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Investigation of the Influence of Emulsification Parameters on the Size Distribution of Superparamagnetic-micellar Nanoparticle

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Abstract - Ultrasonic emulsification is one of preferred methods for the preparation of nano-sized particle. Several process parameters affecting particle size distribution have been reported. In our previous study, superparamagnetic micelles consisting of lipophilic superparamagnetic iron oxide (SPIO) nanoparticles and an amphiphilic chitosan derivative, carboxymethyl-hexanoyl chitosan (CHC), were obtained by this approach. In order to further control particle size of micelle, parameters including energy input, ultrasound duration, stabilizer addition, co-solvent addition, emulsifier concentration and volumes of emulsion were systemically investigated. Material synthesis and characterization were conducted by established processes. Particle size was analyzed by the dynamic light scattering method and confirmed by transmission electron microscopy. Results showed that the amount of stabilizer, lipophilic SPIO, as well as emulsion volume did not significantly affect micelle size. Smaller micelle size was observed when a lower concentration of emulsifier, CHC, was given. However, particle size was decreased with increasing energy input, ultrasound duration and the amount of co-solvent, alcohol, added. Nevertheless, larger micelle size was obtained with higher emulsification temperature. This study might provide valuable information for the manipulation of the size of superparamagnetic micelles for further applications.

Keywords**:** Nanoemulsion, Ultrasonic emulsification, Superparamagnetic iron oxide, Amphiphilic chitosan derivative, Micelle fabrication

1. Introduction

Nanotechnology has received the greatest attention because of its potential that can literally revolutionize each field in which it is being exploited. The importance of nanotechnology in drug delivery is in the concept and ability to manipulate molecules and supramolecular structures for producing devices with programmed functions. In biotechnology and medicine, by controlling the composition, structure, and function of the nanosized polymer materials, such as nanoparticles (Panyam and Labhasetwar, 2003), micelles (Hu et al., 2004), and nanocapsules (Zheng and Liu, 2007), they can serve as effective vehicles for drug delivery, drug controlled release, and gene therapy. Moreover, since first introduced in 2002, the concept of thranostics, the integration of diagnosis and therapy in a single box, has been widely accepted and applied to the clinical treatment of many diseases (Kelkar and Reineke, 2011). Several multifunctional nanoparticles carrying both drugs and contrast agents shows great potential to rapidly assess and adjust treatment to the needs of the individual, therefore the development of theranostic nanoparticles is spurring in recent years (Janib et al., 2010).

In our previous study, an amphiphilic chitosan derivative, carboxymethyl-hexanoyl chitosan (CHC), was successfully synthesized. It had been first developed as a hydrogel with great water-retentive and drug encapsulating abilities (Liu et al., 2006), afterwards had been fabricated to be a hollow, shelled drug carrier (Liu et al., 2008) . By taking the advantage of the self-assemble capability, the CHC was employed for drug encapsulation, especially poorly water-soluble molecules. Also, upon adjusting the composition during fabrication, we observed that micellar structure was generated. Meanwhile, because MR image (MRI) can provide not only structural information at high resolution (i.e. at the cellular and molecular level) but also functional information regarding living bodies in a non-invasive manner, several newly developed drug vehicles have been proposed in order to

demonstrate MR image contrast (Chertok et al., 2008; Jain et al., 2008). For the combination of MR imaging and drug encapsulating functionalities in our designed vehicle, hydrophobic ultrasmall superparamagnetic iron oxide (SPIO) nanoparticles, a well-known MR contrast agent (Xu and Sun, 2009), was introduced into micelle fabrication process.

Particle size distribution of developed nanoparticles has always been taken into consideration for further application in medical use. Suitable size plays an important role for particles to circulate and distribute properly in the body as well as enhance the efficiency of cellular internalization (Maeda et al., 2000; Zhang et al., 2009). Hence in this study, we focused on the influence of several fabrication parameters including energy input, ultrasound duration, stabilizer addition, alcohol addition, emulsifier concentration and volumes of emulsion, on micelle particle size distribution, thereby collected fundamental information to support the design and fabrication of theranostic nanoparticles.

2. Material and Methods

Chitosan (Mw = 215 000 g/mol, deacetylation degree = 85-90%), 2 -Propanol, sodium hydroxide, chloroacetic acid, and hexanoyl anhydride, Iron(III) acetylacetonate were supplied from Sigma-Aldrich. All other chemical reagents in the study were analytical grade.

