, 2008b; Thomar, Benyahia, Durand, & Nicolai, 2014; Thomar, Durand, Benyahia, & Nicolai, 2012). The original data from Nicolai's group (kindly supplied by Taco Nicolai,

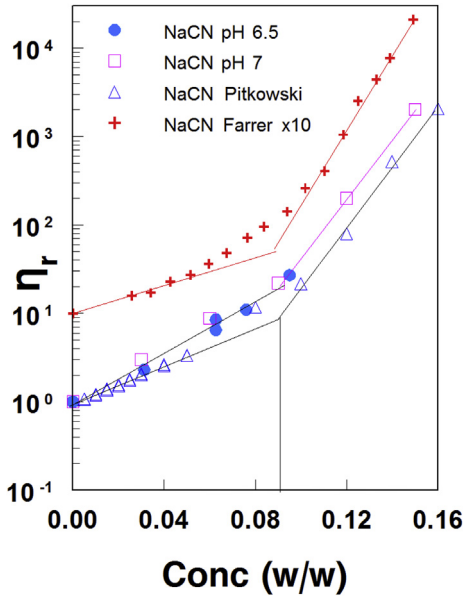

Fig. 1. Relative concentration of sodium caseinate as a function of concentration. Overlap concentration $C^* = 0.088–0.091$ w/w.