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Stability and rheological behaviors of different oil/water emulsions stabilized by natural silk fibroin

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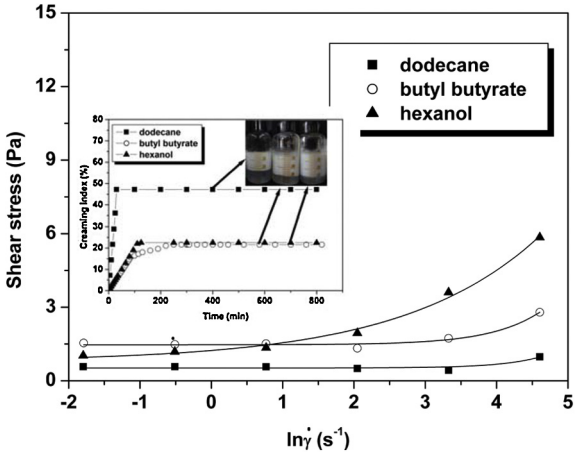
HIGHLIGHTS

- We prepared the oil/water emulsions stabilized by natural silk fibroin.
- Stability and rheological behaviors of the emulsions were investigated.
- Gel-like colloidal networks make emulsions show elastic behaviors.
- Stress overshoot appears due to droplet deformation before association breakage.
- Large modulus, viscosity and yield stress enhance emulsion creaming stability.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, oil/water emulsions stabilized by natural silk fibroin were prepared, and the effects of silk fibroin concentration (φ_{SF}), oil volume fraction (ϕ_o) and oil type on the stability and rheological behavior investigated. After creaming and reaching a stable state, the residual emulsion fraction increases with an increase in φ_{SF}, ϕ_o and oil polarity, while the size of emulsion droplets decreases with an increase in φ_{SF} and oil polarity but a decrease in ϕ_o. Due to the colloidal networks formed by the dispersed oil droplets, the emulsions show a predominantly elastic behavior with a frequency independent modulus and low phase angle. However, the droplet networks are not very strong, and a remarkable shear-thinning behavior occurs for all emulsions during steady shear flow. Moreover, stress overshoot also appears for some emulsions with low φ_{SF} and polar oil during transient shear flows, due to the deformation of droplets before the breakage of their associates. The modulus, viscosity and yield stress become larger with the increase of φ_{SF}, ϕ_o and oil polarity, which is very beneficial for strengthening the creaming stability of the emulsions.

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1. Introduction

Emulsions are thermodynamically unstable systems consisting of at least two immiscible fluids, one of which is dispersed in form of droplets in the other, and it tends to break down over time due to a variety of physicochemical mechanisms, such as gravitational separation or creaming, flocculation, coalescence and Ostwald ripening [1]. However, emulsions can be stabilized kinetically by adding emulsifiers, such as amphiphilic surfactants and proteins or colloidal solid particles, with their capability of absorbing at the interfaces, lowering the interfacial tension and also preventing the droplets from aggregation [2,3]. Due to the amphiphilic property and intrinsic nutritional aspects, proteines become one of the most important and extensively used emulsifiers, and play a significant role in cosmetics, pharmaceuticals, food and agricultural industries. Many researchers have extensively investigated different physicochemical properties of protein stabilized emulsions, such as droplet size, zeta-potential, rheological properties and creaming behavior [4–6], which provide valuable information for the predicting of the physical stability of these kinds of emulsions [1].

Among the characterization methods, rheology is very useful for the product design, product development, quality control, sensory evaluation and long-term stability estimation of the emulsions [7–9]. Generally, dynamic oscillatory, steady and transient shear flow measurements are often used to characterize the rheological behavior of emulsions [10,11]. Dynamic oscillatory tests are expected to obtain the viscoelastic and gel characteristics of emulsions, whereas steady shear flow measurements are applied to characterize their shear-thinning behavior. Transient shear studies, showing time-dependent non-Newtonian flow behavior, imply that the emulsion viscosity gradually changes with time when a constant shear rate is suddenly applied or released. It is demonstrated that when carob, soy and wheat gluten proteins are utilized as emulsifiers, the closed-packing of droplets leads to the formation of a three dimensional flocculated network, and the elastic and loss moduli of the resultant emulsions increase with an increase in protein concentration [12]. As for emulsions stabilized by whey protein, the increased addition of hydrocolloids can obviously enhance the emulsion viscosity [13], while the increased addition of gum can change the emulsion behavior from pronounced shear-thinning to a Newtonian character [14]. High elastic modulus, viscosity and yield stress are very helpful for the stabilization of emulsions.

