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Effect of pH on the interfacial viscoelasticity and stability of the silk fibroin at the oil/water interface

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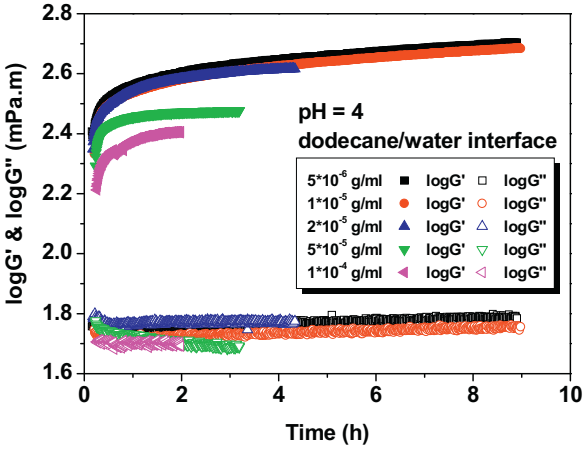
HIGHLIGHTS

- Interfacial rheology of silk fibroin at O/W interface with pH effect was discussed.
- Effect of pH on the stability of silk fibroin emulsions was investigated.
- At pH4, silk fibroin forms compact conformation and adsorbs quickly at the interface.
- At pH4, silk fiborin emulsions exhibit low emulsion stability and large droplet size.

GRAPHICAL ABSTRACT



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ABSTRACT

Effect of pH on the interfacial viscoelasticity of silk fibroin at both nonpolar dodecane/water interface and polar butyl butyrate/water interface was investigated, and the emulsions stabilized by silk fibroin with different pH and different oil phases were also prepared to check the interfacial stability and emulsification effectiveness of silk fibroin. The silk fibroin can adsorb to the oil/water interface, reduce the interfacial tension efficiently and exhibit good emulsifier effects. At pH 4 with respect to isoelectric point, the silk fibroin molecules aggregate more easily, form more compact conformation and have less chances to interact with neighboring molecules, which results in faster molecular adsorption at the interface but lower modulus of interfacial layers. Moreover, the interfacial layers formed by silk fibroin at pH 4 are relatively rigid, rupture more easily and are more likely to allow droplet coalescence upon small emulsion disturbance, and the corresponding emulsions exhibit lower emulsion stability and larger emulsion droplet size.

1. Introduction

Silk fibroin, as a core protein of silk extracted from domesticated silkworm, has attracted more and more attention because of its distinctive mechanical properties, low biodegradability, outstand-

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ing biocompatibility as well as relatively easy fabrication [1]. These excellent properties have led to its wide use in biotechnological and food science applications, such as controlled drug release, tympanic membrane, tissue engineering scaffolds and food additives [2].

Both experimental and computational research work indicate that silk fibroin consists of one heavy chain (H) and one light chain (L) connected by a disulphide linkage. The outstanding mechanical property of silk fibroin originates from its sophisticated hierarchical structure, in which highly organized densely H-bonded β-sheet crystalline protein is arranged in the semi-amorphous matrix with helices and β-turn protein structures [3] . The crystalline region contains largely the repeated sequence of (Gly-Ala-Gly-Ala-Gly-Ser)$_n$, while the amorphous region consists mainly of the amino residues groups as Phe, Tyr and Try [4–6]. Silk fibroin can achieve at least three secondary conformations and the two most commonly known conformations are silk I and silk II. Silk I is a metastable structure of silk fibroin before spinning, while silk II is a heterogeneous structure consisting of two anti-parallel β-sheet folding structures with different inter-molecular arrangements [7–9]. Silk I can be easily converted to silk II by shear force during spinning. The formation of different secondary structures in silk fibroin mainly depends on charge density, pH and salinity of the aqueous solvent as well as processing conditions. When silk fibroin is affected by extensional flow, heat, decrease of pH or addition of metallic ions, it can undergo a transition from helix-form to β-form.

Silk fibroin consists of highly repetitive amino acids with alternating hydrophobic and hydrophilic blocks along the molecular chains, and its amphiphilicity tends to make it rearrange to expose its hydrophobic residues to the hydrophobic phase and hydrophilic residues to the aqueous phase when absorbing at an interphase [10,11]. The adsorption process can be generally divided into four procedures: (i) diffusion from solution towards interface, (ii) adsorption to the interface, (iii) changing molecular structure, (iv) spreading at the interface [12]. The conformational change depends mainly on interface nature, phase conditions (silk fibroin concentration, ionic strength and pH) and the intrinsic properties of silk fibroin. Practically, the pH value when silk fibroin is neutrally charged is called isoelectric point and silk fibroin shows the most compact conformation, which generally results in faster adsorption and lower final interfacial tension [13]. As discussed by Burgess and Sahin [14], both of the interfacial elasticity and tension attain a minimum at the isoelectric pH value for bovine serum albumin (pH 5.3) and human immunoglobulin G (pH 7.4). The lower interfacial elasticity indicates a reduction in interfacial adsorption, while the lower interfacial tension indicates an increase in interfacial adsorption, and this opposite conclusion can be explained by molecular configuration. According to the results of potato protein isolate reported by Romero [15], pH value exerts a negligible effect on its interfacial tension but displays very important differences in the viscoelastic properties of its interfacial films formed at the sun flower oil/water interface, and pH 8 provides a major elastic response at oil/water interface as compared to pH 2, which results in a much higher ability to produce fine and stable emulsions at pH 8.

