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Interfacial and emulsion stabilized behavior of lysozyme/xanthan gum nanoparticles

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ABSTRACT

Interfacial adsorption kinetics and stabilizing emulsion behavior of lysozyme/xanthan gum nanoparticles (Ly/XG NPs) by variation of particle size and energy input. The interfacial rheology indicated that interfacial adsorption behavior of Ly/XG NPs displayed in a particle size manner. Increasing the particle size of Ly/XG NPs hindered its initial diffusion onto the interface. Smaller size was helpful for its fast diffusion to the interface. K_P (rate constant of penetration) and K_R (rate constant of penetration) of Ly/XG NPs were both affected by particle size. The K_P for adsorbed Ly/XG NPs increased as the particle sizes increased. K_R was considerably higher than K_P, indicating the structural rearrangement of adsorbed Ly/XG NPs played an important part in interfacial film formation. The morphology of Pickering emulsion indicated its drop sizes were determined by the oil/aqueous volume fraction and prepared style. The higher energy input the size become smaller. Based on interfacial adsorption kinetics and microstructure of Pickering emulsions, the stabilization behavior was related to particle-particle associations and conformational changes of Ly/XG NPs. This work confirmed Ly/XG NPs could form Pickering emulsion by selecting different particle size and emulsification process, and offer promising prospects in stabilizing emulsion with the demands of surfactant-free.

1. Introduction

Pickering emulsions, stabilized by colloidal or solid particle, were widely concerned and have been important ingredients in food, medicine, cosmetics and other fields [1,2]. Compared with traditional stabilizers, including small molecule and some polysaccharides, Pickering emulsions displayed higher stability and lower toxicity [3,4]. Many kinds of particle stabilizers can be used to stabilize Pickering emulsions, such as calcarea carbonica, silica, nanocrystals, fibrils, and micro-gels [5–8]. While in these particle stabilizers, food-grade particles based self-assemble are one of the most promising candidates for their excellent compatibility with food, extraordinary stability, sustained release of encapsulated ingredients and other outstanding characteristics, and have become the primary source of particle stabilizers [9]. To date, various kind of food-grade particles have been applied in fabrication of Pickering emulsions, including protein nanoparticles [10], protein fibrils [11], modified starch [12], polysaccharide/protein complex [13], and nanocrystals [14]. And not only that, many responsive Pickering emulsions could be prepared owing to the properties of particle stabilizers. For example, simple, reversible emulsion systems switched by pH could be fabricated on the basis of chitosan for its different dissolution and charge characteristics in alkaline and acidic condition [15,16].

Although many protein-based particles have been widely studied in Pickering emulsion. Regulating the emulsion properties and exploring the performance of particle stabilizers were still huge challenge. It confirmed that properties of particles, emulsifying process, the ratio of oil/water and the interaction between interfacial components exhibited remarkable influences on the interfacial stability of emulsions [17]. Among the properties of particles, particle size, surface wettability, morphology and surface charge density decided the fate of its interfacial behavior [18]. Liu et al. have verified that emulsion could not be formed at low soy protein isolate nanoparticles concentration. While the drop size decreased as the concentration improved. The interfacial adsorption behavior of soy protein isolate nanoparticles has further studied, including adsorption kinetics and structural rearrangements at the Interface [19]. Dynamics and interfacial rheology is the basis of particle stabilizers to form Pickering emulsion [20]. Diffusion of particles onto the interface,

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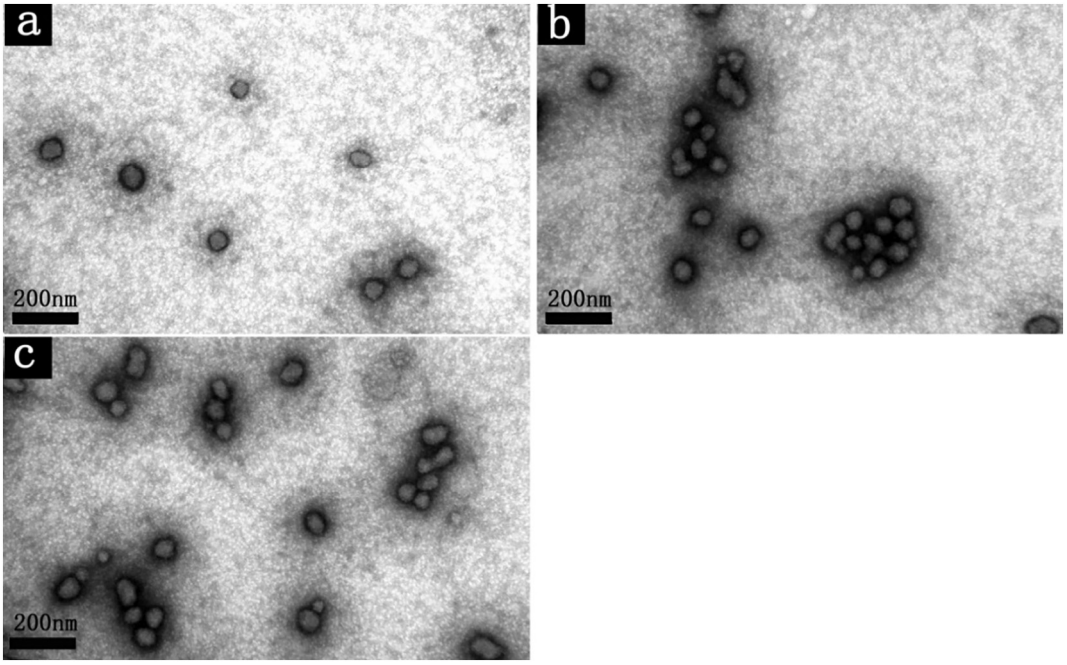

