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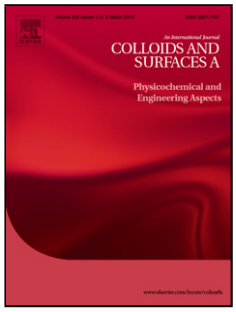

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Interfacial and Emulsifying Properties of the Electrostatic Complex of *β*-Lactoglobulin Fibril and Gum Arabic (*Acacia Seyal*)

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Running title: O/W emulsion stabilized by the complex of *β*-lactoglobulin fibril and gum Arabic

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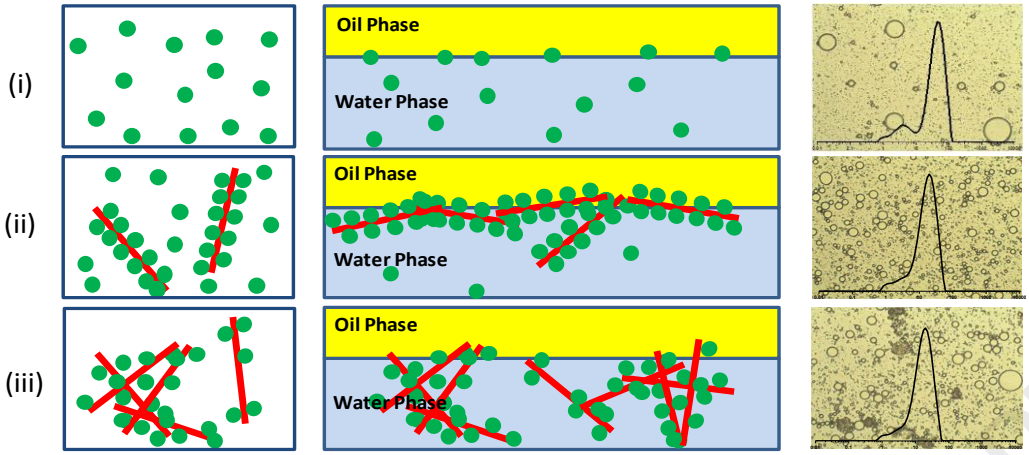
Graphical Abstracts



Proposed mechanisms for the adsorption of BLGF/AS complex at the oil-water interface. Pure AS (i) could adsorb at the oil-water interface but formed a loose film due to its poor surface activity and insufficient adsorption amount. With addition of a small amount of fibrils (ii), soluble electrostatic complexes are formed and they can be adsorbed at the interface to formed a dense viscoelastic film due to the surface activity of the BLGF. With a higher content of fibrils (iii), surface charge of the complex tended to be neutralized, causing the aggregation. Because the presence of protein fibrils, they could also adsorb at the oil-water interface to produce a viscoelastic film. However, with a bigger size and irregular shape, the aggregates were difficult to array at the interface as densely as the soluble complex.

Abstract: Formation, interfacial and emulsifying properties of the electrostatic complex of *β*-lactoglobulin fibril (BLGF) and gum arabic *Acacia Seyal* (AS) were investigated. Necklace-like soluble complex could be formed at pH 3.5, and its charge and interfacial properties depended on the BLGF content. With appropriate amount of BLGF (< 9.09 wt.%), the formed complex possessed a good dispersibility and surface activity. When excessive BLGF (9.09~50 wt.%) existed, surface charge of the complex was gradually neutralized and aggregation occurred. Homogeneous oil-in-water emulsions could be stabilized by the complex and the droplet size decreased with increasing BLGF content. Higher content of BLGF (9.09~50 wt.%) was detrimental for emulsification due to the aggregation of complex, and the formed emulsion tended to flocculate. Compared with AS, the complex formed emulsions were much more stable against heating (90

℃, 30 min) and salting (200 mM NaCl) environments, and the emulsions were stable during long-term storage (46 days).

Keywords: Protein fibril; Anisotropic particle; Gum arabic *acacia seyal*; Pickering emulsion; Emulsion stability

1. Introduction

Electrostatic complexation between proteins and polysaccharides had received extensive attentions during the past decades.[1] Most of the polysaccharides could not easily adsorb onto the oil/water interface to lower interfacial tension, unless a certain amount of protein fraction existed, such as in the case of sugar beet pectin or gum arabic *Acacia Senegal*. However, the surface activity of polysaccharide could be improved via binding with protein or other surface-active substances. Strong attractive electrostatic complexes are typically formed between positively charged proteins (pH<pI) and negatively charged polysaccharides. Compared with the pure protein or polysaccharide, the complex of protein/polysaccharide usually has higher surface activity and better emulsifying stability because of its abilities to form gel-like interfacial layer and to increase the viscosity of dispersion medium.[2] The interfacial properties of the protein-polysaccharide complex were strongly dependant on the conformation of the protein, which were sensitive to the environmental conditions such as pH, temperature and the ratio between protein and polysaccharide. [3, 4]