2. 1. Synthesis of CHC

The synthesis of CHC using N,O-carboxymethyl chitosan (NOCC) as a precursor was reported in our previous study(Liu et al., 2006). The NOCC samples (2 g) were dissolved in distilled water (50 ml) and stirred for 24 h. The resulting solutions were mixed with methanol (50 ml), followed by the addition of hexanoic anhydride at concentrations of 0.5 M. After the reaction time of 12 h, the resulting solutions were collected by dialysis membrane (MWCO = 12,400) after dialysis with ethanol solution (25% v/v) for 24 h.

2. 2. Preparation of Lipophilic SPIO Nanoparticles

Lipophilic SPIO nanoparticles were prepared by following a method developed by Sun et al.(Xu and Sun, 2009). Briefly, 2 mmol of Iron(III) acetylacetonate, 10 mmol of 1,2hexadecanediol, 6 mmol of oleic acid and 6 mmol of olecylamine were dissolved in 20 ml of diphenyl ether and refluxed in 100 ℃ for 30 minutes under a flow of nitrogen. After that, the mixture was heated to 200 ℃ for 1 hour followed by another heating at 265 ℃ for 30 minutes. After centrifugation and washing, the SPIO nanoparticles were collected and re-dispersed into ethanol.

2. 3. Preparation of CHC/SPIO Micelles

The prepared lipophilic SPIO nanoparticles were dispersed with hexane (100 l) and mixed with 15ml CHC aqueous solution (0.125% w/v). The mixture was placed in an ice bath and sonicated by probe sonication (XL2000, Misonix Inc., USA) for 2 min. Co-solvent, ethanol, was added prior to sonication. For the temperature-comparison group, reaction mixture of the 37℃ group was kept in a 37℃ water bath. The resulting CHC/SPIO micelles could be collected by magnetic separation.

2. 4. Material Characterization

Attenuated total reflectance Fourier transformed infrared spectroscopy (ATR-FTIR) spectra were recorded on a spectrometer (PerkinElmer, Spectrum 100S). The ATR-FTIR spectra were taken with a resolution of 2 cm^{-1} in the range of 4000-650 cm^{-1}. Microstructural observations were performed by transmission electron microscopy (TEM) (JEOL, JEM-2000EX Ⅱ) at 100 keV. The size distributions of the drug vehicles were measured by a particle size analyzer (Malvern, ZS90) using a dynamic light scattering (DLS) method (measurement capability 3–5000 nm).

3. Results and Discussion

CHC, SPIO nanoparticle and CHC/SPIO micelles were successfully prepared, as shown in fig. 1. Conjugation of hydrophilic carboxymethyl group (diminish of peak at 2900 cm^{-1} and increment of C=O stretch intensity at 1730 cm^{-1}) in NOCC and hydrophobic hexanoyl group (increment of C -H stretch signal around 2900 cm^{-1}) in CHC was confirmed by ATR-FTIR, respectively. Meanwhile, even size (about 2 nm) of SPIO particle was observed under TEM. Micelle was formed due to the self-

assembly characteristic of CHC that encapsulated lipophilic SPIO nanoparticles into the core, generating a round, solid structure.

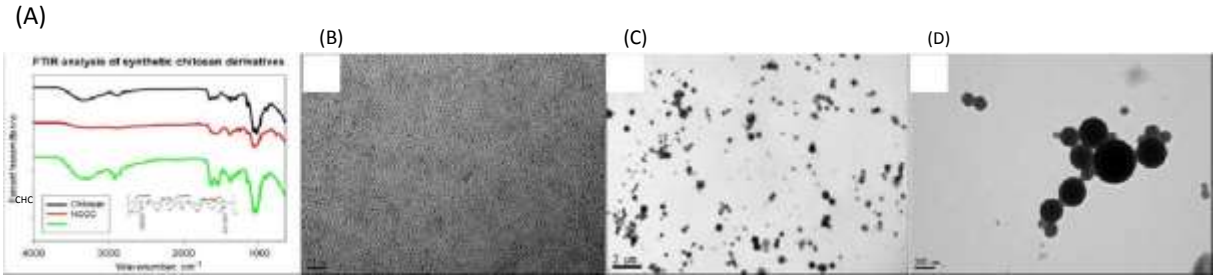

Fig. 1. Characterization of synthetic materials and fabricated SPIO/CHC micelles. (A) Structural formula and FTIR spectrum of amphiphilic chitosan derivatives. (B) TEM image of SPIO nanoparticle. Image was obtained under 500,000x magnification. (C) TEM picture of fabricated SPIO/CHC micelle. Images were obtained under 6,000x and (D)60,000x magnification.