Fig. 1. Morphology of NP1 (a), NP2 (b), and NP3 (c) characterized by TEM.

penetration and structural deformation, and rearrangement of adsorbed nanoparticles at the interface were extensively studied in chitosan/gelatin complex, soy protein nanoparticles, gelatin-based nanocomplex and other particle stabilizers [21,22].

Here, we firstly reported on interfacial adsorption kinetics and stabilizing emulsions behavior of Ly/XG NPs. The both polymers are widely applied in food field. XG, used as stabilizer and thickener, is an extracellular anionic bacterial polysaccharide, consisted of β-1, 4-linked D-glucose backbone [23]. Ly is the main protein of egg white component and has been received attentions in food preservative [24].

Recently, we reported that phase transition of Ly/XG system induced by electrostatic interaction through rheology and micro-structural during the acidification [25]. Based on the self-assembly behavior of Ly/XG system after alkali-coupled heat treatment, we established tunable Ly/XG NPs with controlled organization [26,27]. In the present work, the interfacial adsorption kinetics and stabilizing emulsion behavior of Ly/XG NPs were investigated for the first time by variation of particle size and energy input. The diffusion, penetration and structural deformation, and rearrangement behavior were captured through interfacial rheology. Pickering emulsion stabilized by Ly/XG NPs were further researched and characterized. Finally, a tentative model was established to illustrate the interfacial behavior of Ly/XG NPs in stabilizing Pickering emulsions. This attempt not only confirmed Ly/XG NPs could form Pickering emulsion by selecting different particle size and emulsification process, but also provide some useful information in stabilizing Pickering emulsions for protein/polysaccharide NPs.

2. Materials and methods

2.1. Materials

Xanthan gum (XG, 3.0×10^6–2.0×10^7 Da) was purchased from Shanghai Source Biological Technology co., LTD. Lysozyme (Ly, Mw = 14.3 kDa) from chicken egg white was obtained from Sinopharm Chemical Reagent co., LTD. Other chemicals were reagent grade and used without further purification. All the solutions in the experiments were prepared using ultrapure water through a Millipore (Millipore, Milford, MA, USA) Milli-Q water purification system.

2.2. Preparation of Ly/XG NPs

Ly/XG NPs were prepared by our previous method [26]. Briefly, XG and Ly solutions (1.0 mg/mL) were stirred with purified water using magnetic stirrer at room temperature for 6 h and 2 h, respectively. The pH of mixture with different Ly/XG weight ratios (2:1, 1:1 and 1:2) was adjusted to 11.8. After 1 h stirring, the mixtures were immediately heated at 80 °C for 15 min and cooled to room temperature spontaneously. The obtained Ly/XG NPs were coded as NP1, NP2 and NP3 briefly. Before further experiment, the obtained Ly/XG NPs were filtered using 80 μm membrane to remove impurities.

2.3. Characterization of Ly/XG NPs using TEM

Transmission electron microscopy (TEM) was employed to study the morphology of Ly/XG NPs. A drop of Ly/XG NPs solution was dropped directly onto a copper grid coated carbon, and allowed to dry

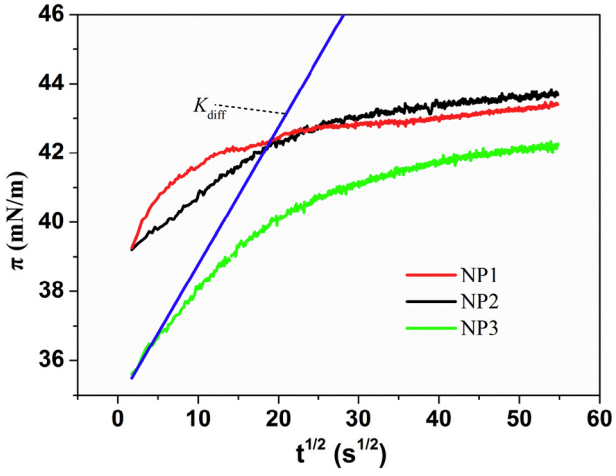

Fig. 2. Time evolution of the surface pressure (π) for the adsorption of Ly/XG NPs at the oil/water interface.