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Uniform chitosan-coated alginate particles as emulsifiers for preparation of stable Pickering emulsions with stimulus dependence

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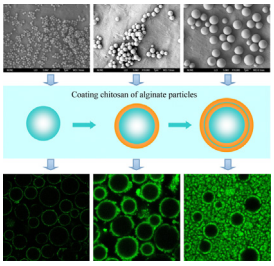
HIGHLIGHTS

- Uniform-sized alginate particles of different sizes were controllably prepared.
- Stable Pickering emulsions were fabricated by chitosan coated alginate particles.
- Particle size, pH and salt concentration had huge effects on emulsion stability.

GRAPHICAL ABSTRACT



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ABSTRACT

Owing to the absence of surfactants, Pickering emulsion shows little toxicity and emerges as an alternative for preparing potential carriers in biomedical area. Alginate particles are attractive stabilizers for this emulsion owing to their biocompatibility and pH sensitivity. However, their performance is still dissatisfactory due to the difficulty in preparing uniform submicron particles and the inappropriate hydrophilic property. In this study, we developed two strategies to circumvent above shortcomings. Uniform alginate particles with submicron to micron size were successfully prepared by premix membrane emulsification. In addition, the uniform particles were coated with relatively hydrophobic chitosan to overcome the high hydrophilicity of alginate particles. These uniform coated particles effectively stabilized the oil-in-water Pickering emulsion. It was found that the stability of emulsions was tremendously affected by particle size, and the least particle concentrations required for emulsion stability were proportional to the particle radii. Besides the particle size, the pH and salt concentration of aqueous phase was demonstrated to impact the stability of emulsions. The underlying mechanism was discussed in detail, concerning with three different-sized particles. Present work provided an effective strategy to prepare Pickering emulsion, which can be applied to design potent carriers in biomedical area.

1. Introduction

Pickering emulsion has been put forward by Ramsden and Pickering for a century [1]. Compared with conventional emulsion stabilized with surfactants, Pickering emulsion requires the self-assembly of colloidal particles at oil–water interface to stabilize the emulsion [2]. It offers many special advantages, such as low

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toxicity without using surfactant, extreme stability, reproducibility and so forth [3]. Therefore, it has been extensively studied for applications in drug delivery, cosmetics, food industries and many other fields [4–7].

Colloids of various materials have been successfully employed to prepare Pickering emulsion, including inorganic and polymeric colloids, such as silica [8], polymer latex [9], magnetic particle [7,10], graphene [11], clays [12], and polymethylmethacrylate particles [13]. However, colloids with high biocompatibility such as alginate particles are required in the applications of drug delivery. The alginate particle (Ca^{2+} as solidification agent) is sensitive to pH, swelling at alkaline pH and shrinking at acidic pH. When active drugs encapsulated in alginate particles were administrated orally, they would be protected from the intense environment in gastric area and delivered into intestines. Thus this particle is highly favored in oral drug applications [14–17]. Nevertheless, there have been very limited studies about alginate particles as emulsion stabilizers because of high hydrophilicity of alginate particles and the difficulty of preparing uniform nano or submicron particles.

Owing to the high hydrophilic property, the alginate particles are hard to arrange at the oil–water interface, leading to unstable emulsions. In order to conquer the instability induced by the high hydrophilicity of alginate particles, conventional surfactant (e.g. span-80) was usually added, which unavoidably resulted in low biocompatibility [18]. In the present study, we proposed a coating method with chitosan to modify the hydrophilicity of alginate particles and prepared stable biocompatible Pickering emulsion in the absence of surfactant, without compromising their biocompatibility and pH sensitivity.

Several methods have been employed for preparing alginate particles, such as spray drying [19], coacervation technique [20], conventional emulsification method [21]. However, the alginate particles prepared by these methods were often widely distributed, leading to decreased preparation efficiency and poor stability. Recently, membrane emulsification technique [22] was developed to prepare uniform alginate microparticles. Albeit promising, the prepared microparticles were too large to perform stability effect. To obtain desired uniform-sized particles in appropriate small size, a convenient premix membrane emulsification method [23] was preferred. The process was conducted by extruding coarse emulsions prepared by conventional emulsification method (such as stirring and homogenization) into porous membrane. And the larger droplets in coarse emulsion could be broken into smaller droplets with narrow size distribution by the shear force of the membrane pores [24]. Using this method, uniform alginate particles with submicron to micron diameters were controllably prepared in present study.

