Journal of Colloid and Interface Science 456 (2015) 206–209

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Liposomes as nanoreactors for the photochemical synthesis of gold nanoparticles

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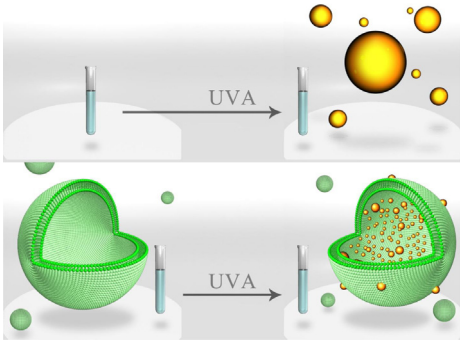
GRAPHICAL ABSTRACT



Photochemical synthesis of gold nanoparticles in bulk solvent (top panel) and within liposomes (bottom panel)

ARTICLE INFO

Article history:
Received 13 May 2015
Revised 22 June 2015
Accepted 22 June 2015
Available online 24 June 2015

Keywords:
Liposomes
AuNPs
Nanoreactors

ABSTRACT

A simple and novel method for the photochemical synthesis of AuNPs in liposomes is described. Gold salt is co-encapsulated with the photoinitiator Irgacure-2959 in POPC liposomes prepared *via* traditional thin-film hydration technique. UVA irradiation for 15 min results in encapsulated AuNPs of 2.8 ± 1.6 nm in diameter that are primarily dispersed in the aqueous interior of the liposomes.

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Gold nanoparticles (AuNPs) have widespread applications in plasmonics [1], biosensors [2], electronics [3], catalysis [4], imaging and drug delivery [5,6]. A large number of methods have been developed and optimized for synthesizing AuNPs with defined sizes and shapes [7,8], and typically involves the chemical reduction of Au (III) to Au (0). The initial small clusters of reduced gold thus obtained serve as nucleation sites for the subsequent growth of nanoparticles. For synthesis of spherical AuNPs, besides a reducing agent, an additional stabilizing or capping agent is sometimes added to the reaction mixture to prevent the AuNPs from aggregating. McGilvray et al. [9], reported a simple and rapid photochemical synthesis of AuNPs to obtain monodisperse spherical nanoparticles that are stable in suspension. In this synthesis,

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http://dx.doi.org/10.1016/j.jcis.2015.06.033
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the reduction of Au (III) was achieved by ketyl radicals generated due to the UVA-irradiation of Irgacure-2959 (Irg), a photoinitiator. Additionally, the 4-hydroxyethoxy benzoic acid generated from irradiated Irg eventually acts to stabilize the AuNPs and thus prevents them from aggregating.

Liposomes are excellent candidates for the compartmentalized synthesis of AuNPs [10], especially if the end-applications are in the field of biological probes and drug delivery. Such compartmentalized synthesis offers the possibility to: control particle size, control the environment of synthesis to include catalytically active components, and carry out synthesis in a highly organized solvent structure [11]. Yet, despite such advantages, previous attempts at synthesizing AuNPs within liposomes have resulted in high size polydispersity, inhomogeneity in shape, or poor yield [12–14]. Recently, the synthesis of AuNPs within liposomes was achieved by the controlled diffusion of stabilizing agents into encapsulated gold salt solutions [15,16], or by doping the lipids that constitute a liposome with glycerol that acts as a reducing agent [17]. However, these approaches have had the need to either modify the method of liposome preparation or use special lipids to achieve controlled synthesis of AuNPs.

In this communication, we describe a simple method (Fig. 1) for the controlled synthesis of spherical AuNPs with an average size of 2.8 ± 1.6 nm, inside palmitoyl oleoyl phosphocholine (POPC) liposomes. The resulting AuNPs are homogeneous in size and shape. Moreover, this simple method circumvents the need for separating unencapsulated AuNPs after their synthesis, which can be challenging when employing the more traditional method of encapsulating preformed AuNPs within liposomes. All these, without having to (1) modify existing liposome preparation techniques, (2) introduce additional stabilizing agents in solution, or (3) dope/-modify lipids that constitute a liposome, with reducing or stabilizing agents.

A freshly prepared solution of $HAuCl_4$ (0.1 mg/mL) and Irg (1 mM) in deionized water was encapsulated into POPC liposomes (2 mg/mL) obtained *via* the thin-film hydration technique [18] followed by extrusion using a polycarbonate filter of 100 nm pore size. After removal of unencapsulated $HAuCl_4$ and Irg by separating them over a Sephadex G-25 spin column, the samples were irradiated in a photoreactor with 108 $\mu W/m^2$ UVA light (365 nm) for 15 min, to generate stable AuNPs within the confines of the liposome.

