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The interaction of casein micelles with κ-carrageenan studied by diffusing wave spectroscopy

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Abstract

The light-scattering properties of mixtures of skim milk and κ-carrageenan in the concentration range 0–0.1% w/v have been studied in detail. The use of diffusing wave spectroscopy (DWS) permitted the study of undiluted mixtures of skim milk and polysaccharide. The results appear to show that the movement of the casein micelles in the mixtures at 25 °C depends on the formation of carrageenan/casein gels, but that within the gels, the casein micelles themselves have considerable mobility. Different methods of mixing milk and carrageenan did not affect the overall conclusions, although the errors of measurement depended on the methods of mixing. Heat treatment of the milk to denature the whey proteins appeared to have no effect on the interactions of the particles and polysaccharides.

Keywords: Casein micelles; κ-carrageenan; Protein-polysaccharide interactions; Gelation

1. Introduction

The stabilization against phase separation or particle sedimentation of food products is an extremely important aspect in the study of physico-chemical properties of food dispersions. Plant and algal polysaccharides such as carrageenans have long been used as gelling agents, thickeners and stabilizers in milk products such as ice cream or chocolate milk (de Vries, 2002; Grindrod & Nickerson, 1986; Xu, Stanley, Goff, Davidson, & Le Maguer, 1992) although their mode of action is still a matter of some debate.

Milk is a suspension of casein micelles dispersed in serum. These particles are large protein complexes containing about up to 80% of the protein in the milk and taking up about 10% of the volume. They are colloidal particles with mean radii of about 100 nm that are at least quasi-stable under a wide range of temperatures and pH values above 5.5 (Holt, 1992). An important part of the stability is provided by the covering of the surface by the κ-casein fraction of the protein, which protrudes its hydrophilic C-terminal macropeptide moieties into solution to confer steric and electrostatic stability on the particles (de Kruif & Holt, 2003). Carrageenans are algal polysaccharides extracted from red seaweed; there are three main types, differing in the number and position of the sulfated groups on the galactose/anhydrogalactose chain. The κ, ι and λ-carrageenans contain one, two and three sulfate groups, respectively, per disaccharide repeating unit. During heating and under specific ionic conditions (Chronakis, Doublier, & Piculell, 2000), ι- and κ-carrageenans undergo a reversible helix (ordered)–coil (disordered) transition while λ-carrageenan always adopts a coil conformation and is unable to form gels. In their helical conformations, ι and κ-carrageenan can self-associate to form gels. The temperature at which the helix–coil transition occurs depends on the conditions, especially on the concentrations of K^+ and Ca^{2+} ions (Doyle, Giannouli, Philip, & Morris, 2000).

Addition of κ-carrageenan to milk is believed to cause interactions between the casein micelles and the polysaccharide under specific conditions of temperature, ionic strength and concentration (Hemar, Hall, Munro, & Singh, 2002; Mleko, Li-Chan, & Pikus, 1997; Puvanenthiran, Goddard, McKinnon, & Augustin, 2003). Although the casein micelles and the κ-carrageenan both carry a net

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negative charge, the interactions between them have been suggested to occur via localized electrostatic attractions between the sulfated groups of the κ-carrageenan and a very short positively charged region of the κ-casein (Snoeren, Payens, Jeunink, & Both, 1975). Increases in the diameters of casein micelles in the presence of κ-carrageenan have been interpreted as being a result of the binding of the polysaccharide to the micelles (Spagnuolo, Dalgleish, Goff, & Morris, 2005), and direct evidence for the formation of micelle/polysaccharide networks has been demonstrated by electron microscopy (Martin, Goff, Smith, & Dalgleish, 2006; Spagnuolo et al., 2005). In addition to these direct interactions, the κ-carrageenan may also induce stabilization in milk systems by forming a weak gel which prevents the casein micelles from diffusing freely (Bourriot, Garnier, & Doublier, 1999).