Not only native protein molecules but also their aggregates could bind electrostatically with polysaccharide.[5] Unfortunately, the interfacial and emulsifying properties of such complex has taken a few attentions. Compared to native protein, the protein aggregates have desirable advantages, such as maintaining their stability under various environments (such as pH, surfactant and heating)[6-8]. Furthermore, quite amount of researches have shown that surface-active solid particles (e. g. protein aggregates) are more effective interfacial stabilizers

compared with the protein monomer or other small molecule surfactant, due to their higher detaching energy from the interface, especially the anisotropic solid particles.[9-11] Therefore, it is probable that the complex of protein aggregate/polysaccharide might have more stable physicochemical properties and would be more effective in stabilizing an oil/water emulsions, compared with that of native protein/polysaccharide complex.

Highly ordered superstructures could be obtained when the electrostatic complex formed by protein aggregates and flexible polysaccharide,[12, 13] while an amorphous structures were most likely formed by native protein and flexible polysaccharides.[14] Interestingly, it seems that the ordered structure of protein aggregates/polysaccharide electrostatic complex depends on the charge property and conformation of the polysaccharide. For example, *β*-lactoglobulin fibril (BLGF) and sulfated *κ*-carrageenan could form a necklace-like complex via electrostatic interaction along the fibrils' contour length.[12] In contrast, the electrostatic complexation between BLGF and low molecular weight pectin produced nanotapes.[13] A bottle-brush structure was formed when BLGF interacted with linear and terminal charged polyethylene glycol.[15] In view that BLGF formed electrostatic complexes of various shapes with charged polymers, a wide range of applications of these complexes could be expected. However, the physiochemical properties, especially interfacial and emulsifying properties of the complexes, have not been clarified so far.

BLGF is a self-assembly product of *β*-lactoglobulin (BLG) under acidic and heating conditions, as described in other research,[16] and it's shown to be surface active because it can adsorb onto the oil-water interface to decrease the surface tension. [17] The viscoelasticity of the interfacial film formed by BLGF was much higher than that by native BLG, although the kinetics of interfacial adsorption and resulting interfacial tension were not so different with native protein.[17] Gum Arabic *Acacia seyal* (AS) is a negatively charged polysaccharide which has been widely used in the food industry. It contains about 1% (w/w) of protein, compared with that of 2.5% in gum Arabic *Acacia Senegal*.[18] Due to the low protein content, the emulsifying capacity of AS are usually unsatisfactory, and therefore AS has a lower application value than gum Arabic *Acacia Senegal*.[18] In this study, BLGF was choose as the protein aggregates and AS were choose as polysaccharide, to form a electrostatic complex. The aim of

this study was to investigate the effect of the BLGF content on the formation and interfacial properties of the complex.

2. Materials and methods

2.1. Materials. BLG (protein content 97.4 %) was kindly supplied by Davisco Foods International, Inc. (Minnesota, USA). *Acacia seyal* gum (AS) (M_W=0.8 × 10^6 as showed in our previous measurement [19]) with a purity of >96.0%, was obtained from Elie Group Holding Co. Ltd. (Sudan). Soy oil was purchased from a local market. All solutions were prepared with ultra pure water (Millipore Corporation, Billerica, Massachusetts, USA). All other chemicals were of analytical grade.

2.2. Preparation of Freeze-dried BLGF. Freeze-dried BLGF was prepared according to the method used by others[20,21] and with little modifications. BLG was dispersed in Millipore water at a concentration of 20.0 mg/mL, and left overnight at 5 ℃ for full hydration. After adjusted to pH 2.0 with 2.0 M HCl, the BLG solution was incubated in water bath at 80 ℃ for 15 hours under magnetic stirring at 400 rpm. The sample was then cooled and dialyzed against pH 2.0 de-ionized water by a 20 kDa dialyzer at 5 ℃ for 48 hours, followed by freeze-drying using an Alpha-4 freeze-dryer (CHRIST, Germany).

2.3. Preparation and Characterization of the Complex of BLGF and AS. Firstly, BLGF and AS were separately dispersed in acetate buffer (pH 3.5) at a concentration of 5 mg/mL. The pH 3.5 was choose because our previous study showed that the complexation between BLGF and AS (data not shown) could happen at this pH. The dispersions were mildly stirred at least 2 hours to ensure full hydration. BLGF/AS complex were formed by mixing BLGF and AS dispersions at various ratios (the BLGF content in the mixtures ranged from 0% to 50% by weight) under continuous stirring for 2 hours. The content of BLGF in the paper refers to the weight percentage of BLGF in the complex.

Transmission electron microscope (TEM, FEI Electronic Optics, Holland) was used to visualize the morphology of BLGF, AS and BLGF/AS complex. Two μL of samples was deposited onto a carbon-coated copper grid (230 meshes) and the superfluous sample was sucked away by a capillary pipe. The samples were dried for 72 h at room temperature in

desiccator. The samples were negatively stained by phosphotungstic acid (10 mg/mL) for 60 s and air-dried prior to TEM observation.

