INFLUENCE OF HIGH INTENSITY ULTRASONICATION ON THE FABRICATION OF POLY(LACTIC-CO-GLYCOLIC ACID)/ CHITOSAN NANOCOMPOSITES BASED ON PICKERING EMULSIONS

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Keywords: Nanocomposite, Pickering emulsions, High Intensity Ultrasonication, Nanospheres, Microcapsules

ABSTRACT

Previously, by introducing High intensity ultrasonication (HIU) technique into Pickering emulsions, a modified Pickering emulsions is proposed by our group to facilely fabricate biodegradable composite nanospheres with narrow size distribution. In this study, in order to further study the influence of HIU in Pickering emulsions on the formation of monodisperse nanospheres, comparisons between HIU-assisted and non-HIU-assisted emulsions stabilized by chitosan colloids based on different initial molecular weight were conducted. It was found that in non-HIU-assisted emulsions, no matter the difference of the molecular weight of the used stabilizers, microcapsules were formed instead of nanospheres. Moreover, in contrast to the obtain of monodisperse nanospheres from HIU-assisted emulsions, it was observed that the sizes of the resulting microcapsules are widely distributed. The illustration of the solidification process of solvent evaporation further revealed the formation of microcapsules based on non-HIU-assisted emulsions. The results therefore suggest that the assistance of HIU as a generator of strong shear force is effective in reducing emulsion droplet sizes as well as improving monodispersity and without the assistance of HIU, the formation of hollow structure can be led within larger emulsion droplets due to the deposition of the polymer crust on the wall of stabilizers as outer coating.

1 INTRODUCTION

Pickering emulsions are emulsions stabilized by colloids instead of molecular surfactants, which were firstly proposed by Ramsden and Pickering [1]. Different from conventional emulsions, the use of colloids as stabilizers instead of molecular surfactant distinguishes Pickering emulsions. Therefore, it has attracted considerable interest and recently been studied for biomedical applications, such as the fabrication of DDSs [2]. Specifically, its superiorities mainly lay in (i) better biocompatibility due to the no use of molecular surfactant, (ii) enhanced stabilized and droplet size controllability because of the deposited wall of colloidal stabilizers, (iii) facile process and scalable productivity under mild processing conditions [3].

Although efforts have been continuously invested to the study of Pickering emulsions for the fabrication of various DDSs to cater requirements under different circumstances, one of the most prominent limitations of current approaches based on Pickering emulsions is the lack of facile control of system sizes and size distributions, which hinder the expansion of its application scope. The majority of the resulting systems fabricated from Pickering emulsions is in micron-scale and the realization of nanosized systems with narrow size distribution rely heavily on the use of complex devices and custom-made auxiliaries [4-6]. To overcome the aforementioned limitations, HIU-assisted Pickering emulsions based on the use of soft colloids as stabilizers was proposed recently by our group [7]. The successful fabrication of monodisperse drug-loaded nanospheres can be attributed to the incorporation of the HIU technique. Investigations of the influence of processing conditions and material characteristics on the

proposed fabrication method were carried out. By simply varying the applied amplitude, nanospheres ranged from 255.1 to 824.8 nm can be facilely obtained. Specifically, by applying a low amplitude of 20% of the total power of the HIU and stabilizers based on polymers with low molecular weight (M_w) of 3000 to 6000, drug-loaded systems with a size of 255.1 nm and a very low level of polydispersity index of 0.078 can be obtained.

In fact, the use of HIU technique mainly contributes to two aspects: (i) depolymerization of the biodegradable soft colloidal stabilizers and (ii) well-dispersion of emulsion. In this study, in order to further confirm the influence of HIU in Pickering emulsions, investigations were carried out by comparing the emulsions prepared with and without the assistance of HIU. Moreover, in order to illustrate the role between HIU and the size of the stabilizers in Pickering emulsions, the differences among non-HIU-assisted emulsions based on the use of stabilizers with different initial M_w were launched and discussed.

2 MATERIALS AND METHOD

2.1 MATERIALS

Chitosan with molecular weights (M_w) of 500,000 (degree of deacetylation ≥ 95%) and with Mw of 3000 to 6000 (degree of deacetylation ≥ 90%) were purchased from HeFei BoMei Biotechnology (China). PLGA 50:50 (Mw = 50,000) was obtained from Jinan Daigang Bio-Technology (China), dichloromethane (CH_2Cl_2) was purchased from Sigma Aldrich, acetic acid (AA) and sodium hydroxide (NaOH) were supplied by the International Laboratory (United States), and ethanol was purchased from Merck. All chemicals and reagents were of analytical grade and used without further purification. The water used in all experiments was purified by deionization and filtration with a Millipore purification apparatus to a resistivity greater than 18.0 MΩ cm.