Photochemical synthesis of AuNPs within liposomes was confirmed by the presence of the localized surface plasmon resonance (LSPR) band centered around 530 nm (Fig. 2). While this LSPR band is typically centered around 520 nm for small (~10 nm) and dispersed AuNPs, its position is affected by factors such as particle size, shape, refractive index of the medium, temperature and by species adsorbed on the nanoparticle surface [1]. The position of the LSPR band for AuNPs within liposomes appears red shifted due to the presence of liposomes that scatter significantly at lower wavelengths. The effect of liposome scattering on the LSPR peak position was confirmed using liposome encapsulation of commercial AuNPs. In the absence of liposomes they show λ_{max} at ~520 nm, and when encapsulated, the peak shifted to λ_{max} ~550 nm (Fig. S1). On the other hand, very small AuNPs (<2 nm) lose their plasmonic properties and hence will not show an LSPR band [19–21]. This loss of plasmonic properties may explain to some extent, the relatively weaker LSPR band from samples where AuNPs were synthesized within liposomes.

Interestingly, transmission electron microscopy (TEM) revealed significant differences in nanoparticle size and the overall size distribution of the AuNPs synthesized within liposomes and in bulk solvent (Fig. 3) using otherwise identical conditions. The AuNPs synthesized within liposomes are very homogeneous in shape and size (Fig. 3A). Based on the TEMs and size distribution analysis

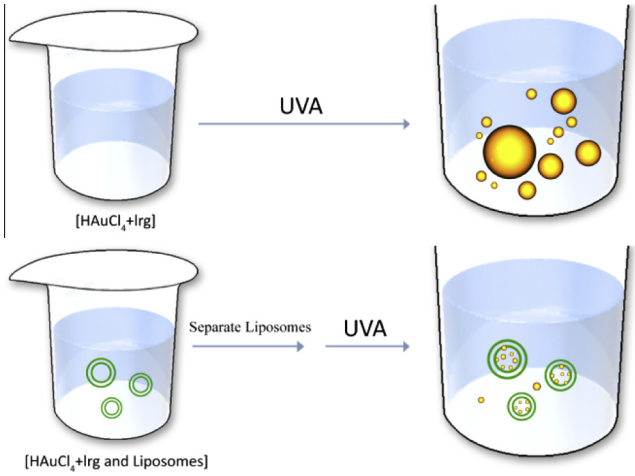

Fig. 1. Schematic illustration of the photochemical synthesis of AuNPs in bulk solvent (top panel) and within liposomes (bottom panel). Green circles represent liposomes and golden spheres represent AuNPs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

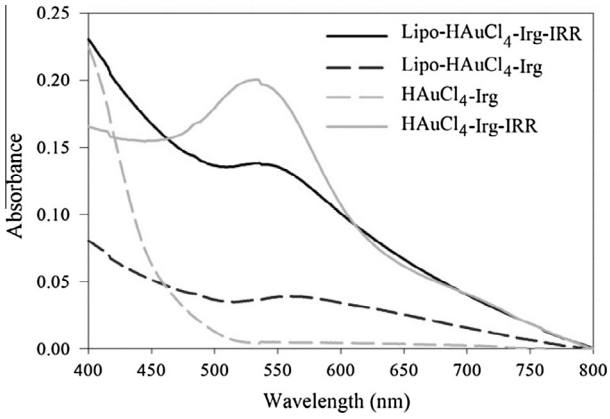

Fig. 2. UV–Vis spectra of 0.1 mg/mL $HAuCl_4$ + 1 mM Irg samples unencapsulated (gray lines) or encapsulated within POPC liposomes (black lines). Samples were prepared in the dark (dashed lines) followed by UVA-irradiation (IRR) for 15 min (solid lines) to initiate AuNP synthesis.

performed on these images, the AuNPs are spherical and have an average size of 2.8 ± 1.6 nm (Figs. 3A and 4A). In comparison, there is a significant difference in the size range of AuNPs synthesized in bulk solvent when using the same conditions but excluding liposomes (Figs. 3B and 4A). These nanoparticles are significantly larger in size and more polydispersed.

The large difference in the AuNP sizes between those synthesized within liposomes and those in the bulk solvent is presumably due to an effect of confinement as well as the presence of a lipid bilayer. The inner membrane of the liposome offers a convenient surface for adsorption, formation and growth of nuclei but affects the diffusion coefficient of the nuclei [22,23]. Hence, when compared to the bulk solvent, the nuclei formation within the liposomes is fast but the smaller diffusion coefficient of the nuclei along with the physical barrier which inhibits NP diffusion, encounter and agglomeration, slows down the rate of the particle growth [24]. Such reduced growth rates allow for other processes such as the capping/stabilization of AuNPs to occur and thus limits and controls the size of the particles formed.

We investigated the effect of $HAuCl_4$ + Irg concentrations and time of irradiation on AuNP synthesis in suspension (Fig. S2). We

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