Zeta-potentials of the complex were determined using a Nano ZS Zetasizer apparatus (Malvern Instruments, Worcestershire, UK). Samples with different BLGF content were diluted to 1 mg/mL before loading to cuvettes. All measurements were carried out at 25 ℃ in triplicate.

Turbidity measurement was performed on a UV/visible spectrophotometer (TU-1900, PERSEE, China), followed the method of Lee et al. [22] The absorbance at 600 nm of each sample represented the turbidity.

2.4. Dynamic Surface Properties at the O/W Interface. The interfacial adsorption kinetics and dilatational rheological properties of the samples at the O/W interface were measured by a drop profile tensiometer (Teclis Tracker, France). The experiments were carried out at 25 °C. BLGF, AS and BLGF/AS complex dispersions were diluted to a concentration of 0.01 wt.% using 20 mM acetate buffer (pH 3.5), and then were loaded into the syringe. A drop of the dispersions was delivered and allowed to stand at least for 3 hours to achieve their adsorption at the O/W interface. The soy oil used for the surface properties measurements was purified by the method of Gaonkar,[23] and surface tension measurements were performed to check the absence of surface-active contaminants in the buffer solution and oil phase. The surface tension (σ) was calculated according to the fundamental Laplace equation. The surface pressure is $\pi = \sigma_0 - \sigma$, where σ_0 is the surface tension of distilled water.

To obtain surface dilatational parameters, sinusoidal interfacial compression and expansion were applied by decreasing and increasing the drop volume at 5% of deformation amplitude ($\Delta A/A$) and 0.1 Hz of frequency. The surface dilatational modulus (E^*) derived from the change in interfacial tension (σ), resulting from a small change in surface area (A), can be described as follows:[24]

$$E^* = d\sigma/(dA/A) = -d\pi/d\ln A = E' + iE''$$

The dilatational modulus (E^*) is a complex quantity and is composed of real and imaginary parts ($E' + iE''$). The real part E' of dilatational modulus or storage component is dilatational elasticity. The imaginary part E'' of dilatational modulus or loss component is surface dilatational viscosity.

2.5. Preparation and Characterization of Emulsions. Emulsions stabilized by BLGF, AS and their complexes were prepared by mixing BLGF, AS, and the complex dispersions (5 mg/mL, pH 3.5) with purified oil phase using a high-speed blender at 20,000 rpm/min for 3 min (T25 digital ULTRA TURRAX®, IKA, German). The oil fraction were 10 % v/v in all the samples. Sodium azide was added (0.4 mg/mL) to prevent bacteria growth. The microstructure of the emulsions were observed using a conventional optical light microscope (Nikon YS100), equipped with a CCD camera (HV2001UC). A magnification of 10 times was applied. The particle size of the emulsions was measured using laser diffraction on MasterSizer 2000 (Malvern Instruments Ltd., UK). Particle size distribution (PSD) and Sauter mean diameter ($d_{3,2}$) were reported. The stability of the emulsions was evaluated by stress treatment and long-term storage. For stress treatment, the emulsions were added with 200 mM NaCl and then heated under 90 ℃ for 30 min. The changes in droplet size and microstructure of the emulsions were analyzed. For long-term storage, the emulsions were kept under 25 ℃ for 46 days and the change in $d_{3,2}$ was monitored during the storage.

2.6. Statistical Analysis. Unless otherwise specified, three independent trials were carried out for each experiment, and the data were analyzed using the software of Origin 8.0. The results were given as mean ± standard deviation.

3. Results and discussion

3.1. Formation of BLGF/AS complex. TEM observations of AS, BLGF and their complex at pH 3.5 are presented in Fig.1. AS appeared as clusters under TEM observation, and single AS molecule seemed to be spherical with a diameter around 30 nm (Fig. 1A). This phenomenon might be induced by the aggregation of the molecules during sample preparation as reported by others [25, 26]. BLGF was shown to be linear as reported previously (Fig. 1B) [17, 21, 27]. The

of 1.96 wt. % at pH 3.5. (scale bar = 100 nm)

complex of AS and BLGF with a BLGF content of 1.96 wt. % exhibited a necklace-like structure, in which spherical AS molecules were bound along linear BLGF. There was no free fibrils observed in the field of view, indicating that all the fibrils have engaged in the interaction with AS. The observation is similar with the microstructure of the complex of BLGF and

sulfated polysaccharide (*k*-carrageenan),[12] in which *κ*-carrageenan interacted with neighboring BLGF, acted as cross-linking agents and led to a clustering of the fibrils.