Most important parameters of ultrasonic liquid processing are amplitude, pressure, temperature, viscosity, and concentration, as described by Hielscher (2007). Here we found that two energy-related parameter-power output (fig. 2A) and time of exposure (fig. 2B) gave rise to narrow down the particle size. Factors related to surface intensity have consequent effect on the power output into the system. In this study, co-solvent used and concentrated emulsifier might lead the difference viscosity of system, thus resulted significant change of particle size distribution (fig. 2C and 2D). Temperature is one of surface intensity generating factor, which resulted alteration of particle size as expected (fig. 2E). While reactor volume also plays an intensity-related factor, no obvious change of particle size was observed (fig. 2F). It might due to the relatively small volume of reaction that generated slight, non- considerable effects. Lipophilic SPIO nanoparticles act as stabilizer that performs structure support into the CHC molecules during micelle formation. Since the amount of fundamental structural material, CHC, remains equal in the comparing group, no significant effect by the amount of stabilizer was observed (fig. 2G).

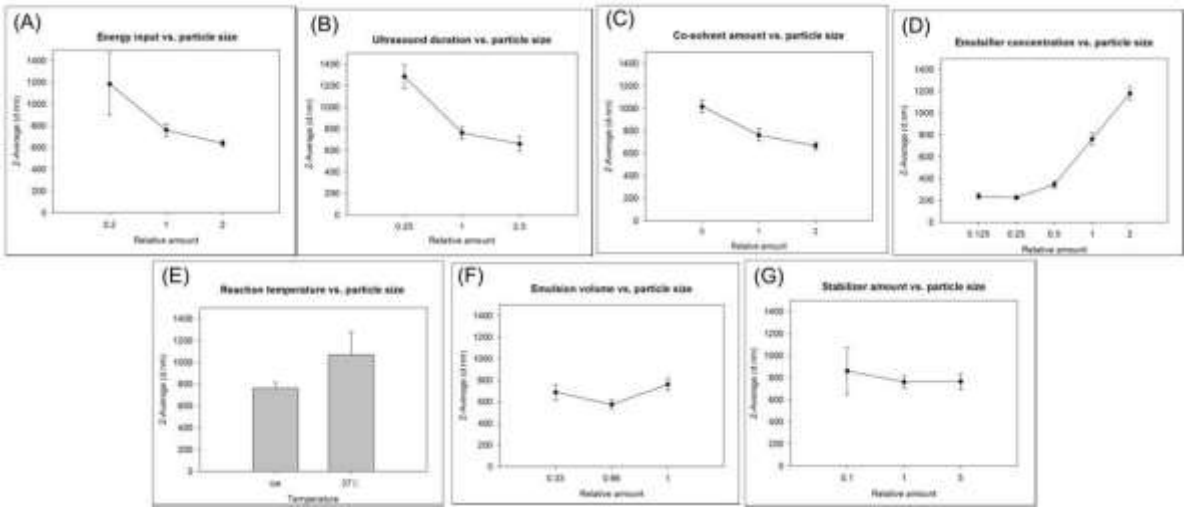

Fig. 2. Comparative analysis of SPIO/CHC micelle particle size distribution between different process groups. (A) Comparison of energy output. (B) Differences between time of exposure. (C) Effect of addition of co-solvent. (D) Influence by increment of emulsifier concentration. (E) Temperature effect on particle size distribution. (F) Resulting size distribution with changes of reaction volume. (G) Particle size generated with different stabilizer amount. All significant differences of results were statistically inferred by one-way ANOVA analysis.

4. Conclusion

In this study, the relationship between size distribution of micelle and several fabrication parameters were systemically analyzed. The volume of reaction as well as the amount of stabilizer, SPIO nanoparticle, did not affect the size of micelle. Positive correlation was observed in the

increment of emulsifier, CHC, concentration and temperature of emulsification groups. Negative correlation was obtained among the decrement of energy input, ultrasound duration and co-solvent addition groups. The fundamental structure forming information collected provides supporting evidence for the design and fabrication of theranostic nanoparticles with suitable particle size.

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