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Liposome-Permeability Templating of Gadolinium Hydroxide Nanostructures

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With the growing attention in building novel nanostructures, there is a need for general approaches to controlling the sizes and shapes in precipitation processes. Here, precipitated gadolinium hydroxides ($Gd(OH)_3$) were made in the form of hollow spheres by control of the liposome permeability. Addition of $Gd(NO_3)_3$ to the acidic solution containing the liposomes entrapping OH^- creates a self-precipitating template in the precipitation process. The control-formation of these nanostructures takes place through a liposome-membrane phase change. Hybrid nanocapsules are obtained when entrapped hydroxyl ions (OH^-) permeates from the liposome and shell material precipitates by forming $Gd(OH)_3$. The morphology of the final product is molded on the original liposome. This synthesis strategy might have implications in significant applications, such as drug delivery systems, and in mineralization, where many processes are also membrane-assisted.

Introduction

Lipid vesicles (liposomes) have proved to be a very useful and exciting medium for nanotechnology.[1] This is a consequence of the tunable surface properties and specificity through the modification of lipid membrane composition, combined with the well-defined size-structure that occurs via different synthesis conditions.[2] The development of entrapping methods has enabled the encapsulation of drug, enzymes and nanoparticles inside liposomes to produce functional vehicles of high quality at nanometer scale.[3] In a separate but related thread, in recent years there has been a remarkable interest in creating new methodologies to achieve hollow inorganic structures.[9] This interest originates from their many technological and scientific applications, including the development of selective catalysts, optical probes and drug delivery systems. In particular, hybrid systems consisting of lipid based vesicles stabilized by inorganic shells, nanoparticles or nanodiamond have also been developed.[5] These conventional strategies are based on using the liposomes as an inert template to create hybrid nanocapsules. The inclusion of specific phospholipids into lipid membrane composition results in a temperature sensitive system, wherein molecule permeability through that membrane can be controlled.[3a,6] We combined this liposome interface physics with a standard oxide precipitation method to create a novel and straightforward approach for making inorganic hollow structures. Here, the control of permeability in lipid interface exerts morphological control during the formation of the inorganic shell. In the basic synthesis, Gd ions are the Gd compound source. Gd was chosen for its variety of uses that span from biomedical research[7] to optical applications.[8] It precipitates under basic pH conditions to form the $Gd(OH)_3$. The OH^- are retained and released from the liposomes that are surrounded by a Gd solution. The Gd ions precipitate around the liposome membrane (because of the OH permeation) and there form the shells, which reflect the size and shape of the liposomes because the inorganic material solidifies around them.

Results and Discussion

Lipid vesicles of desired composition (DPPC/Cholesterol 1:0.2) and size (400 nm) were prepared by the lipid film hydration and extrusion method.[3a,9] The gel to fluid phase transition temperature (Tm) of vesicles was seen at approximately 40 °C (Figure S1), in agreement with literature values.[6b,10] With temperature approaching Tm, liposome membrane is consequently more permeable to ions. With this in mind, we exploited this permeability control for the synthesis of gadolinium hollow particles based on precipitation of the $Gd(OH)_3$ by controlled reaction between gadolinium ions and hydroxide.

A typical synthesis was carried out as follows: above Tm, a liposome solution was adjusted to a basic pH by small additions of NaOH and incubated for 30 min. Afterwards, this dispersion was aged for 15 min below Tm after which a small aliquot of HCl (pH 2.3) was added to lower the pH. To this solution, $Gd(NO_3)_3$ was added rapidly to start precipitation stage. This dispersion was then aged for 30 min at different temperatures (above and below Tm). Addition of $Gd(NO_3)_3$ to the acidic solution containing the liposomes entrapping OH^- while stirring creates a self-precipitating template in the precipitation process. The formed particles were then separated by centrifugation, washed thoroughly and re-suspended in water; these colloidal dispersions remained stable for months. Control experiments, i.e., without liposomes, were also carried out, where no precipitation was observed.