2.2 PROCESSING

2.2.1 PREPARATION OF CHITOSAN COLLOIDS

First of all, chitosan solutions were prepared based on polymer protonation. Chitosan powder was dissolved in a 1% (v/v) aqueous solution of AA under magnetic stirring at a rate of 600 rpm for 12 h and stored overnight to allow complete hydration and dissolution. To further form chitosan colloidal solutions, the pH of chitosan solutions was adjusted to 6.5 by adding NaOH solutions (1 M) and stirred at 600 rpm for 15 min. Thereafter, to depolymerize the obtained chitosan colloids, chitosan colloidal solutions were treated with a high-intensity ultrasonic processor (SKL-250W, Ningbo HaiShu Sklon Electronic Instrument Co) equipped with an ultrasonic probe (SKL-IIN, 4 6), under 40% amplitude for 8 min, using an ice bath to avoid over- heating during the ultrasonication. The concentrations of the HIU-treated chitosan colloidal solutions were 0.1% w/v. Unless otherwise specified, all chitosan colloids were prepared under the same conditions, and the HIU treatments were conducted with the same equipment as described above.

2.2.2 FABRICATION OF CHITOSAN COLLOID-STABILIZED EMULSIONS

Oil-in-water (O/W) emulsions stabilized by HIU-treated chitosan colloids were prepared by mixing 0.1% w/v HIU-treated chitosan colloidal solution with 2% w/w PLGA/CH_2Cl_2 at an oil volume fraction (Φ) of 0.05, with magnetic stirring for 15 min at 600 rpm.

To prepare HIU-assisted emulsions, the obtained coarse emulsions were homogenized by the same high-intensity ultrasonic processor as described at an amplitude of 40% for 8 min. An ice bath was placed to avoid over-heating.

To prepare non-HIU-assisted emulsions, the obtained coarse emulsions were continuously stirred at a stirring rate of 600 rpm for 8 min.

2.2.3 FABRICATION OF PLGA/CHITOSAN NANOCOMPOSITES

To obtain solid systems, solvent evaporation was further carried out. The obtained emulsions were magnetically stirred at 600 rpm for 3 h in a 37 ℃ water bath to remove volatile solvents. Afterwards, to collect the resulting systems, at least three centrifugation-redispersion cycles were applied in deionized water. Each centrifugation was conducted at 12,000 rpm for 10 min.

2.3 CHARACTERIZATION

The optical microscopy images of emulsions were captured by the use of a Leica optical microscope. Specimens were prepared by first using a dropper to withdraw a small amount of corresponding emulsions or samples. Then, each specimen was dropped onto a glass slide and placed on the specimen stage of the microscope before examination.

3 RESULTS AND DISCUSSIONS

As one of the dominant factor of the resulting system size in Pickering emulsion is the size of the stabilizers, the influence of HIU on chitosan colloids as stabilizers was firstly discussed. HIU-treated chitosan colloids have been comprehensively studied for the preparation of long-term stable Pickering emulsions [8]. It was demonstrated that, under the treatment of HIU, the M_w of HIU-treated chitosan colloids was significantly smaller. Such result further confirms the important role of the size of stabilizers in Pickering emulsions, that is, the smaller the size of stabilizers, the smaller the size of emulsion droplets can be obtained. Similarly, in our previous study, by using HIU-treated chitosan colloids as stabilizers, smaller nanospheres with narrow size distribution can be obtained from the application of stabilizers with smaller initial M_w (Figure 1). The influence of processing parameters and material characteristics have been studied and discussed in the previous study.

