Journal of Molecular Liquids 268 (2018) 849–853

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

A novel and simple microemulsion method for synthesis of biocompatible functionalized gold nanoparticles

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ARTICLE INFO

Article history:
Received 9 May 2018
Received in revised form 27 July 2018
Accepted 29 July 2018
Available online 02 August 2018

Keywords:
Gold nanoparticles
Microemulsion
Functionalization
Colloid
Biocompatible nanoparticles

ABSTRACT

Synthesize of functionalized gold nanoparticles has attracted a great deal of interest due to their favorable optical properties and broad range of biomedical applications. One challenge for biomedical application is to find a simple method in the manufacturing of biocompatible and stable water soluble gold particles with a well-controlled shape and size. In the current study, a novel methodology based on microemulsion system has been introduced to prepare biocompatible gold nanoparticles protected by a monohydroxy thioalkylated PEG ligand. The morphology and size distribution of gold nanoparticles was determined by different methods including dynamic light scattering (DLS), UV–visible spectrophotometry, Energy-dispersive X-ray analysis (EDX) and transmission electron microscopy (TEM). It is verified that the prepared water-based functionalized gold colloidal system has particles size in the range of 7–9 nm with no cytotoxicity effect to HeLa cells.

1. Introduction

In recent years, noble metal nanoparticles (NPs) are being extensively realized in various biomedical applications [1–4] with potential use in different areas including: diagnostics and sensing, *in vitro* and *in vivo* imaging, and therapeutic techniques [5,6]. Gold NPs are attracting interest due to their amenability of synthesis and functionalization, the non-reactive and relatively bio-inert nature, less toxicity and ease of detection [7–9]. It is obvious that the potential applications of gold NPs, especially in biomedical and bioanalytical areas, can be limited due to their instability in aqueous medium. To overcome this drawback, Brust et al. [10] fabricated gold clusters protected by a monolayer of thiolalkylated polyethylene glycol (PEG) ligand. After that this type of ligands were used successfully in the manufacturing of stable water soluble functionalized gold NPs [11–14].

Up to now, different protocols have been proposed for preparing of functionalized gold NPs. Singh et al. [15] presented a remarkable review, with a comprehensive study covering over a hundred published papers focuses on several methods for functionalization of gold NPs and their application in biomedical areas. Recently, some researches have focused on functionalization of gold NPs by different capping agents. Chen et al. [16] prepared highly water-soluble, stable and biocompatible polyamidoamine (PAMAM) dendrimers to entrap gold nanoparticles for catalysis application. A colloidal system of water-soluble N-heterocyclic carbene-protected gold NPs was introduced by Salorinne et al. [17]. This proposed water-soluble biocompatible NPs system is promising candidate for using in photoacoustic imaging. An aqueous colloidal suspension of non-toxic and biocompatible spherical gold nanoparticles stabilized by branched polyethylenimine produced photochemically by Teixeira et al. [18]. Their method was based on photoexcitation process of an aqueous chloroauric acid containing polyethylenimine at room temperature.

Some other research that has been done to prepare water soluble gold NPs are based on polyethylene glycol ligand. PEGylated polyethylenimine (PEI)-entrapped gold nanoparticles modified with folic acid (FA-Au PENPs) as a nanoprobe for targeted tumor CT imaging studied by Shi et al. [19]. They reported that the FA-Au PENPs are water soluble, stable, and cytocompatible in a given concentration range and are able to target cancer cells over expressing FA receptors. Reznickova et al. [20] proposed a simple, reproducible and green method for preparation of functionalized gold NPs by PEG ligand. The PEGylated gold nanoparticles have been fabricated by direct deposition into various polyethylene glycol solutions. Synthesis of two types of biocompatible colloidal gold nanoparticles for applications in drug delivery and therapy was also demonstrated by Coman et al. [21]. These nanocolloids are containing particles coated with polyethylene glycol or coated with PEG and bovine serum albumin. Many other researches are also published in recent years to confirm effective applications of functionalized gold nanoparticles in biomedical areas [22,23].

In our previous research work [24], a new method was introduced for preparing organic soluble thiol functionalized gold NPs based on reverse microemulsion as reaction vessel and compared with two phase

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https://doi.org/10.1016/j.molliq.2018.07.112
0167-7322/

liquid-liquid Brust's method. In progressing of our previous research studies on microemulsion systems [25–28], the aim of the present study is fabrication of biocompatible and water soluble gold NPs functionalized by a monohydroxy thiolalkylated polyethylene glycol ligand, in microemulsion system as a soft template. The ligand used in this research was (11-mercaptoundecyl)tetra(ethylene glycol), so that the shorter ethylene glycol chain making gold NPs amenable to ligand exchange reactions for further functionalization [29]. To the best of our knowledge, no papers have been reported on reverse microemulsion method, using tetraoctylammonium bromide (TOAB) as surfactant, to produce functionalized water soluble gold NPs. This makes it a promising research area to be explored and developed further. The morphology and size distribution of gold nanoparticles were obtained by different approaches including dynamic light scattering (DLS), UV–visible (UV–vis) spectrophotometry, transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDX). The cytotoxicity effect of the prepared functionalized gold nanoparticles was also investigated.