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Temperature is the mechanism for controlling permeability properties through modification of phospholipid interactions and is crucial in the development of the hollow particles. Under high temperature, in precipitation step, predominantly bunched-type morphologies are revealed by electron microscopy (Figure S2a) and some small particles (≈ 180 nm) were also found by dynamic light scattering (DLS) (Figure S2b). The bunch consists of several hollow particles (≈ 520 nm) that intertwine during the process of Gd-hydroxide precipitation. With decreasing temperature, more and more isolated particles are formed until the bunch-morphology completely disappears.

By carefully tuning the synthesis conditions in the precipitation stage, especially at high temperature (50 °C), we prepared samples that consisted entirely of rather spherical particles with a relatively narrow size distribution. However, the particles had agglomerated into lumps (Figure S2a). This aggregation was probably due to fast precipitation of different spheres when they came into contact with each other in the stage of synthesis. This agglomeration can be avoided if the precipitation temperature is 4 °C (below Tm). Using such a procedure, we produced almost exclusively isolated particles (Figure 1a). The thickness of the coating was determined by DLS measurements (~15 nm; Figure S3). These particles are in fact hollow after the gadolinium hydroxide has been precipitated (inset Fig 1a) and their shells are amorphous (See Figure S4). As an indication of the robust nature of the capsules, they were stable enough to outlast drying and vacuum when the samples were prepared for electron microscopy. We suggest a model for the formation of such particles according to the process sketched in Figure 1c: addition of Gd ions to the acidic solution containing the liposome-entrapped OH^- creates $Gd(OH)_3$ hollow particles. Gd^{+3} and OH^- together are enriched at the liposome interface. Then Gd is precipitated under this basic condition at that interface and there forms the shell by the influence of liposomes. A lower ion permeability of the liposomes ($T < Tm$) was a key to maintain the individuality of the capsules.

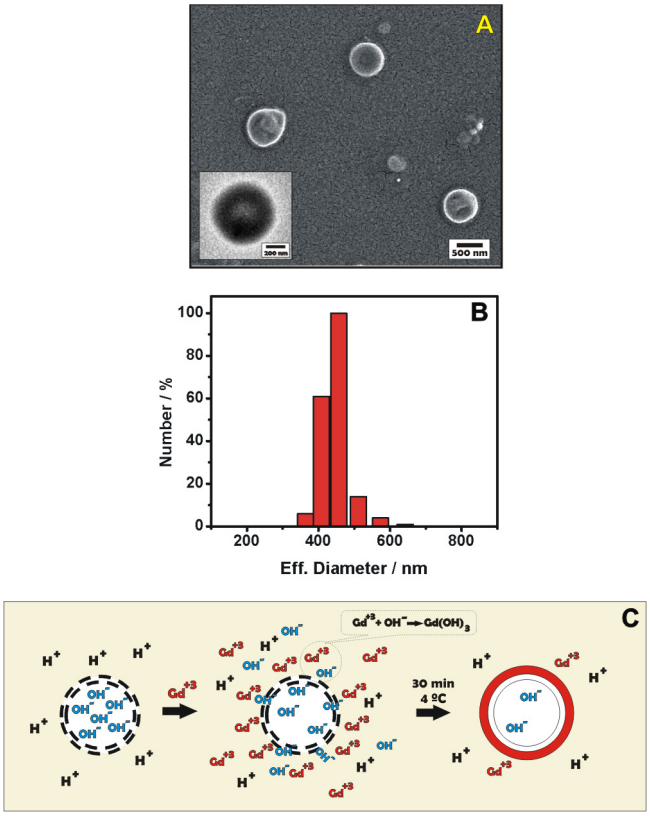

Figure 1. (A) SEM and TEM (inset) images of the $Gd(OH)_3$ hollow spheres synthesized at low temperature. (B) Size distribution of the nanocapsules. (C) Schematic drawing of the suggested formation process of the spheres. The liposomes in the Gd^{3+} solution contain the entrapped OH^-. At the interface, gadolinium is precipitated and solidifies there as a shell mediated by hydroxyl ions. The morphology of the final product is molded on the original liposome.