Zeta potential of AS/BLGF complex with different BLGF content was shown in Fig. 2 A. Since AS and BLGF were oppositely charged, the incorporation and increasing content of BLGF resulted in a decrease in surface charge of the complex due to the charge neutralization. In details, when BLGF content was below 9.09 wt.%, the surface charge decreased gradually from -15.9 ± 0.75 mV to -14.1 ± 0.36 mV. Further addition of BLGF led to a more dramatic decrease in surface charge (-14.1 ± 0.36 mV - 0.22±0.17 mV). When BLGF content reached ca. 50 wt. %, the complex was nearly neutralized. In parallel, the addition of BLGF led to an increase in turbidity of the complex as shown in Fig. 2 B. At lower BLGF content(≤ 9.09 wt. %), the turbidity increased slowly from 0.04 to 0.07. At higher BLGF content, the turbidity increased more rapidly. The increasing in turbidity indicating an aggregation and increasing of the particle size. This phenomenon was well explained by the fact that higher surface charge usually leads to higher dispersibility and stability. Visual observations of the complexes are displayed in Figure 2C. Serious sedimentation/precipitation of the complex was found when BLGF content reached 50 wt. %.

(C) of BLGF/AS complex at pH 3.5 as a function of BLGF content.

When a small amount of BLGF mixed with an excessive amount of the polysaccharide molecules (i.e., BLGF content ≤ 9.09 wt. %), the surface of BLGF could be nearly completely covered by AS via electrostatic interaction. This coverage enabled the surface charge of the complex to be dominated by AS and to be maintained in a narrower range. At higher BLGF content (9.09~50 wt. %), AS molecules were gradually insufficient to saturatedly cover the exposed positive charged BLGF surface. The expose BLGF surface contributed significantly the neutralization of AS/BLGF complex. Therefore, the surface charge of the complex decreased conspicuously with increasing BLGF content. When BLGF interacted with *k*-carrageenan at pH 3.0,[12] the maximum complexation efficiency was found at a protein content of 62.5 wt. %, which was higher than that of AS/BLGF. Different charge properties of the polysaccharides and pH value might be the reasons.

3.2. Interfacial Properties of the Complex of BLGF and AS. Interfacial adsorption and dilatational rheological behaviors of the complex were investigated, and the results are presented in Fig. 3. AS was able to adsorb at the oil-water interface and the equilibrium was reached in short time (within 50 seconds). However, the saturated surface pressure was low (around 7 mN/m), even when the adsorption duration was prolonged to 3 hours (Fig. 3 A). The low surface pressure should be due to the low surface activity of AS, particularly the extremely low protein content intrinsically contained in AS.

With the incorporation of BLGF, the saturated surface pressure of the complex increased correspondingly, indicating improved surface activity and more materials being adsorbed onto the interface. The highest surface pressure (about 17 mN/m) was achieved when BLGF content was 33.3 wt. % in the complex. Further addition of BLGF to 50 wt. % resulted in the decrease of the saturated surface pressure (around 15 mN/m). This transition should be attributed to the neutralization of the surface charge, which led to the aggregation of the complex at higher BLGF content. Aggregation led to bigger molecular size and subdued diffusion capacity of the complex, thus lower surface pressure. Bigger molecular size of the adsorbed material also inhibited their effective arrangement at the interface and reduced the surface pressure.

Dilatational modulus of the pure AS at the oil-water interface was as expected very low (around 15 mN/m) (Fig. 3 B), due to the insufficient adsorption at the interface and the weak interactions between the molecules. Incorporating a small amount of BLGF (e. g. 5 wt. %) did not produce apparently higher modulus, despite that the surface pressure was much higher than that of AS. At higher BLGF content (e. g. 9.09 wt. %), the viscoelastic moduli increased markedly (about 25 mN/m). Unlike the surface pressure which rose firstly and then fell with the incorporation of BLGF, dilatational moduli of the complex increased continually with increasing BLGF content, even at a BLGF content of 50 wt. %. At low content of BLGF (e. g. 5 wt. %), free AS molecules in the dispersion were sufficient to cover the surface of BLGF completely, therefore the formed complex was less surface active. With higher BLGF content (e. g. 9.09 wt. %), the uncovered surface of BLGF increased and therefore the surface activity of the complex increased, led more adsorption of the complex on the interface and produced higher moduli. Further increasing the BLGF content resulted in a continuous ascent of the

interfacial moduli. It could be observed that the slopes of the plot of viscoelastic modulus vs. surface pressure was shown to be greater than 1 (broken line in Fig. 3). This means that the increase in the modulus of the complex at the interface was faster than its adsorption speed, indicating the strong interaction between the adsorbed materials. The modulus of the complex with 50 wt. % BLGF content reached 37.8 mN/m, almost the same with that of pure BLGF (38.1 mN/m, data not shown). It implies that the interfacial strength composited by the complex was comparable to that of the pure BLGF. This would be meaningful for its applications in foods.

