Controlled Synthesis of Monodisperse Magnetic Porous/Hollow Nanostructures for Biomedical Applications

Kyo-Seon Kim and Dung The Nguyen

Department of Chemical Engineering, Kangwon National University, Chuncheon, Kangwon-Do, 200-701, Republic of Korea, kkyoseon@kangwon.ac.kr

ABSTRACT

Monodisperse magnetite Fe₃O₄ porous/hollow nanoparticles were successfully synthesized through onepot solvothermal process without any surfactant and template. The Fe₃O₄ porous/hollow nanoparticles were synthesized controllably with tunable particle size and porosity by adjusting the initial concentrations of Fe precursor and ammonium acetate. The porous/hollow structure exhibited a great potential to encapsulate small drug molecules. Once inside the porous structures, small drug molecules would be shielded by the shell from fast reaction/deterioration in biological solutions. The porous/hollow nanoparticles could be further coupled with a specific targeting agent and be concentrated around the area of interest, where drug molecules would be released either chemically via a pH control or physically through a magnetic stimulation and activation.

Keywords: magnetite, porous/hollow structure, hyperthermia, drug delivery.

1 INTRODUCTION

In recent years, the magnetic Fe_3O_4 nanoparticles have been of great interest to worldwide researchers for biomedical applications. The particle size, shape and surface structure of magnetic Fe_3O_4 nanoparticles can be prepared controllably to match with the interest of study. Unique applications of the Fe_3O_4 nanoparticles come from their ability to be manipulated by an external magnetic force. This action at a distance provides tremendous advantages for magnetic hyperthermia and targeted drug delivery as well as controlled drug release [1].

It should be emphasized that it is still a great challenge to synthesize Fe_3O_4 nanoparticles of customized size and shape. Among various nanostructures, formation of Fe_3O_4 nanoparticles with porous/hollow structures is expected to integrate the valuable characteristics of porous/hollow structure and unique magnetic property of Fe_3O_4 material in a single platform which can provide opportunities to tune their properties for specific applications. Generally, the synthetic strategies for the magnetite hollow structures can be roughly categorized into two methods: template and template-free approaches. The template synthesis is an effective and common method for synthesizing porous/hollow nanostructures. Preparation of porous/hollow structures by template synthesis method involves the functionalization of template surface, deposition of shell materials or their precursors with subsequent treatments to form solid shells, and selective removal of the templates [2,3]. Even though the template method has been proven as very effective and versatile method to synthesize the hollow structure, it exhibits the difficulties in term of achieving high product yield, removing the template completely and refilling the hollow interior with functional species simultaneously. Other disadvantages are related to high cost and tedious synthetic procedures which have impeded the large-scale production.

In our research, we synthesized monodisperse magnetite Fe₃O₄ porous/hollow nanoparticles without any surfactant and template. This synthesis method is facile and straightforward through a one-pot process. Only one iron source is required. The magnetic-induced heating characteristics of those Fe₃O₄ porous/hollow nanoparticles were studied for different process variables. Because of a possibility to overheat in hyperthermia by a continuous heat generation, we investigated performance of the Fe₃O₄ porous/hollow nanoparticles under an oscillating magnetic field which was designed to maintain the sample temperature at a certain value. Finally, we investigated the potential application of Fe₃O₄ porous/hollow nanoparticles as drug carriers by using Rhodamine 6G as drug model. The release profile of R6G was recorded for different releasing environments. This demonstration could be considered as a practical option to combine remote control drug delivery, drug release and magnetic hyperthermia.

2 EXPERIMENTAL SECTION

Monodisperse magnetite Fe_3O_4 porous/hollow nanoparticles were successfully synthesized through onepot solvothermal process without any surfactant and template. A solution of ethylene glycol (C₂H₄(OH)₂, J.T.Baker, AR) containing 0.1 M of FeCl₃·6H₂O (Sigma-Aldrich, \geq 98%) and 1 M of ammonium acetate (CH₃COONH₄ or NH₄Ac, Sigma, \geq 98%) was well mixed and then transferred to a Teflon-lined autoclave cell. The autoclave cell was kept inside an oven of high temperature to guarantee the uniform temperature inside the cell. The temperature of solution inside the cell was maintained at 200°C. After the scheduled processing time, the autoclave cell was cooled to room temperature by using tap water. The product particles were obtained by centrifuging and washing with ethanol and water for several times and then were dried in a vacuum oven at 60° C for 6 h before characterization.