The composition and structure of the nanocapsules were characterized using thermogravimetric analysis (TGA) and X-ray photoelectron spectroscopy (XPS). Figure 2 shows the TGA

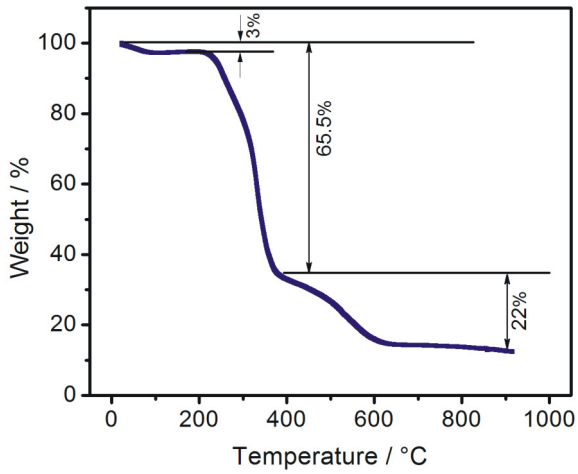

Figure 2. TGA curve of $Gd(OH)_3$ nanocapsules recorded in N_2 atmosphere.

curve recorded by heating the dry powder of the microcapsules under a nitrogen flow. The initial mass loss of ~ 3% at low temperatures corresponds to desorption of physically adsorbed water molecules.[7a,11] The major weight loss of 65.5% spans from 200 to 380 °C and involves the removal of confined water into the capsule interiors[5a] and the conversion of gadolinium hydroxide to gadolinium oxide hydroxide because of the removal of structural water according to the following reaction:[11c,12]

$$Gd(OH)_3 \rightarrow GdOOH + H_2O$$

The combustion of the liposomal DPPC is also produced during this temperature interval and can be followed up to about 500 °C.[7a,13]

The subsequent transition from 380 to 600 °C suggests that the majority of the gadolinium oxide hydroxide is transformed into the gadolinium oxide form.[11c,12,14]

Figure 3 shows the XPS spectra taken from a sample of nanocapsules after Ar^+ ion sputtering. The survey XPS spectrum clearly shows the presence of the characteristic Gd peaks, particularly the $3d_{5/2}$ and $3d_{3/2}$ peaks at binding energy of ~1185 and 1218 eV,[8a,11c,15] and the $4d_{5/2}$ and $4d_{3/2}$ peaks at 140.64 and 146.23 eV (see Figure 3b), together with C, N and P peaks of the liposome template.

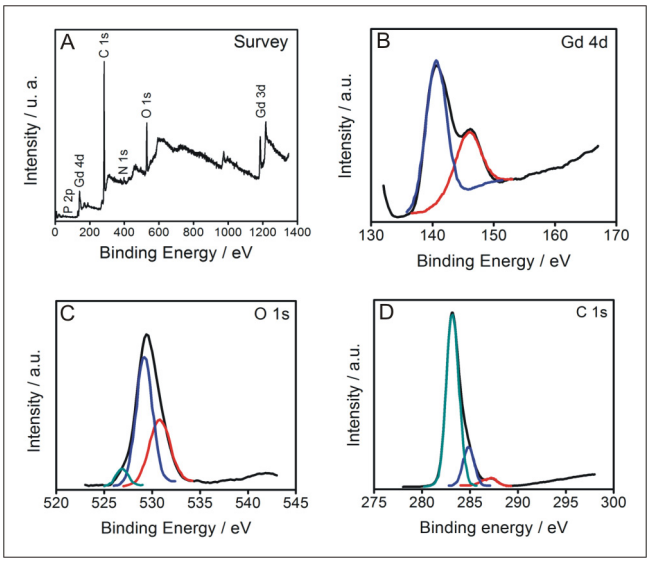

Figure 3. (a) Survey spectrum of as-prepared $Gd(OH)_3$ hollow spheres. Core level high-resolution XPS spectrum of the Gd 4d (b), O 1 s (c) and C 1 s (d) energy levels.