To better understand how the BLGF content affects the interfacial behavior of the complex, the mechanisms are illustrated in Fig. 4. Pure AS could adsorb at the oil-water interface but formed a loose film due to its poor surface activity and insufficient adsorption amount (Fig. 4 i). As a result, the surface pressure was low and the formed interfacial film was weak. With addition of a small amount of fibrils ($\leq$ 9.09 wt. % wt.), soluble electrostatic complexes were formed and they can be further adsorbed at the interface and formed a dense viscoelastic film due to the surface activity of the BLGF (Fig. 4 ii). As a result, the surface pressure increased apparently. Meanwhile, the dilatational modulus could attain a high value. With a higher content of fibrils (such as 50 wt. %), surface charge of the complex tended to be neutralized, causing the aggregation of the complex. Since the aggregates were surface active because of the presence of protein fibrils, they could also adsorb at the oil-water interface to produce a viscoelastic film (Fig. 4 iii). However, with a bigger size and irregular shape, the aggregates were difficult to array at the interface as densely as the soluble complex. This might be the reason why the surface pressure of the complex with 50 wt. % fibrils content turned to decrease.

(i) represents the AS adsorbed at the interface. (ii) represents the interfacial adsorption of BLGF/AS with a small content of BLGF (0~9.09 wt. %). (iii) represents the interfacial adsorption of the BLGF/AS complex with excessive content of BLGF (~50 wt. %).

3.3. Emulsifying Properties of the Complex. Microstructure and droplet size distribution of the oil-in-water emulsions prepared with the complexes are shown in Fig. 5 A. All the samples, including pure AS and the complex, could form an oil-in-water emulsion. The droplet size distribution of the AS-stabilized emulsion was composited by two main peaks, which were big

size (around 45 μm) and small size (around 4 μm). As incorporation and increasing of the BLGF, the big size peak shifted toward to a smaller size, and the small size peak disappeared gradually, indicating the emulsion became to be more homogeneous. The average droplet size ($d_{3,2}$) of the AS-stabilized emulsion was 15 μm as showed in Fig. 5 B. In the presence of 4.76 wt. % BLGF, the emulsion droplets became to be apparently smaller ($d_{3,2}$=10.5 μm). Further increasing BLGF content could continually decrease the droplet size and meanwhile maintain the size distributing in a narrow range. When the BLGF content was 50 wt. % in the complex, the formed emulsions were found to be mildly flocculated. Changes in average droplet size ($d_{3,2}$) of the emulsions as influenced by the BLGF content is shown in Fig. 5 B. At the first stage (BLGF < 9.09 wt. %), the $d_{3,2}$ decreased sharply with increasing BLGF content. However, further addition of BLGF resulted in a slightly increase of $d_{3,2}$.

As was well known, the key factors affecting the formation and droplet size of the emulsions during emulsification are the surface activity and adsorption speed of the emulsifiers. Higher surface activity and faster adsorption resulted in smaller droplet size. In the present experiment, incorporation of BLGF could improve the surface activity of AS obviously, therefore the droplet size formed by complex was much smaller than that of pure AS. When the BLGF content is low (e. g. <9.09 wt. %), BLGF addition makes the complex more surface active, therefore continually decreasing the droplet size of the emulsions. Further increasing of BLGF content (> 9.09%) would result in the decrease of the surface charge of the complex as observed in Fig. 2, which led to the aggregation of the complex and decreasing of the adsorption speed of the complex at the interface. Therefore, at high BLGF content (≥ 9.09%), a rising of the average droplet size occurred with addition of the BLGF. Moreover, because of the low surface charge, emulsion droplets stabilized by the complex with high BLGF content were prone to flocculate, as observed in Fig. 5 A (50% wt. BLGF content). In brief summary, the formation of BLGF/AS complex was beneficial for improving the emulsifying properties of AS, as long as BLGF content was in an appropriate range.

complex with different BLGF content (A). Change of the average droplet size ($d_{3,2}$) of the emulsions stabilized by BLGF/AS complex as a function of BLGF content. (Scale bars in A are 50 μm)

3.4. Emulsion Stability. To evaluate the emulsion stability against heating and salting, all the samples were treated in 90 ℃water bath for 30 min in the presence of 200 mM NaCl. Emulsion droplet size distributions before and after heating are shown in Fig. 6 (A, B, C and D). AS-stabilized emulsion was changed obviously after heat treatment because the droplet size distribution curve moved toward the bigger droplet size (Fig. 6 A). Polysaccharides were known to be relatively more resistant against heating as compared with protein, but they are sensible to salting. Under 200 mM NaCl, surface charge of the droplets was shielded seriously, inducing flocculation and coalescence. The BLGF/AS complex stabilized emulsions did not show an obvious change according to the size distribution before and after heat treatment (Fig. 6 B, C, and D), indicating that the interface formed by the BLGF/AS complex was more stable against heating and salting. This might benefit from the high viscoelasticity and high steric repelling effect of the complex film on the droplet surface. Changes of $d_{3,2}$ of the emulsions before and after heating is summarized in Fig. 6 E. After heating, the average droplet size of AS emulsion was almost as 3 times big as that of the original emulsion. While in the presence of fibrils, this increase in droplet size was significantly inhibited. Especially for the complex containing 16.67 % BLGF, there was no change in the average droplet size before and after heating. These results were consistent with the size distribution patterns.

distribution of BLGF/AS complex stabilized emulsions. BLGF content are 0 (A), 4.67 % wt. (B), 16.67 % wt. (C), and 50 % wt. (D), respectively.

