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Microstructural evolution of viscoelastic emulsions stabilised by sodium caseinate and xanthan gum

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Abstract

The time-dependent evolution of the phase-separated microstructure of a caseinate-stabilised emulsion containing xanthan gum added before emulsification has been investigated by confocal laser scanning microscopy, image analysis and rheology. Moderately low levels of xanthan addition lead to depletion flocculation and gravity-induced phase separation. Increasing the polysaccharide concentration causes immobilisation of the microstructure due to an increase in the local viscoelasticity: that is, the emulsion structure cannot easily rearrange to expel xanthan-enriched aqueous serum phase because a weak gel-like network is generated. The effect of xanthan on the evolving microstructure of phase-separated regions, which reflects indirectly the local emulsion micro-rheology, has been estimated from image analysis of time sequences of confocal micrographs. A comparison has been made between object shape analysis using four different shape descriptors. The roundness parameter has been found to be a convenient descriptor for reliably quantifying the structural change in terms of the relaxation rate of xanthan-rich aqueous drops. The Taylor parameter has been used to link the kinetics of drop relaxation to the time-dependent small-deformation rheological behaviour. The analysis of the combined experimental data reveals the difficulty of relating the evolving microstructure to bulk rheological measurements.

Keywords: Confocal scanning laser microscopy; Image analysis; Phase separation; Depletion flocculation; Sodium caseinate; Xanthan gum; Emulsion rheology

1. Introduction

Proteins and polysaccharides are the two most important functional biopolymers used as ingredients in food emulsions [1]. Together they have the ability to control the texture, structure, and stability of dairy emulsions.

Sodium caseinate is widely used as an emulsifying agent in many dairy products, and it imparts stability to emulsions by a combination of steric and electrostatic mechanisms [2]. However, despite excellent coalescence stability above a certain critical protein concentration, caseinate-based emulsions can exhibit pronounced creaming or serum separation due to depletion flocculation induced by excess unadsorbed protein in the aqueous continuous phase [3,4].

Polysaccharides are predominantly hydrophilic and therefore are not particularly surface-active [1]. Hence, they are usually added to the aqueous phase of emulsions as thickening agents in order to modify the rheological behaviour of the aqueous phase and thereby to retard instability mechanisms. However, under specific conditions, they can cause phase separation and flocculation [1,5]. Xanthan gum is one such example; it is an extracellular high-molecular-weight anionic polysaccharide produced by the microorganism *Xanthomonas campestris*. The structure and conformation of xanthan explain many of its unique solution properties [6,7]. It has been found [6–9] that a xanthan solution has pseudoplastic flow properties even at low concentrations. The large molecules of xanthan form aggregates through hydrogen bonding and polymer entanglement, resulting in a high 'Newtonian' viscosity at low shear-rate. However, under the influence of a high shear-rate, the viscosity of the

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xanthan solution decreases, with the disentanglement of the network and the (partial) alignment of the individual macromolecules in the direction of the shear, resulting in a low 'Newtonian' viscosity region at high shear-rate. Upon the removal of shear, the initial viscosity is recovered almost instantaneously [6,7]. These rheological features make xanthan a suitable thickener for many food products.

Thermodynamic incompatibility [10,11] and isotropic/anisotropic phase separation [12], as well as depletion flocculation [13–19], are all possible phenomena potentially leading to phase separation in emulsions containing xanthan. Phase separation (and associated depletion flocculation) can readily occur in a binary mixture of isotropic particles (such as spherical oil droplets coated with adsorbed protein) and anisotropic particles (such as rod-like xanthan molecules). If the length-to-diameter ratio of the rods is high, as in case of xanthan, then the driving force for phase separation becomes more pronounced. Even if there is no specific interaction energy between the particles, phase separation is thermodynamically favourable in such systems above a certain concentration of both species, because it is an entropy-driven process [12].