Under given ionic strength conditions and protein concentrations, and at high κ-carrageenan concentrations (above 0.2%) and at temperatures above the helix–coil transition, depletion flocculation occurs and the casein micelles sediment from the milk, whereas below the transition temperature the milk/carrageenan mixture forms a gel (Langendorff, Cuvelier, Launay, & Parker, 1997). Using small-deformation dynamic rheology, it has also been shown that at high polysaccharide concentrations the amount of protein in solution does not have a marked effect on the gelling capabilities of the milk, but when the κ-carrageenan concentration is below 0.018%, gelling is hampered, possibly by interactions of the casein micelles with the small numbers of molecules of carrageenan available in solution, thereby interfering with the ability of the carrageenan to form a gel (Thaiudom & Goff, 2003; Vega, Andrew, & Goff, 2004).

When milk is heated, the whey proteins denature and interact with the casein micelles to give a mixture of soluble whey protein/κ-casein complexes, and micelles with quantities of whey proteins attached to them (Guyomarc'h, Law, & Dalgleish, 2003; Vasbinder, van Mil, Bot, & de Kruif, 2001). This change in the composition and possibly the structure of the casein micelles suggests that the properties of milk–carrageenan mixtures may be affected by the order of heating and carrageenan addition. However, Tziboula and Horne (1999) studied the influence of whey protein denaturation in milk on κ-carrageenan gelling capabilities, and concluded that it did not greatly affect the κ-carrageenan gelation; conversely, the κ-carrageenan present in solution during heat treatment of the milk did not influence the rate of whey protein denaturation.

This paper describes a study of the milk-κ-carrageenan system using the light-scattering technique of diffusing wave spectroscopy (DWS), which is well adapted to the study of undiluted turbid suspensions. The study basically concentrated on measuring the mobility and size of the casein micelles, at different concentrations of carrageenan, and also studied the influence of mixing conditions and prior denaturation of the whey proteins on the behavior of the polysaccharide–protein system.

2. Principles of DWS

Light scattering has been used for many years in the study of colloidal systems. However, it requires that any scattered photon undergoes only one scattering event before detection (i.e., they must be in the single scattering regime). This has limited the use of the technique to samples that are highly diluted, and this has generally precluded the study of interparticle interactions. In recent years, a variant of light scattering, DWS has been gaining acceptance as a method for studying highly turbid media such as milk (Alexander & Dalgleish, 2004; Hemar, Singh, & Horne, 2004). The basic principle of DWS is that of dynamic light scattering; however, the path of the photon of light in DWS is treated as a random walk through the sample. In such a case, the temporal autocorrelation function can be written as (Weitz & Pine, 1993)

$$g_{(1)}(t) \approx \frac{\left(\frac{L}{l^*}+\frac{4}{3}\right)\sqrt{\frac{6t}{\tau}}}{\left(1+\frac{8t}{3\tau}\right)\sinh\left[\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right]+\frac{4}{3}\sqrt{\frac{6t}{\tau}}\cosh\left[\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right]}, \qquad (1)$$

where $\tau = (Dk_0^2)^{-1}$, in which D is the particle diffusion coefficient, $k_0 = 2\pi n/\lambda$ is the wave vector of the light, with n the refractive index of the medium and L the thickness of the sample being measured. This correlation function is only valid when the thickness of the sample $L \gg l^*$ (i.e., $L/l^* > 10$) and $t \ll \tau$. The parameter l^* is the photon transport mean free path, and can be defined as the length scale over which the direction of the scattered light has been completely randomized; it is essentially a turbidity parameter and is directly related to the total scattered intensity of the system (Weitz & Pine, 1993). It depends on the sizes and concentrations of the particles and the indices of refraction of the particles and the dispersion medium. For completely non-interacting scatterers, only the physical characteristics of the scatterers determine the scattering behavior. In concentrated and turbid suspensions, however, the spatial positions of the particles may become significant, and the scattering profile depends not only on the physical properties of the colloids but on the interactions between them. These correlations in position affect the angular distribution of the scattered light and hence the turbidity and l^*. Therefore, changes in this parameter can be taken as indications of changing organization within the suspension, such as gelation and crystallization.

In suspensions where the particles are freely diffusing and spherical, the value of the diffusion coefficient (D) obtained from the DWS measurement can be used to calculate the apparent particle radius using the Stokes–Einstein equation. However, in gelling suspensions this procedure is not valid. A further parameter obtainable from the DWS measurement is the mean square displacement (MSD) of the particles. This can be calculated from the correlation time τ obtained with DWS via Eq. (1) above