A lab-made device was used to induce magnetic hyperthermia. The magnetic hyperthermia device consists of the RLC (resistor, inductor, and capacitor) circuit causing the induction of an alternative current field with a theoretical frequency range of 220 kHz to 500 kHz. The magnetic field is adjusted by changing power supply from 0 to 10 kW. The magnetic field is generated by a working coil with diameter of 4 cm and having 4 turns. To characterize the magnetic heating efficiency of the magnetic porous/hollow nanoparticles, a sample volume (Vs) of 0.3 mL containing the Fe_3O_4 porous/hollow nanoparticles was placed in the center of the coil where the magnetic field reaches the highest value. The initial linear rise in temperature versus time dependence, dT/dt, was measured. The specific loss power (SLP) was calculated with the volumetric specific heat capacity of the sample C =4185 (J $L^{-1} K^{-1}$).

The drug loading and in vitro release profiles were carried out to determine the potential application of Fe₃O₄ nanoparticles porous/hollow as а drug carrier. Rhodamine6G (R6G) as drug model was performed to demonstrate the loading capacity of Fe₃O₄ porous/hollow nanoparticles and the sustaining release property. The R6G was incorporated into porous/hollow structure of Fe₃O₄ particles and then the particles were immerse in phosphate buffer solution. The amount of released R6G was determined for an interval of 1 h by fluorescence analysis. The fluorescence spectra of R6G were recorded at excitation and emission wavelengths of 350 nm and 480-650 nm, respectively, showing a fluorescence peak at approximately 560 nm.

3 RESULT AND DISCUSSION

Figure 1 shows the XRD patterns of the product particles synthesized at 200°C and their morphology. The XRD diffraction peaks can be well indexed to the facedcenter cubic structure of Fe₃O₄ (reference code: 01-088-0315) with lattice constants of 8.375 Å. No peak corresponding to the hematite or other impurities was detected, indicating the formation of pure magnetite products. Figure 1b shows a formation of a large quantity of spheres with an average diameter of about 300 nm. It should be noticed that the spheres were composed of many smaller grains. Some broken spheres and many holes on the surface of particles were clearly observed, confirming the formation of particles with solid shell. The hollow structure of the product particles was observed by the TEM measurements as shown in Figures 1c. An intensive contrast between the black margin and the bright center of the particles indicates the existence of hollow structure in the resulting spheres.

The plausible mechanism for the formation of Fe₃O₄ porous/hollow nanoparticles was supported by several experimental observations. It was recognized that the Ostwald ripening should be the underlying mechanism for the formation of hollow microspheres. Since this process involves matter relocation, it can occur within a crystallite aggregate for generation of interior space. The dissolutionrelocation of grains which built the aggregates could be rationalized by considering the different chelation modes between the outer and inner grains or by the existence of bubbles inside the aggregates [4,5]. However, on the basis of formation of Fe₃O₄ from a sole Fe (III) precursor, a series of chemical conversion processes was proposed. Acetate groups derived from NH₄Ac might coordinate with iron ions derived from FeCl₃ to form Fe (III) acetate compounds. Ethylene glycol which was reported as a mild reducing agent could partially reduce the Fe (III) compounds to generate Fe (II) compounds. The respective Fe (III) and Fe (II) compounds were then hydrolyzed to form Fe(OH)₃ and Fe(OH)₂, followed by the generation of Fe₃O₄ nanoparticles via dehydration of these hydroxides. A general chemical reaction can be proposed as follows.

3 FeCl₃.6H₂O + C₂H₄(OH)₂ + 9 NH₄OOCCH₃ → Fe₃O₄ + 0.5 C₄H₆O₂ + 9 NH₄Cl + 9 CH₃COOH + 15 H₂O (1)

The chemical conversions of solid material caused a little shrinkage of the grain size and thus made more voids between the grains inside the aggregates and led to the formation of loose package of aggregates. The inner grains would dissolve into the solution and then diffuse to the outer stable shell by the Ostwald ripening process, resulting in continuous expansion of cavity space inside the aggregates [6,7]. Finally, the hollow structure was well developed with the complete chemical conversions of core grains.

The Fe₃O₄ porous/hollow nanoparticles could be synthesized controllably with tunable particle size and porosity by simply adjusting the initial concentrations of Fe precursor and additive or varying other process variables such as processing time and temperature [8]. The higher the initial concentration of Fe precursor was supplied, the larger the Fe₃O₄ nanospheres were obtained. the concentration of NH₄Ac and the molar ratio of Fe precursor to NH₄Ac also played critical roles to control the porosity of the Fe₃O₄ porous/hollow nanospheres. Ammonium acetate was hydrolyzed into acetic acid and NH₃·H₂O, and partial NH₃·H₂O was evaporated at elevated temperature and formed little gaseous bubbles [9]. The gaseous bubbles provided centers for the small grains to aggregate around the gas-liquid interface. The spherical aggregates were then formed by aggregation of numerous small grains, and thus the bubbles were trapped inside the aggregates, forming the spherical loose structures. Increasing the concentration of NH₄Ac would result in generation of higher bubble

concentration remaining inside the aggregates and thus facilitate the formation of higher porous/hollow structure.



Figure 1: (a) XRD pattern (b) SEM and (c) TEM analyses of the magnetic nanoparticles prepared through solvothermal process.

