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Mechanical stability of hollow spherical nano-aggregates as ultrasound contrast agent

Kunn Hadinoto*

School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637459, Singapore

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ABSTRACT

Gas-filled hollow nanoparticulate aggregates designed for use as an ultrasound contrast agent and as an ultrasound-mediated nanoparticulate drug delivery vehicle are manufactured by spray drying of nanoparticulate suspension at a fast convective drying rate. The gas outward diffusion from the hollow particles during insonication reduces the shell mechanical stability hence shortening the lifespan of the ultrasound contrast agent. The present work aims to develop a formulation method to produce micron-size hollow nanoparticulate aggregates with high shell mechanical stability by controlling the shell thickness-to-particle radius (S/R) ratio. The impacts of changing (1) the spray drying parameters, (2) nanoparticulate suspension concentration, and (3) surfactant inclusion (i.e. phospholipids) on the particle morphology and the S/R ratio are investigated. Biocompatible PMMA-MeOPEGMA nanoparticles of varying sizes (i.e. 50 ± 20, 110 ± 40, and 230 ± 80 nm) are used as the model nanoparticles. The results indicate that the S/R ratio increases with decreasing particle size and the shell mechanical stability is linearly dependent on the S/R ratio. The effects of the spray drying parameters and nanoparticle concentration are found to be minimal in the absence of the phospholipids. The S/R ratio can be significantly increased by using larger size nanoparticles with the phospholipids inclusion.

1. Introduction

Ultrasound imaging is one of the most widely performed non-intrusive medical diagnostic tests, whose operation relies on its ability to detect and quantify the backscattered acoustic signal from the organ tissue being imaged. The traditional ultrasound imaging, however, lacks the capability to image microvascular blood flow in an organ tissue due to the similar echogenicity levels of the backscattered acoustic signals from the tissue and the blood capillary. For that reason, hollow micron-size particles acting as an ultrasound contrast agent are locally delivered by an intravenous injection, prior to the tissue perfusion imaging, to enhance the signal amplitude of the blood flow relative to that of the tissue. Ultrasound contrast agents typically consist of a gas core that is encapsulated by a solid shell of polymer or lipid materials to form hollow gas-filled particles. In addition to their use as an ultrasound contrast agent, the hollow particles can also be employed as a targeted ultrasound-mediated drug delivery vehicle in which ultrasound-mediated destruction of the shell, which is loaded with the drug, triggers the therapeutic release (Chen et al., 2006; Lum et al., 2006).

The ultrasound contrast enhancement in the presence of the hollow particles is attributed to (1) a strong acoustic signal generated by the compressible gas, and (2) the large density difference between the gas-filled particles and the surrounding tissue (Lindner, 2004). The effectiveness of the ultrasound contrast agent is governed by (1) the particle size, (2) the gas aqueous diffusivity, and (3) the shell mechanical stability. A large particle size is preferred as it generates a strong acoustic signal. Nevertheless, the particle size must not exceed the blood capillary size (i.e. <10 μm) to ensure its capillary passage. To prolong the lifespan of the contrast agent, low diffusivity gases (e.g. perfluorocarbons) are often used in place of air to minimize the gas outward diffusion from the shell that is often defected during insonication.

The shell can be fragmented or even completely destructed under a strong ultrasound field allowing the gas to diffuse out through the shell defects. A polymeric shell is found to exhibit a higher mechanical stability than a lipid shell, though the lipid shell is easier to form via its self-assembly process and it exhibits a higher echogenicity level compared to the polymer shell (Ferrara et al., 2007). The shell mechanical stability of a hollow particle is governed in the classical mechanics theory by the ratio of the shell thickness to the particle radius (S/R), where S is the shell thickness and R is the hollow particle radius (Landau and Lifshitz, 1976). Hollow particles with a high S/R ratio are less prone to the fragmentation and consequently a higher ultrasound frequency is needed to destabilize the shell (Raisinghani and DeMaria, 2001).

* Tel.: +65 6514 8381; fax: +65 6794 7553.
E-mail address: kunnong@ntu.edu.sg.

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Nevertheless, the use of a polymeric shell with an excessive S/R ratio has its drawbacks. First, the high S/R ratio indicates that a less amount of gas is being encapsulated resulting in a lower echogenicity level. Second, a rigid polymeric shell significantly increases the resonance frequency of the hollow particles, which necessitates the use of high-intensity ultrasound to achieve an optimal echogenicity level that is typically obtained at the resonance frequency of the ultrasound contrast agent (Goertz et al., 2007). The use of high-intensity ultrasound may negatively affect the polymeric shell integrity hence limiting the use of the hollow particles as a drug delivery vehicle (Rossi et al., 2008). Therefore, the ability to produce hollow particles with a controllable shell thickness is crucial in the design of ultrasound contrast agents.

A two-step process of emulsification followed by drying is typically employed to produce the hollow particles in the dry-powder form, which are later dispersed in an aqueous solution to create an injectable form of the ultrasound contrast agent. The process consists of (1) an emulsification step in which a liquid/solid core of a volatile foaming agent (e.g. ammonium bicarbonate) is encapsulated by a polymeric shell, and (2) a drying step in which the liquid/solid core is removed by either freeze or spray drying to create the hollow structure (Bjerknes et al., 1997; El-Sherif and Wheatley, 2003; Narayan and Wheatley, 1999; Straub et al., 2005). Alternatively, Pisani et al. (2006) describe a one-step emulsification technique to encapsulate liquid perfluorocarbons inside a polymeric shell to produce an aqueous suspension of microcapsules, whose S/R ratio is controlled by modifying the polymer-to-perfluorocarbons ratio. On a similar note, a production of nanocomposite hollow particles by homogenization of surfactant-coated polymeric nanoparticles using micron-size bubbles as a template is described by Schmidt and Roessling (2006).