The core level spectrum of the O (1 s) (Figure 3c) shows three peaks. The most intense peak at 531.22 eV is a combination of the signal of the oxygen of $Gd(OH)_3$[8a,11c,16] with the signal of the oxygen doubly bound to carbon and phosphorous (O=P and O=C) of DPPC.[17] A component at higher binding energy (532.69 eV) corresponds to the oxygen singly bound to carbon in the carboxyl of phospholipid.[17] The peak that appears at 529.1 eV is attributed to P–O bond.[17] The N (1 s) signal which appears at 398 eV is in accordance with previous data[15,17] and the peak of P (2p) is lumped with the Gd (4d) peak.[18] The C(1 s) high-resolution spectrum (Figure 3d) was decomposed into three different peaks corresponding to DPPC of liposomes. The major component appearing at 283.18 eV corresponds to carbon only bound to carbon and hydrogen (C–C/C–H). The peak at 284.73 eV is attributed to carbon making a single bond with oxygen or nitrogen (C–O/C–N) and the peak at higher binding energy (286.88 eV) includes carbon making one double bond and one single bond with oxygen (O=C-OR).[17,18,19]

Fourier transform infrared spectroscopy (FTIR) was performed to further characterize the composition of the hollow nanostructures. Figure 4 shows the spectrum taken from dry powders of nanocapsules. The multiple bands between 1900 and 2500 cm^{-1} can be assigned to various vibrational modes of $Gd(OH)_3$,[20] and the relatively intense band at 3345 cm^{-1} is attributable to the stretching and bending O–H vibrations of $Gd(OH)_3$.[21] The bands at 813 and 744 cm^{-1} again confirms the presence of Gd–O–H bonds,[7a,22], which lead to the conclusion that the shells are composed of $Gd(OH)_3$. Some characteristic bands such as the symmetric and asymmetric stretching vibrations of CH_2[23] at 2854 and 2923 cm^{-1} and the C=O stretching band of the ester group at 1740 cm^{-1} indicate the existence of the DPPC and cholesterol molecules. This shifted C=O band from 1726 cm^{-1} indicates the presence of intermolecular cholesterol between DPPC molecules.[23b] In addition, the bands at 1324 and 1474 cm^{-1} can be assigned to CH_2 wagging and scissoring modes of the extended acyl chains of DPPC, respectively. The band at 977 cm^{-1} can be attributed to stretching vibration of the choline group of DPPC and asymmetric and symmetric stretching bands of DPPC phosphate are noticed at 1208 and 1041 cm^{-1}, respectively. The bands centered at 3218 cm^{-1} and 1635 cm^{-1} are due to O–H vibrations of adsorbed water.[7a,11a,b]

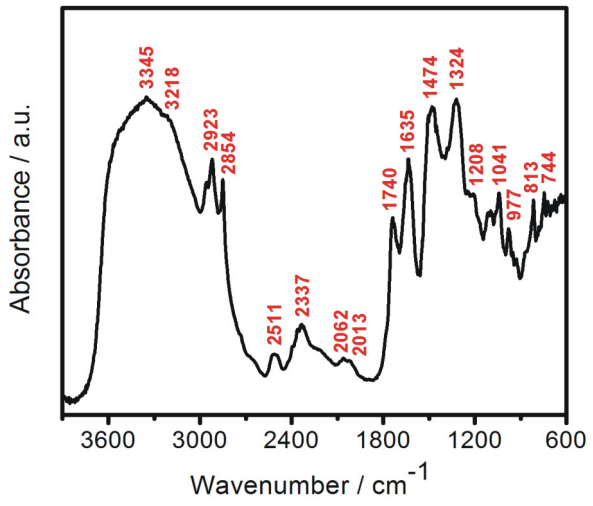

Figure 4. FTIR spectra of $Gd(OH)_3$ nanocapsules.

By combining the results of TGA, XPS, and FTIR and other experimental observations, such as electronic microscopy measurements, we can conclude that the nanocapsules are mainly liposomes covered with $Gd(OH)_3$.

Another interesting aspect of these nanocapsules is their magnetic behavior. Field-dependent magnetism at room temperature showed a linear relationship between the magnetization and applied field with no hysteresis loop (Figure 5a), which represented that nanocapsules exhibited the paramagnetic characteristics of gadolinium compounds,[7] desirable for their applications as contrast enhancement agents in magnetic resonance imaging (MRI).

