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Facile preparation of biocompatible polymer microgels with tunable properties and unique functions to solely stabilize high internal phase emulsions

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HIGHLIGHTS

- Crosslinking chitosan with genipin generated microgels with finely tunable size.
- The microgels showed unique performance to prepare HIPEs and macroporous materials.
- The HIPEs and macroporous materials have a cellular and tunable pore structure.
- The HIPEs could resist mechanical perturbation and were maintained for months.
- The microgels stabilized HIPEs due to the reduced size and increased lipophilicity.

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ABSTRACT

Simply crosslinking chitosan (CS) aggregates with genipin in an aqueous solution was found to form biocompatible polymer microgel particles with a finely tunable size and unique emulsifying property for solely stabilizing high internal phase emulsions (HIPEs). The size of the microgel particles was controlled by changing the degree of crosslinking. Such microgel particles showed a concentration advantage of more than 100-fold in emulsifying HIPEs compared with other CS analogs. In addition, the fabrication process avoided the drawbacks of labor-intensive and usage of toxic chemical reagents and solvents in other previous studies. The HIPEs and macroporous polymer materials thus formed had a cellular and tunable pore structure that could resist mechanical perturbation and were maintained for months. The strong emulsifying performance of the microgel particles was attributable to a simultaneous reduction in the size and size distribution and increase in lipophilicity during the crosslinking. This study paves the way for a promising green approach to facilely fabricate macromolecule microgel particles with tunable properties and functions for the development of HIPEs and macroporous polymer materials, which are expected to have extensive applications as biocompatible materials for delivery and scaffold design.

1. Introduction

High internal phase emulsions (HIPEs) have diverse applications in food and cosmetic formulations, drug delivery, porous scaffold fabrication and pollution treatment [1–5]. HIPEs are typically formed by emulsifying immiscible mixtures of oil and water, wherein the volume fraction of the internal phase is usually higher than 74%. HIPEs are used as a template to produce macroporous polymers that retain the structure of the emulsion after the liquid phase is removed. The macroporous polymers thus formed have a tailored pore size and interconnectivity, leading to a myriad of potential applications, including catalyst support, filtration, oil recovery, drug delivery and tissue engineering [6–10].

The core used to prepare HIPEs is an interfacial stabilizer, i.e., emulsifier. Conventional HIPEs are usually stabilized against coalescence with large amounts (5–50 wt%) of low-molecular-weight or macromolecular surfactants [11,12]. However, a large amount of surfactants is undesirable, especially in food and biomedical applications, as they have been shown to be toxic to living organisms or to have deleterious impacts on health [13]. Over the past decade, breakthroughs have been made in the fabrication of solid

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particle-stabilized Pickering-HIPEs [14–17], which require a comparatively small amount of interfacial stabilizers. Inorganic and/or synthetic organic particles, such as functionalized titania, silica, or poly(N-isopropylamide-co-methacrylic acid) (PNIPAM-co-MAA) microgel particles, have been successfully applied as stabilizers for Pickering-HIPEs [16–18]. Microgels are colloidal gel particles that consist of chemically crosslinked three-dimensional polymer networks and have recently drawn attention as emulsion stabilizers [19]. Several naturally occurring polymer particles have recently shown potential in stabilizing HIPEs, including chitin nanocrystal particles [20] and cellulose nanocrystals [21], which were prepared through acid hydrolysis of the amorphous regions of chitin and cellulose fibers, as well as gelatin [22] and bovine serum albumin [23] microgel particles formed via the anti-solvent method followed by glutaraldehyde crosslinking.

Although the preparation parameters of HIPEs (e.g., particle concentration, aqueous phase pH and salt concentration, oil type, water and oil volume fractions, etc.) have been extensively investigated in previous studies [1,18,20–23], the formation mechanism of the microgel particles and the relationship between structure and emulsifying properties have yet to be fully clarified [24–27].

Chitosan (CS) is a chitin-derived water-soluble polymer formed by a disruption of the chitin crystalline structure by electrostatic repulsion as the degree of deacetylation exceeds 60%. CS has a pKa near 6.5, and it becomes positively charged when the pH is less than 6. CS is the only natural cationic polysaccharide with widespread food and biomedical applications [28]. Recently, CS-based graft copolymers were synthesized solely to stabilize HIPEs [29,30]. However, the synthesis process was labor-intensive, and toxic chemical reagents and solvents were used in the grafting reactions. Furthermore, a high concentration of the synthesized copolymers, 10–40 wt%, was required in the aqueous continuous phase to stabilize HIPEs with an internal phase volume fraction of 0.60–0.90.