The long-term storage stability of the emulsions at ambient temperature was also evaluated by monitoring the changes of $d_{3,2}$ during 46 days storage (Fig. 7). AS stabilized emulsions were unstable and the $d_{3,2}$ increased apparently from the 4^{th} day. The emulsion almost collapsed after storage for 11 days, and the measurement had to be stopped. In the presence of 1.96% BLGF in the complex, the emulsion stability could be well improved as showed in Fig. 7. Although the initial droplet size was relatively bigger than the other complex-stabilized emulsions, the complex with a low BLGF content was still able to maintain the stability of the emulsions during the storage. Excessive BLGF might be inappropriate for the emulsion stability, because the droplet size of the emulsion stabilized by 50% BLGF complex had been increasing

gradually during the storage. Flocculation between the emulsion droplets might be the reason because the surface charge of the emulsion droplets was nearly neutral.

emulsions during long-term storage at ambient temperature (25 ℃).

4. Conclusions

A soluble electrostatic complex could be formed by mixing the BLGF and AS at pH 3.5. with the BLGF content below 9.09% wt. Higher BLGF content (from 9.09% wt. to 50% wt.) led to the fast decreasing of surface charge and increasing of turbidity of the complex. When the BLGF content arrived 50% wt., neutralization and aggregation of the complex happened. The BLGF/AS complex have good surface activity, especially when the BLGF content above 9.09% wt., the complex could adsorb onto the interface and produce high surface pressures and viscoelastic modules. The emulsifying capacity and emulsion stability of the complex could be improved apparently by increasing the BLGF content in the complex. When BLGF content below 9.09% wt., the emulsion droplet size deceased steeply with BLGF content increasing, further increasing BLGF content is ineffective for the droplet size decreasing. The BLGF/AS complex stabilized emulsions could keep stable under high temperature, high salt content or long-term storage when BLGF content located during 9.09% wt. and 50 % wt.

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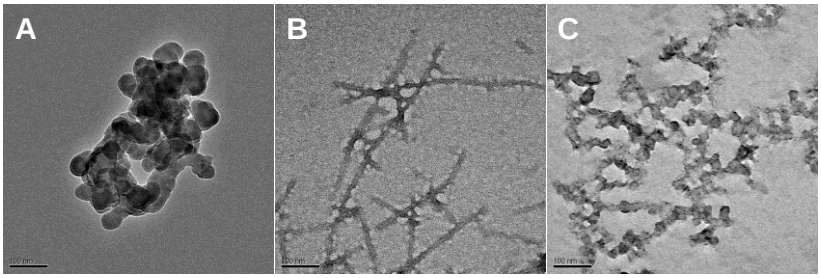

Figure 1 TEM images of AS (A), BLGF (B) and their complex (C) with a BLGF content