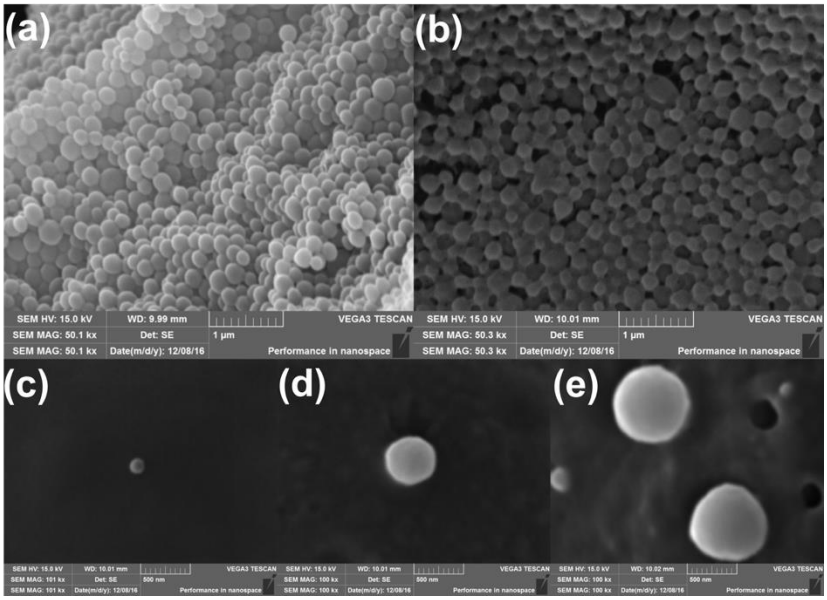


Figure 1 SEM images of PLGA nanoparticle coated by different chitosan colloids based on chitosan precursors with Mw of 3000 to 6000 (a, b, c), 50,000 (d) and 500,000 (e), where (c) was treated with 0.1% (v/v) AA solution for the dissolution of the outer coating of chitosan. Reproduced from Reference [7], Copyright 2017, Elsevier.

However, the aforementioned results may not be obtainable without the assistance of HIU on emulsification. By using HIU-treated chitosan colloids as stabilizers, emulsions prepared with no assistance of HIU for emulsification lead to the formation of microcapsules with wide size distribution as shown in Figure 2. It can be observed that by using HIU-treated chitosan colloids based on chitosan

with M_w of 3000 to 6000, the size of the obtained microcapsules was around 200 μm (Figure 2a). Moreover, the sizes were widely distributed (Figure 2b).

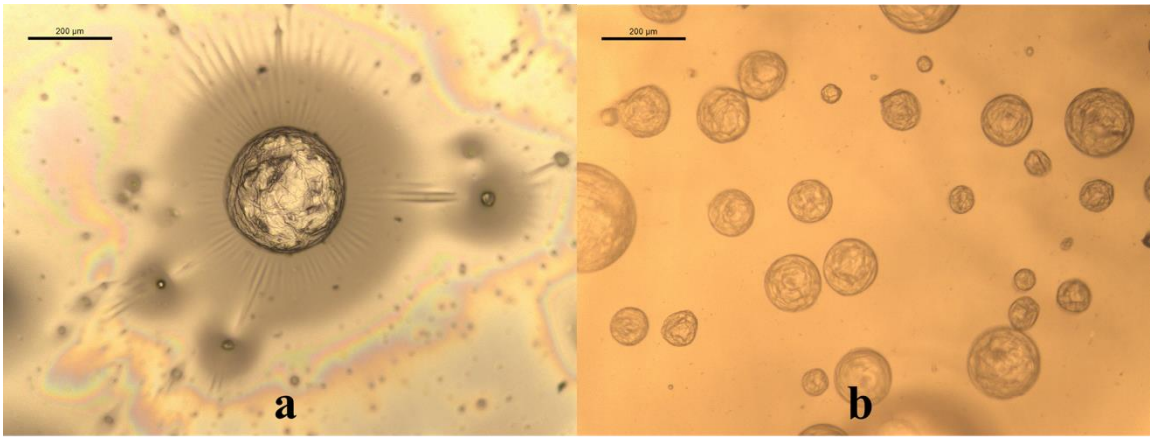
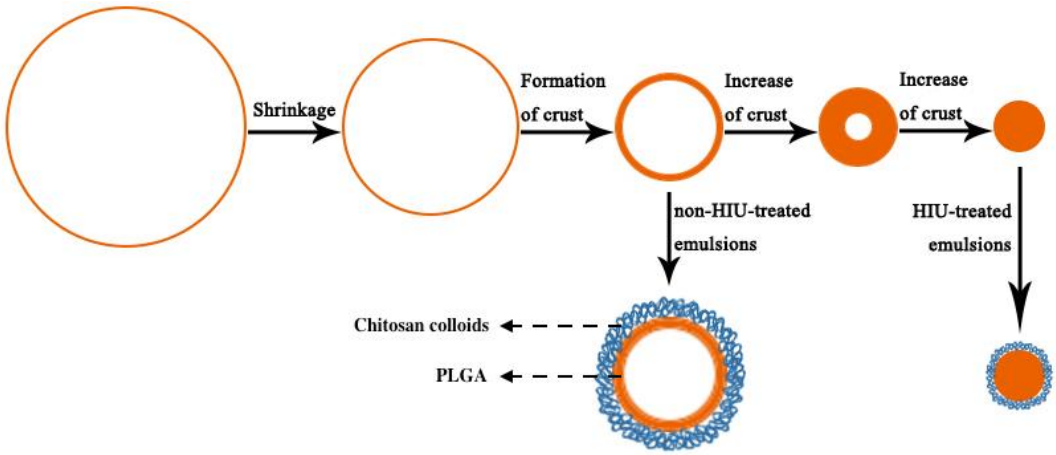


Figure 2 Optical images of PLGA/chitosan microcapsules prepared by chitosan colloids based on chitosan precursors with Mw of 3000 to 6000. The scale bar in this image is represented 200 μm.