In contrast, CS microgel particles with unique emulsifying properties for stabilizing HIPEs were prepared in the present study by simply crosslinking CS aggregates with genipin in an aqueous solution (Fig. 1). Genipin, one of the key bioactive ingredients extracted from the flower of *Gardenia jasminoides E*, is a natural, water-soluble, bifunctional crosslinking reagent that reacts rapidly and spontaneously with amines in the CS chain [31]. The dynamic light scattering (DLS) and transmission electron microscopy (TEM) were combined to confirm and characterize the structure of the CS microgel particles. In addition, the confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were combined to investigate the morphology and microstructure of the formed HIPEs. Furthermore, the contact angles between the crosslinked CS microgel particles and water, the particles and the oil, respectively were measured to calculate the corresponding interfacial tensions. Finally, the dependence of the interfacial energies on the partition ratio of the microgel particles within the oil phase was calculated to further elucidate their high-performance emulsifying efficiency in producing the HIPEs with high stability.

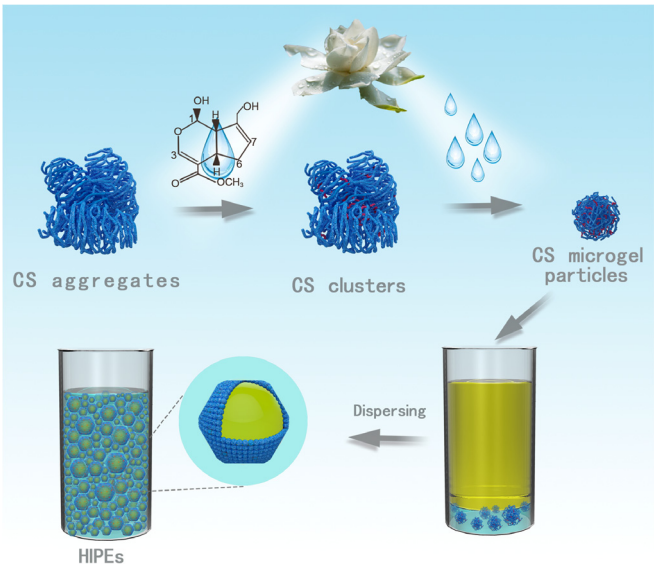

Fig. 1. The schematic for the formation of the chitosan microgel particles and the high internal phase emulsions (HIPEs).

2. Experimental section

2.1. Chemicals and reagents

Chitosan (CS) of the molecular weight 100 kDa with a 90% deacetylation, derived from crab shell, was obtained from Golden-Shell Biochemical Co. Ltd (Hangzhou, China). Genipin (purity > 98%) was purchased from Linchuan Zhixin Biotechnology Co., Ltd (Jiangxi, China). Dodecane and cyclohexane were purified by extraction with deionized water three times, which involved mixing equal volume of water and dodecane (cyclohexane) in a separation funnel by shaking and then discharging the water after phase separation. Fluorescein Isothiocyanate Isomer (FITC) was purchased from Sigma Co. (St. Louis, MO). Acetic acid was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals were of analytical grade.

2.2. Preparation of microgel particles through crosslinking of CS with genipin

For preparation of the CS microgel particles, different amounts of genipin were added to the CS solution (0.50 wt%) under pH 5.5, forming a set of crosslinked CS with the DCs of 0%, 1.6%, 2.2%, 3.3%, 8.2%, 16.4%, 32.7% and 65.5%. During this process, the solid content of the reaction system was kept consistent at 0.50 wt%. The reaction mixture was incubated at 37 °C for 24 h. The genipin crosslinked CS microgel particles were also prepared with different polymer concentrations (solid contents) of 0.05 wt %, 0.1 wt%, 0.25 wt%, and 1.0 wt%.

2.3. DLS experiments

The measurements of particle size of the genipin crosslinked CS microgel particles were performed on a Zetasizer Nano-ZS (Malvern Instruments) on the basis of DLS techniques. All measurements were made in triplicate at 25 ± 1 °C.

2.4. TEM experiments

The morphological characteristics of the CS microgel particles were examined by a high performance digital imaging TEM machine (JEM-1011, JEOL Ltd., Japan). One drop of the suspension was placed on a copper grid and the droplet was wicked away from the edge with a piece of filter paper and air dried. The fully dried samples were placed in a TEM for imaging. The accelerating voltage used was 100 kV.

2.5. Preparation of HIPEs stabilized by the CS microgel particles

The HIPEs were prepared by mixing a CS microgel particle solution and dodecane or cyclohexane with various internal phase volume fractions higher than 74% by mechanical shearing with a