In the present work, a novel yet simple technique to produce polymeric hollow particles with a controllable shell thickness, by means of spray drying of polymeric nanoparticulate suspension, is developed. The spray-dried particles exhibit a hollow structure, whose shells are composed of nanoparticulate aggregates that are held together by physical attractive forces (i.e. capillary and van der Waals forces). The simplicity of a spray drying process makes this technique significantly less time-intensive compared to the aforementioned techniques involving both emulsification and drying steps. Furthermore, the use of polymeric nanoparticles offers a versatile platform in ultrasound-mediated nanoparticulate drug delivery as the nanoparticles can be specifically formulated to suit the hydrophobicity and therapeutic release requirement of the drug.

The present technique was first developed by the authors to manufacture micron-size carrier particles of nanoparticulate drug for pulmonary drug delivery using a dry powder inhaler (Hadinoto et al., 2007). The specific aim of Hadinoto et al. (2007) was to manufacture hollow spherical nanoparticulate aggregates with a large geometric diameter ($d_G \approx 10$–$15\,\mu m$) to improve the aerosolization efficiency and therapeutic efficacy of the inhaled particles. The shell mechanical stability of the hollow nanoparticulate aggregates, however, was not examined by Hadinoto et al. (2007) because the particles were not to be exposed to high-intensity destabilizing forces in a dry powder inhaler. On the other hand, the shell mechanical stability is of significant importance in the present work as the hollow nanoparticulate aggregates are to be used as an ultrasound contrast agent that is routinely exposed to high-intensity ultrasonication.

The detailed physical mechanism behind the hollow nanoparticulate aggregate formation is illustrated in Fig. 1. Evaporation of the liquid from the droplet surface causes the nanoparticles at the receding liquid–vapour interface being exposed to the vapour phase. As the surface energy of a solid–vapour interface is greater than that of a liquid–vapour interface, the exposed nanoparticles migrate toward the droplet centre to minimize their surface energy. To produce the hollow nanoparticulate aggregates, a fast convective drying rate, where the time for the liquid evaporation is shorter than the time needed by the nanoparticles to diffuse back toward the droplet centre, is required. A fast convective drying rate is obtained when the local Peclet number (Pe) is significantly larger than unity. Pe defined in Eq. (1) signifies the relative importance of the time scale of the nanoparticle diffusion (R^2/D_S) with respect to that of the convective drying rate (τ_D).

$$Pe = \frac{r^2}{\tau_D D_S} \tag{1}$$

where r, τ_D, and D_S are the droplet radius, drying time, and nanoparticle diffusion coefficient, respectively.

For $Pe \gg 1$, the nanoparticle diffusion rate toward the droplet centre is slower than the convective drying rate hence resulting in the shell formation. As the shell begins to form, the capillary force generated by the meniscus formed in the gap between the nanoparticles drives the nanoparticles closer forming the nanoparticulate aggregates. This attractive capillary force, however, is resisted by the repulsive electrostatic force acting as a stabilizer against the aggregation process. The competing interaction between these two forces leads to a shell buckling phenomenon, which influences the resultant shell thickness and is manifested in the formation of dimpled spherical hollow particles (Tsapis et al., 2005).

Fig. 1 displays the three types of particle morphology that are typically formed due to the shell buckling phenomenon. They are (A) dimpled spherical hollow nanoparticulate aggregates with geometric diameter between 5 and 15 μm, (B) 2–5 μm convex-shaped solid (i.e. non-hollow) aggregates, and (C) very fine crumpled solid aggregates (<2 μm). The ideal morphology for the ultrasound contrast agent is the type A morphology with a controllable S/R ratio and geometric size smaller than 10 μm. The types B and C morphology are likely caused by (1) an excessive shell buckling that breaks the hollow nanoparticulate aggregates into non-hollow fragments, or (2) the presence of a surfactant that reduces the spray droplets stability causing their initially spherical shape to be easily distorted as the droplets travel along the drying chamber.

The specific aim of the present work is to develop a formulation method to produce hollow spherical nanoparticulate aggregates with controllable shell thickness and to examine the impact of varying shell thickness on the shell mechanical stability. For that purpose, the effects of (1) the spray drying operating condition, (2) the nanoparticulate suspension concentration, and (3) the surfactant inclusion (i.e. phospholipids) on the S/R ratio and particle morphology are investigated for a wide range of nanoparticle sizes. Biocompatible PMMA-MeOPEGMA nanoparticles (i.e. poly(methyl-methacrylate) conjugated with methoxy(polyethylene-glycol)methacrylate) are used as the model nanoparticle because acrylic-based polymer, such as PMMA-MeOPEGMA, is well known to exhibit a high mechanical strength suited for the spray drying process, where a high shear force is exerted by the drying gas on the spray droplets containing the polymeric nanoparticles.

2. Materials and methods

2.1. Materials

The monomers for the synthesis of the polymeric nanoparticles i.e. methyl methacrylate (MMA), butyl acrylate (BA), the initiator 4,4-azobis(4-cyanovaleric acid) (carboxy ADIB, purity ≥75%), ethanol, and ethyl acetate are purchased from Sigma–Aldrich. The methoxy(polyethylene glycol)methacrylate (MeOPEGMA, MW = 2000) is kindly supplied by Cognis Performance Chemicals (UK). Phospholipids S100 (95% phosphatidyl-