As Gd based nanocapsules do not show any emission in the visible region[24] Eu:$Gd(OH)_3$ nanocapsules were synthesized by the same liposome-assisted approach. The resulting luminescent hollow particles exhibited a strong red emission (Fig-

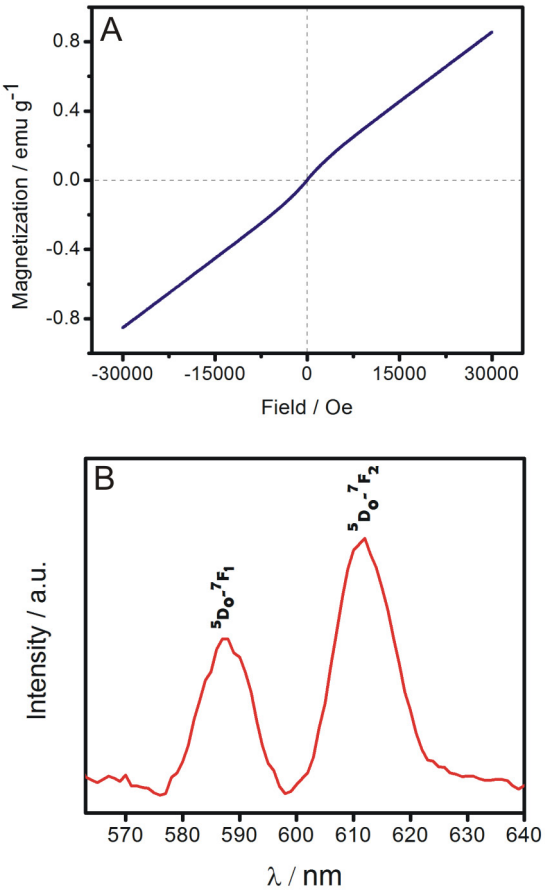

Figure 5. (A) Magnetization curve of $Gd(OH)_3$ nanocapsules at room temperature. (B) Emission spectrum of Eu:$Gd(OH)_3$ nanocapsules, recorded under 394 nm illumination, showing the main transitions $5D_0 \rightarrow 7F_1$ (589 nm) and $5D_0 \rightarrow 7F_2$ (612 nm).

ure 5b) under UV irradiation (394 nm) and the spectral properties are typical of the well-known $5D_0$–$7F_J$ transitions ($J = 0$-6) of trivalent europium ion (Eu^{3+}),[8b,25] indicating that Eu^{3+} ions have been successfully incorporated in the shell.

A wide variety of nano-objects can be encapsulated in liposomes, including metal and oxide nanoparticles, quantum dots and enzymes.[3a,26] Thus, it should be possible to trap species inside the lipid vesicle and grow a shell around the liposome, resulting in caged desired species. As an example, we formed a $Gd(OH)_3$ shell around liposomes containing small numbers of well-characterized magnetic nanoparticles (Figure S5). After synthesizing the inorganic shell, some entrapped magnetite nanoparticles into the hybrid nanocapsule were obtained (dark feature in Figure S6).

Together, these results illustrate that this advanced procedure can be easily extrapolated to produce a diversity of new multifunctional nanocapsules that combine the versatile properties of lanthanide-shells with those from the incorporated nanomaterials.

Conclusions

In summary, we have developed a novel liposome-templated approach for the synthesis of $Gd(OH)_3$ nanocapsules. Temperature-tuning was the tool for controlling gadolinium precipitation through modification of permeability of liposomes and was the key in the formation of the hollow particle morphology. This process produces nanocapsules directly from the Gd precipitation on liposomes, avoiding the need of sacrificial templates and time-consuming dialysis steps associated with conventional procedures. We believe that the current procedure might be extended to produce nanocapsules from other materials because a number of lanthanides have been found to precipitate by a mechanism similar to the gadolinium one. Also, it is expected that the size of the capsules can be reduced to tens of nanometers by starting with nanometer-scale liposomes. These nanostructures may have technological as well as fundamental implications. The process described here for structuring inorganic materials provides new approaches to multi-integrated systems that could be important in various fields of application such as controlled drug-delivery systems and nanoscale reactors in catalysis. From a more fundamental point of view, it is by now almost certain that interfaces play a crucial role in mineralization and the study of self-precipitation control on biological membranes is certainly necessary to develop intricate structures mimicking nature.

Supporting Information

The Supporting Information includes the Experimental Section and further characterization of the liposome-templates and the $Gd(OH)_3$ hollow spheres.

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Keywords: Nanocapsules · Liposomes · Multifunctional Nanostructures · Encapsulation

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