2. Experimental section

2.1. Materials

The hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4$) (99.9%) as source of gold nanoparticles, tetraoctylammonium bromide ($N(C_8H_{17})_4Br$) (98%) as a cationic surfactant, sodium borohydride as reducing agent and (11-Mercaptoundecyl)tetra(ethylene glycol) as a monohydroxy thioalkylated PEG (MUTEG) ligand (90%) with linear formula of $HS(CH_2)_{11}(OCH_2CH_2)_4OH$ as a functionalizing reagent were purchased from Sigma-Aldrich. Toluene as continues phase in the microemulsion system, 2-propanol and diethyl ether were received from Merck. All aqueous solutions of $HAuCl_4$ were prepared with deionized water (0.055 μS/cm) which was produced in our lab with PKA (Smart two pure) instrument.

2.2. Preparation of functionalized gold nanoparticles by microemulsion method

Based on our own experience and obtained ternary phase diagram in previous research work [24] the microemulsion system was prepared by mixing yellow aqueous solution of hydrogen tetrachloroaurate (0.03 M) and a solution of TOAB in toluene (0.05 M). The same microemulsion system containing freshly prepared aqueous solution of $NaBH_4$ (0.4 M) was also prepared separately. The reductant-containing microemulsion was added into another microemulsion containing gold precursor drop-wise, while the system strongly stirred over a period of 5 min. Au nanoparticles were formed by reducing the Au(III) ions with sodium borohydride and the resulting microemulsion mixture changed to a stable light ruby red colour. The microemulsion was left stirring for a further 1 h. To remove residual TOAB surfactant from the AuNPs surfaces, the AuNPs were pipetted into a 10 ml vial along with 5 ml ethanol and then centrifuged. The AuNPs were then suspended in toluene. At this stage a small fraction of TOAB surfactant may be still exists on the gold nanoparticles surface which helps to much more dispersion of nanoparticles in toluene phase. After that, about 0.5 mg of MUTEG ligand, dissolved in 2-propanol, was added to 1 ml of the AuNPs suspension and stirred for 10 min. A slight colour change of the colloidal system from wine red to purple was obtained during stirring, indicating MUTEG attachment to the surface of AuNPs. After 10 min, excesses water was added to the mixture and stirred vigorously. Subsequently the organic phase became completely colourless while the colourless aqueous phase became deep wine red indicating that functionalized AuNPs have been transferred to the aqueous phase. Finally, the aqueous phase was separated and washed with diethyl ether. This process was repeated six times and further purified by centrifugation for 20 min (13,000 rev min^{-1}). A feasible preparation procedure of the biocompatible and water soluble gold NPs, functionalized with MUTEG ligand, is shown schematically in Fig. 1.

2.3. Characterization

As the first characterization step to confirm formation and stability of the functionalized AuNPs disperse in aqueous phase the UV–Visible method was used. A double beam UV–Vis spectrophotometer (Perkin Elmer lambda 15) was applied for this purpose. Afterward, morphology of the synthesized AuNPs was observed using transmission electron microscope (Zeiss- EM10C-80 kV) and distribution of the nanoparticles determined by image analyzer software. Sample for TEM analysis was prepared by dropping aqueous colloidal solution of Au-MUTEG nanoparticles onto carbon-coated copper grids.

Dynamic light scattering instrument (Nano zeta size-90, Malvern) was used to determine particles distribution and zeta potential of Au-MUTEG nanoparticles.

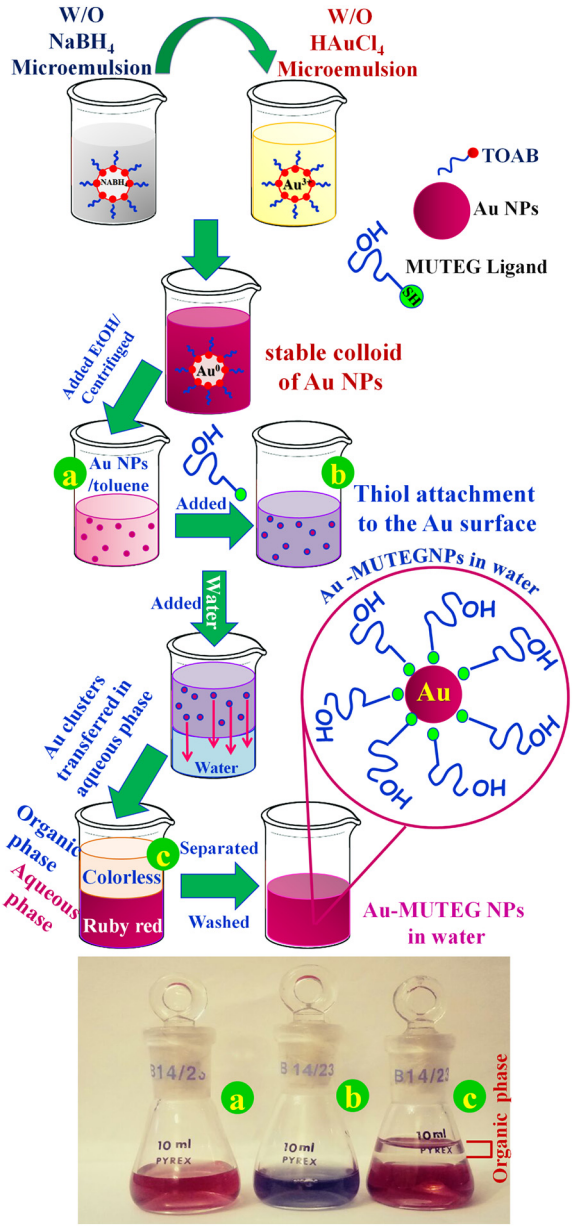

Fig. 1. Schematic illustration of the preparation procedure of water soluble AuNPs stabilized by MUTEG ligand using w/o microemulsion system.