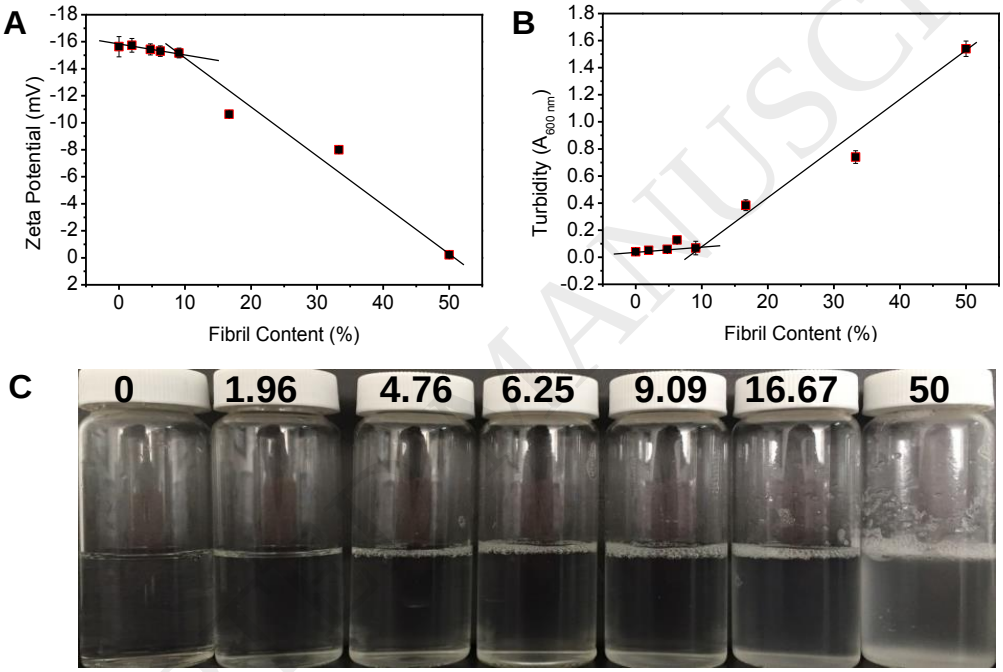

Figure 2 Changes of the zeta-potential (A), turbidity (B) and appearance

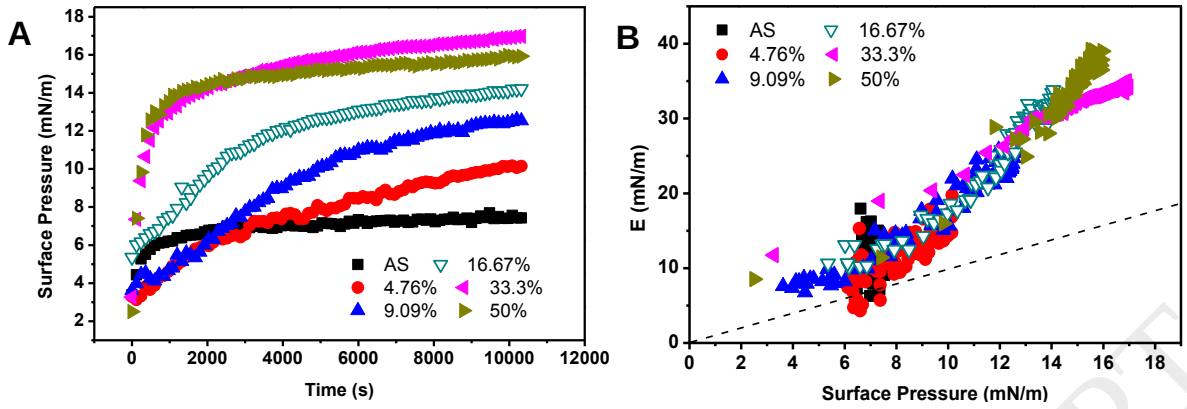

Figure 3 Interfacial dilatational rheological properties of BLGF/AS complex with various BLGF content.

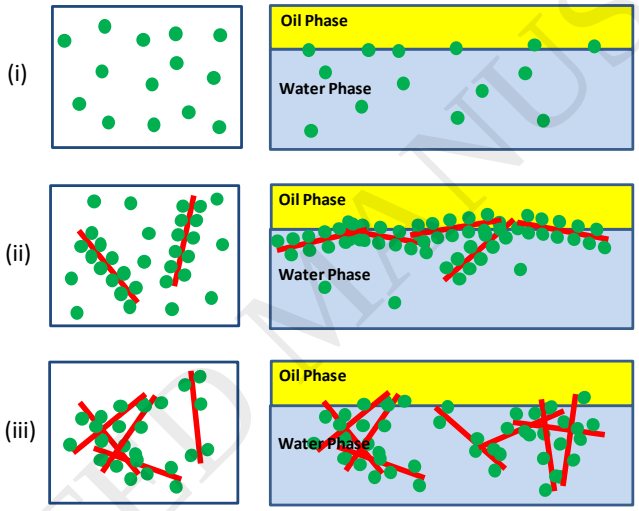

Figure 4 Proposed mechanisms for the adsorption of BLGF/AS complex at the oil-water interface.

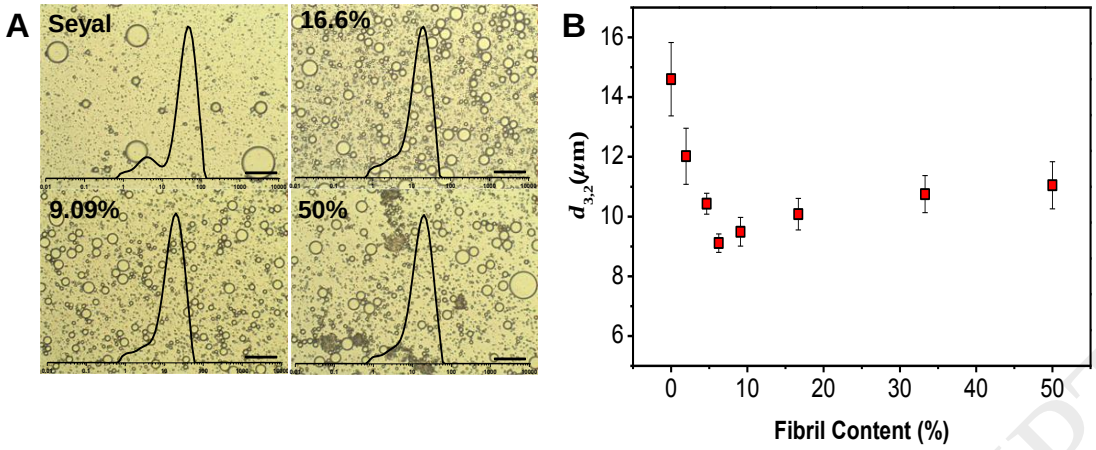

Figure 5 Optical microscope pictures and size distribution of the emulsions stabilized by BLGF/AS