Silk fibroin, as a core protein of silk produced by the domesticated silkworm, has attracted more and more attention due to its high mechanical properties, great biocompatibility with living tissues, low biodegradability and minimal inflammatory reaction [15,16]. These properties have led to exploring its use in biotechnological and biomedical applications, such as controlled drug release and tissue engineering scaffolds [17–19]. Silk fibroin is composed of highly repetitive amino acid sequences with alternating hydrophobic and hydrophilic blocks along the molecular chains and can be regarded as nature's counterpart of a synthetic, multi-block polyelectrolyte. The coexistence of distinct hydrophobic and hydrophilic regions provide the silk fibroin molecules an amphiphilic character and hence a respective surface activity, i.e. silk fibroin self-assembles at fluid interfaces and forms stable viscoelastic surface or interfacial layers [20–22]. These stable viscoelastic layers can prevent the bubbles or droplets from coalescence, thus making silk fibroin a possible new good macromolecular surfactant with potential applications in drug delivery systems and cosmetics as a novel biocompatible emulsion stabilizer. Actually, silk fibroin has been verified to be a successful stabilizer for oil–water emulsions, and the effects of pH, thermal processing, and salt concentration on the properties and stability of emulsions were investigated [20,21]. Taking advantage of emulsions stabilized by silk fibroin, biodegradable microparticles and microspheres for controlled-release drug delivery applications and electrospun nanofibers for tissue engineering applications were successfully prepared [23,24]. However, there is still very little work reported on the use of silk fibroin as a stabilizer of emulsions and foams, and the rheological behaviors of the resultant emulsions have not been previously reported.

In this study, emulsions stabilized by silk fibroin were prepared, and effects of silk fibroin concentration (φ_{SF}), oil volume fraction (ϕ_o) and oil type on the stability and rheological behavior were investigated in order to gain further insight into the stabilization mechanisms.

2. Experimental

2.1. Materials

Fresh domestic *Bombyx mori* cocoon shells were supplied by a farm cooperative in China. Analytical grade sodium carbonate (Na_2CO_3) and lithium bromide (LiBr) were purchased from Sinopharm Chemical Reagent Co. (China), dodecane (≥99%) and butyl butyrate (98%) were supplied by Shanghai Aladdin Reagent Co. (China), and hexanol (98%) was purchased from Sigma–Aldrich. The densities of the three oils were 0.7808 g/cm^3, 0.8692 g/cm^3 and 0.8136 g/cm^3, respectively. All chemicals were used as received. All aqueous solutions were prepared with Milli-Q water. By using a K100 tensiometer (Krüss tensiometer, Germany) with a Du Noüy ring arrangement, the interfacial tension between the water and oil phases were measured as 55.3, 18.8 and 5.0 mN/m for the dodecane/water, butyl butyrate/water and hexanol/water interfaces, respectively. The larger the interfacial tension is, the lower is the polarity of the oil [23].

2.2. Preparation of aqueous solution of silk fibroin

The preparation of aqueous silk fibroin solutions from fresh domestic *B. mori* cocoon shells generally involves three steps: extraction of sericin, dissolution of the cocoon fiber, and dialysis of the aqueous silk fibroin solutions against de-ionized water. The first step is the removal of sericin (degumming) from the cocoon shells by maintaining them in 100 times by weight of boiling 0.02 M Na_2CO_3 aqueous solution for 30 min. The resulting silk fibroin material was then rinsed with de-ionized water. The degumming process was repeated at least three times, and the thoroughly degummed silk was then dried at 50 °C. The dissolution of the silk fibroin was accomplished by stirring the resulting material in an aqueous 9.3 M LiBr solution at 70 °C until it was dissolved completely. This solution was then dialyzed against water (volume ratio of 100) for at least 3 days, while refreshing the outer de-ionized water six times. This procedure yielded very reproducible products of typically aqueous solution of 20 mg/ml silk fibroin, which was determined by weighing the remaining solid after completely drying a known volume of the silk fibroin solution. Before use, the fibroin solution was centrifuged at 13,000 rpm for 15 min to remove any aggregates, and then the solution was diluted with Milli-Q water to the desired fibroin concentration for emulsion preparation. The diluted fibroin solutions were freshly prepared for each experiment.

2.3. Preparation of emulsions stabilized by silk fibroin

Emulsions were prepared with an FM200 high shear dispersing homogenizer (Fluko Equipment Shanghai, China) by mixing a silk fibroin aqueous solution with oil at 10,000 rpm for 2 min. No pH adjustments were made for the final emulsions because neither the silk fibroin nor oil volume affected the pH. Seven emulsions