This phenomenon may be attributed to the well emulsion dispersity of HIU. Different from common agitation, HIU has been widely applied in biomedical applications for emulsifications in generating smaller emulsion droplets due to the intensive compression and shear waves generated by HIU [9]. As shown in Scheme 1, during solvent evaporation, the polymer inside emulsion droplets is continuously deposited on the coating of stabilizers and a wall crust can be therefore formed [10]. With the assistance of HIU, smaller emulsion droplets can be formed and while bigger emulsion droplets may be formed due to the absence of HIU. Hence, under the same polymer concentration, hollow structure is formed inside droplet with larger size and solid spheres can be formed with in droplet with smaller size. Such process was illustrated in Figure 3 as well. Based on non-HIU-assisted emulsions, it can be clearly observed that droplets experienced shrinkage during the process of solvent evaporation and microcapsules were eventually formed.



Scheme 1 Evolution of droplet sizes during solvent evaporation for system solidification.

In fact, Wang et al. reported a similar result by using chitosan colloids as the stabilizers for the fabrication of PLGA/chitosan nanocomposite microcapsules [11]. In that study, the chitosan colloids were applied directly without the pre-treatment of HIU and the emulsions were prepared by either simple hand-shaking or agitation at 8000 rpm. As a result, chitosan colloid-coated PLGA microcapsules were obtained after solvent evaporation and their sizes mainly ranged from tens to hundreds of microns based on the calculation of 200 droplet sizes.

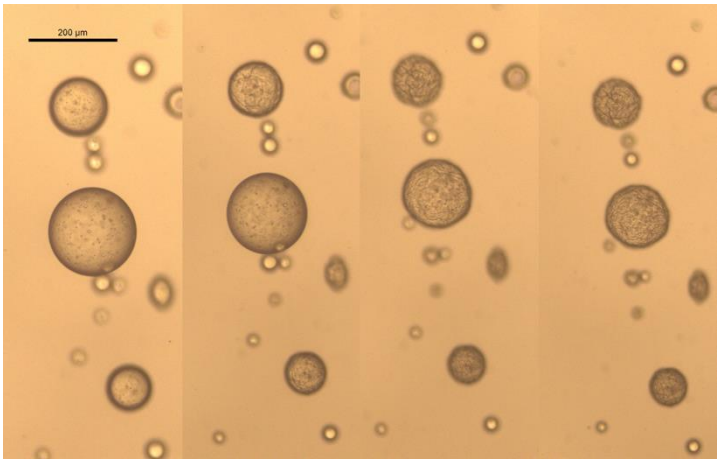


Figure 3 Optical image of the solvent evaporation process for the formation of microcapsules fabricated from the use of chitosan colloids based on chitosan precursors with M_w of 500,000. The scale bar in this image is represented 200 µm.

Interestingly, by comparing the system sizes obtained from using chitosan colloidal stabilizers with different initial M_w, as shown in Figure 2 and 3, their sizes were within the same range, which is of several hundreds of microns. Hence, it can be discovered that without the assistance of HIU for emulsification, the influence of the size of the applied stabilizers on the obtained system sizes is little.

4 CONCLUSIONS

In this study, the influence of HIU on fabrication of PLGA/chitosan nanocomposites based on Pickering emulsions has been further studied. According to the experimental results, it is found that although the size of the colloidal stabilizers play an important role on the resulting system sizes, without the assistance of HIU for Pickering emulsification, nanocomposites with different structures and size distribution, i.e. microcapsules with wide size distribution instead of nanospheres with narrow size distribution, will be obtained. In combination with the results that we obtained from our previous study, it can be understood that only in HIU-assisted emulsions, the resulting system sizes can be controlled by the size of the colloidal stabilizers. Moreover, in non-HIU-assisted emulsions, the M_w of the used colloidal stabilizers has little influence on the sizes of the obtained microcapsules.

ACKNOWLEDGEMENTS

The work described in this paper was supported by the Research Committee of the Hong Kong Polytechnic University (Project code: RUF0).

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