Due to the coexistence of distinct hydrophobic and hydrophilic regions, silk fibroin can reside at the fluid interfaces and quite effectively stabilize dispersed systems like emulsions by efficiently reducing the interfacial tension and forming steric and highly viscoelastic films at the interface [16,17]. The reduction of interfacial tension facilitates droplet breakup during a homogenization process, while the viscoelastic film assists in stabilizing emulsion droplets against coalescence during emulsification and long-term storage. The interfacial deformation includes compression and shear deformation, in which the former relates to the variation of area at a constant shape and is far more relevant to short-term emulsion stability, and the latter accounts for changes in shape at a constant area and plays an important role in long-term emulsion stability [18,19]. Globally, previous studies reveal that the complex corn oil/water emulsions stabilized by silk fibroin presents a bigger droplet size and more droplet aggregation at pH 4 as compared to other pH values [20], while other studies show that the emulsions stabilized by sodium caseinates, bovine serum albumin as well as whey proteins also exhibit an increased droplet size at the isoelectric pH, because the low surface charge and weak electrostatic repulsion between droplets are insufficient to overcome the van der Waals and hydrophobic interactions [21–23].

In this study, the effect of pH on the interfacial rheological behaviors of silk fibroin at the nonpolar dodecane/water interface and polar butyl butyrate/water interface was investigated respectively, and the stability of the corresponding emulsions stabilized by silk fibroin at different pH was also discussed. The present work aims at establishing a relationship between the interfacial viscoelasticity and emulsion stability for silk fibroin and providing support for its real emulsification process.

2. Materials and methods

2.1. Materials

Fresh domestic *Bombyxmori* cocoon shells were kindly supplied by a farm cooperative in China. Analytical grade sodium carbonate (Na_2CO_3), lithium bromide (LiBr), sodium phosphate dibasic dihydrate (Na_2HPO_4) and citric acid ($C_6H_8O_7$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Butyl butyrate (≥99%) and dodecane (≥99%) were purchased from Sigma-Aldrich. All chemicals were used as received. Buffer solutions with different pH values were prepared by mixing suitable amount of 10 mM Na_2HPO_4 aqueous solution and 10 mM citric acid aqueous solutions. All aqueous solutions were prepared with deionized water.

2.2. Preparation of silk fibroin aqueous solution

The preparation of silk fibroin aqueous solution from raw domestic *Bombyxmori* cocoon shells includes three steps: extraction of sericin, dissolution of cocoon fiber and dialysis of silk fibroin aqueous solution against deionized water. The first step was to remove sericin from the cocoon shells by boiling them in 0.02 M Na_2CO_3 aqueous solution (100 times by weight) for 30 min, and the obtained silk fibroin was then rinsed with deionized water. This degumming process was repeated at least three times and the thoroughly degummed silk was dried at 50 °C. The resulting dried silk fibroin was then magnetically stirred in 9.3 M LiBr aqueous solution at 70 °C for 4 h until it was completely dissolved. This solution was dialyzed against water (100 times by volume) for 3 days, while refreshing the outer deionized water six times to remove all the salt and contaminants. This procedure yielded reproducible 20 mg/ml silk fibroin aqueous solution, which was determined by weighing the residual solid after completely drying a known volume of the silk fibroin solution. The regenerated silk fibroin solution was stored at 4 °C to avoid premature gelation. Before use, the silk fibroin solution was centrifuged at 13,000 rpm for 15 min to remove any aggregates, and then the solution was diluted with deionized water or buffer solutions to the desired concentration for measurements and emulsion preparation. The diluted silk fibroin solutions were freshly prepared for each experiment.

2.3. Interfacial dilatational rheology

Interfacial dilatational rheology measurements were performed at 25 °C using a PAT-1 Profile Analysis Tensiometer (Sinterface Technologies, Berlin, Germany) according to the oscillation bubble method (Fig. 1(a)). During the measurements, the silk fibroin solution droplet was formed at the tip of a capillary which was