Generally, in the case of emulsions exhibiting gravity creaming, a low xanthan concentration induces visible phase separation which can be attributed mainly to reversible depletion flocculation of protein-coated emulsion droplets by the non-adsorbing polysaccharide [10]. On the other hand, a relatively high xanthan concentration (still rather low in relation to many other polysaccharides) produces a very high low-stress viscosity in the aqueous phase, which greatly retards the mobility of the emulsion droplets, thus preventing aggregation or coalescence, and thereby inhibiting macroscopic phase separation [6,7]. Stability is observed at extremely low polymer concentrations and at reasonably high polymer concentrations. In the former case, this is because the polymer concentration is not high enough to cause phase separation by depletion flocculation, and in the latter case it is because an aggregated particle network is formed, which, when combined with the high viscoelasticity of the aqueous phase, requires an extremely long timescale for reorganisation [5].

Previously in our laboratory [13,14] we studied flocculation of hydrocarbon oil-in-water emulsions stabilised by sodium caseinate in the presence of several polysaccharides, including xanthan gum, and found a general destabilising effect caused by the added polysaccharide. Flocculation occurred at very low xanthan concentration (0.01%, w/w). Increasing the polysaccharide level resulted in gradually slower creaming, until at concentrations above 0.125% (w/w), the emulsions did not exhibit any discernible serum separation over timescale of a few days. We could not detect any complexation or association between the xanthan and the droplets coated by sodium caseinate, thereby ruling out bridging flocculation as the source of the instability. The inhibition of creaming at relatively high xanthan concentrations was attributed to immobilisation of dispersed oil droplets in a weak gel-like network with a high low-stress shear viscosity [13−15].

Most of the above research was concerned with macroscopic measurements of stability and rheology. We still know little about the structural evolution within such systems at the microscopic level. The aim of the present work is to investigate the kinetics of evolution of the microstructure during the destabilisation of sodium caseinate-based emulsions (30 vol% oil) in the presence of xanthan gum. The concentration of caseinate emulsifier (1.4%, w/v) was chosen as the appropriate amount required for full surface coverage whilst leaving the minimum amount of excess protein in the aqueous phase [20,21]. The dense oil phase (1-bromohexadecane) was selected as being suitable for diminishing buoyancy effects. The main experimental technique used here is confocal laser scanning microscopy (CLSM) combined with image processing to quantify the data. The CLSM technique provides images of better resolution than conventional light microscopy or fluorescence microscopy, and it provides an opportunity for combining time-series of micrographs with image analysis, so that dynamic processes such as phase separation may be examined quantitatively [22,23]. Complementary rheological measurements have been made in an attempt to relate the microstructural changes to rheological and stability properties.

2. Materials and methods

2.1. Materials

Spray-dried sodium caseinate (>82 wt% dry protein, <6 wt% moisture, <6 wt% fat and ash, 0.05 wt% calcium) was obtained from DeMelkindustrie (Veghel, Netherlands). The caseinate powder was stored in a hermetically sealed container placed in separate desiccators. The hydrocarbon oil, 1-bromohexadecane (99%, density 0.9899 g/ml, refractive index (n_D^{20}) 1.4609), and the hydrochloric acid were purchased from Fisher Chemicals (Loughborough, UK). Imidazole (99.0%), 1,2-propanediol (99%) and Nile Red were obtained from Sigma Chemicals (Gillingham, UK). The Nile Red was stored in a dry and dark place. A food-grade xanthan gum powder (Kelzan 'S' F850414) from Kelco (San Diego, CA) was used in all the experiments. Milli-Q water (water purified by treatment with a Milli-Q apparatus, Millipore, Bedford) was used for the preparation of the solutions.

2.2. Emulsion preparation

Aqueous solutions of sodium caseinate (2%, w/v) and xanthan (0–1.5%, w/v) were prepared by adding protein powder and xanthan gum powder to buffer solution (imidazole) and then gently stirring overnight at room temperature to ensure complete dispersion. The 20 mM imidazole buffer was adjusted to pH 6.8 using hydrochloric acid. Sodium azide (0.01%, w/v) was added to the buffer as an antimicrobiological agent.