Besides property of particle material, particle size was also reported to influence the stability of Pickering emulsion. Binks et al. [9] have employed different sizes of polystyrene particles to stabilize water-in-cyclohexane Pickering emulsion and found that the droplet diameter initially increased with particle size and then remained constant. Moreover, particle sizes were reported highly related to the permeability and elasticity of solidified colloidosome [25]. To clarify this event, uniform particles with different sizes were fabricated to explore emulsion performance under different particle sizes.

Apart from property of particle material and particles size, external environmental factors, such as pH and salt concentration, are of great importance for Pickering emulsion [2,26–28]. When using lightly cross-linked poly (4-vinylpyridine)/silica nanocomposite microgel particles as stabilizer, the emulsion remained stable at high pH but exhibited demulsification below pH 3.4. It was likely due to that the microgel particles became more charged and swelling at low pH. The salt addition would strengthen the degree of ionization of the microgel particles, therefore the emulsion coalescence stability was reduced [29]. Thus in this study, we investigated the effects of pH and salt concentration on emulsion stability and delineated the underlying mechanisms of the effects on emulsion stability. In virtue of both the superior stabilizer and the optimized environment, we prepared Pickering emulsion with favorable stability and biocompatibility that caters for further biomedical application.

2. Materials and methods

2.1. Materials

Sodium alginate (molecular weight 450–550 kDa) was purchased from Acros Organics (NJ, USA). Chitosan (deacetylation degree 89%, molecular weight 50 kDa) was supplied by Zhejiang Jinke Co., Ltd. (Zhejiang, China). PO-5S (hexaglycerin penta ester) was bought from Sakamoto Yakuhin Kogyo Co., Ltd. (Japan). Paraffin and petroleum ether (boiling range 60–90 °C) were provided by Sinopharm Chemical Reagent Co., Ltd. (China). Shirasu Porous Glass (SPG) membranes were purchased from SPG Technology Co., Ltd. (Japan). The SPG premix membrane emulsification equipment (FMEM-500M) was designed by National Engineering Research Center for Biotechnology (NERCB, Beijing, China). Acetic acid and ethyl acetate was purchased from Beijing Chemical Plant (China). All the reagents were analytic grade.

2.2. Methods

2.2.1. Preparation of alginate particles

Alginate particles were prepared using premix membrane emulsification technique. Oil phase was the mixture of petroleum ether and liquid paraffin with a volume ratio of 2:1 (PO-5S as surfactant with the mass fraction of 4%). Aqueous phase (alginate solution, 1.0 wt%, 2 ml) and oil phase (60 ml) were emulsified by homogenization at 3600 rpm for 1 min to prepare coarse emulsion. Then the coarse emulsion was poured into the reservoir of premix membrane emulsification equipment and extruded by nitrogen gas through the SPG membrane to achieve uniform-sized emulsion. $CaCl_2$ solution (5 mol/L, 12 ml), as solidification agent, was dispersed into the oil phase (24 ml) by ultrasonication at 120 W for 1 min to form a mini-emulsion. This mini-emulsion and the uniform-sized emulsion prepared above were mixed together and kept in a water bath (37 °C, 5 h) to solidify the emulsions into alginate particles. After that the alginate particles were collected by washing them with petroleum ether, ethanol and water [30].

2.2.2. Preparation of chitosan-coated alginate particles

Firstly, the alginate particles were dispersed into 0.7 wt% chitosan solution and stirred for 1 h. Chitosan molecules were adsorbed onto alginate particles by electrostatic interaction. The particles were obtained and washed with acetate buffer solution (pH 4 and pH 5.5) and distilled water.

To further improve the hydrophobicity of particles, the second coating chitosan was conducted after a layer of alginate was coated. Firstly, the particles with a chitosan layer just obtained were dispersed into 0.5 wt% alginate solution and stirred for 1 h. The particles were washed with distilled water, and then they were dispersed into 0.7 wt% chitosan solution and stirred for 1 h. The final chitosan-coated particles were gained and washed with acetate buffer solution (pH 4 and pH 5.5) and distilled water.

2.2.3. Preparation of Pickering emulsions

The emulsions were prepared by mixing the particle dispersion with oil phase (ethyl acetate, the volume ratio of oil to water was 1:1), and emulsified by a vortex for 2 min. The emulsion type was determined by adding one drop of the emulsion to water or the oil