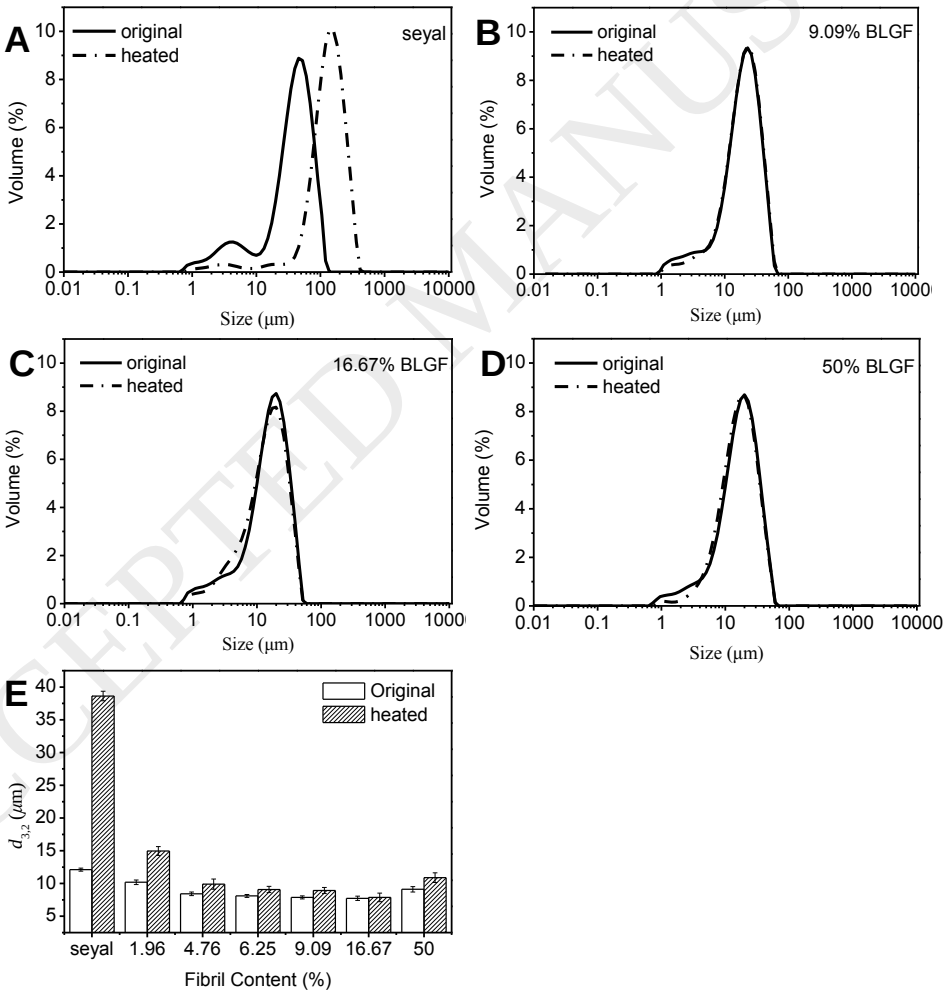

Figure 6 Impact of the heating treatment (90℃, 30min, with presence of 200 mM NaCl) on the droplet size

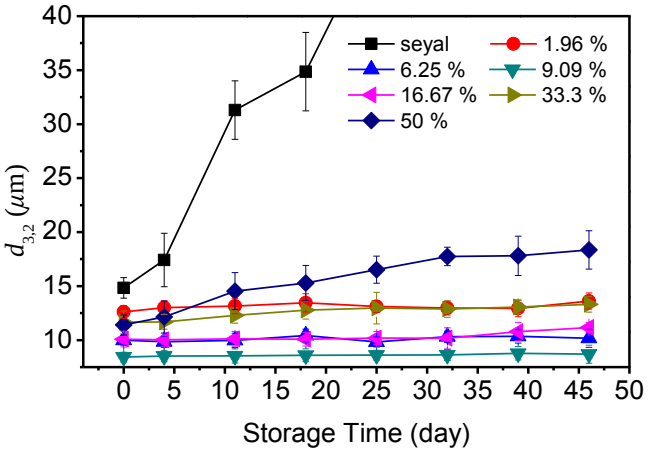

Figure 7 Evolution of the average droplet size ($d_{3,2}$) of BLGF/AS complex stabilized