Magnetic measurements of the Fe_3O_4 porous/hollow nanoparticles is shown in Figure 2a. The porous/hollow spheres showed a saturation magnetization of 81 emu g⁻¹, a remanent magnetization of about 22 emu g⁻¹ and coercivity of about 200 Oe. The high magnetization saturation of Fe₃O₄ porous/hollow nanoparticles can be exploited to achieve magnetically induced heat generation for the thermal treatment of cancer and other diseases. Figure 2b shows an example of the temperature rise as a function of time for several heating cycles. For each cycle, an applied amplitude field of 240 Oe was initially supplied and automatically adjusted to maintain the sample temperature at 42°C which was considered as the required therapeutic threshold for cancer hyperthermia. The temperature profile exhibited that the sample temperature was precisely controlled at $42^{\circ}C \pm 0.2^{\circ}C$. The oscillation of temperature was observed around 42°C due to the time lag of measurement as well as the oscillation of applied magnetic field to control the sample temperature. A so-called hysteresis loss mechanism dominates heat generation of ferromagnetic materials whose sizes exceed the domain wall width. Under application of an alternative field, the magnetic moments oscillate and cause domain wall displacement which generates heat. Heat generation is also caused by the rotational Brownian motion within a dispersed media because of the torque exerted on the magnetic moment by the external alternating magnetic field. In this case, the thermal energy is delivered through shear stress in the surrounding fluid.



Figure 2: (a) Magnetic property and (b) temperature growth of the Fe_3O_4 porous/hollow nanoparticles heated for four continuous cycles .

Once the magnetic carrier was concentrated at the target, the therapeutic agent was then released from the magnetic carrier, leading to increased uptake of the drug by the tumor cells at the target sites. The loaded drug could be released by changes in the local physicochemical environment. For example, drug molecule was loaded into the Fe₃O₄ porous/hollow nanoparticles and its release was studied as a function of the pH with a higher release rate in acidic conditions [10]. It was supposed that the acidic environment could weaken the binding between drug molecule and polymer layer on the surface of Fe₃O₄ porous/hollow nanoparticles. The drug release from Fe₃O₄ porous/hollow nanoparticles was also significantly enhanced by temperature as shown in Figure 3. The amount of R6G was very low at temperature of 25°C. A significantly higher concentration of R6G release was reached at 45°C. Within the first hour, an amount of 7.7% R6G was released at 25°C in comparison with that of 15.6% R6G was released at 42°C. After 5 hours, the accumulation releases of R6G were about 23.3% at 25°C and 38.9% at 42°C. By increasing temperature from 25°C to 42°C, the R6G release increased for approximately 2 timesAs the reverse process of the drug loading, diffusion was the most common mechanism in controlled drug release. The release of R6G from a hollow structure of Fe₃O₄ occurred by diffusion through the pores on the shell and was driven by the concentration gradient of R6G from the hollow cavity to the medium surrounding the nanoparticles. Higher temperature could weaken the binding of P123 polymer layer on the surface of Fe₃O₄ porous/hollow nanoparticles, facilitating the outward diffusion of R6G. The higher drug release could be also attributed to the higher mobility of all chemical compounds at higher temperature.



Figure 3: R6G release profile from Fe₃O₄ porous/hollow nanoparticles for different evironment temperatures.

Since the changes in local environment temperature could enhance drug release, the thermal energy from magnetic nanoparticles by magnetic-induced heating could be used as an external and remotely controlled trigger for controlled drug release. If a drug molecule is attached to magnetic nanoparticles through a linker under application of an alternative magnetic field, the linker is heated and then is detached from the nanoparticle surface, resulting in releasing of the drug molecule. In another manner, if both drug molecules and magnetic nanoparticles are encapsulated within a polymeric matrix, by applying a magnetic field, local heat generated by the magnetic nanoparticles results in formation of crevices or cracks of nanometer scale within a polymeric matrix, thereby encapsulated drugs are released. A combination of hyperthermia-based therapy and controlled drug release has strong potential to develop an intelligent therapy for cancer treatment.

4 CONCLUSION

Monodisperse magnetite Fe₃O₄ porous/hollow nanoparticles were successfully and controllably synthesized through one-pot solvothermal process without any surfactant and template. The use of those magnetic nanoparticles for hyperthermia has shown great promise in the field of nanobiomedicine. By applying an alternative magnetic field with sufficient amplitude and frequency, the magnetization of the particles was continuously reversed, which converted magnetic to thermal energy. A combination of hyperthermia-based therapy and controlled drug release has strong potential to develop an intelligent therapy for cancer treatment. The biomedical uses of magnetic nanoparticles are not restricted to controlled drug delivery and hyperthermia, and, indeed, they are also applicable to magnetic resonance imaging, bioseparation, sensing, enzyme immobilization, immunoassays, and so on.

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