Colloids and Surfaces B: Biointerfaces 143 (2016) 472–480

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Biosurfactant templated quantum sized fluorescent gold nanoclusters for *in vivo* bioimaging in zebrafish embryos

S. Chandirasekar[a], C. Chandrasekaran[b], T. Muthukumarasamyvel[a], G. Sudhandiran[b], N. Rajendiran[a,*]

[a] Department of Polymer Science, University of Madras, Guindy Campus, Chennai 600025 Tamil Nadu, India
[b] Department of Biochemistry, Cell biology laboratory, University of Madras, Guindy Campus, Chennai 600025, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form 22 March 2016
Accepted 23 March 2016
Available online 24 March 2016

Keywords:
Gold nanocluster
Fluorescence
Biosurfactant
Zebrafish
Bioimaging

ABSTRACT

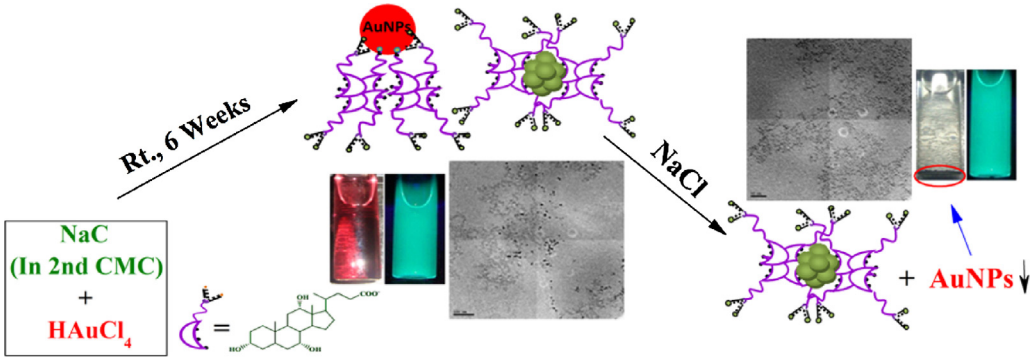
We report the biosurfactant (sodium cholate) templated bright bluish-green emitting gold nanoclusters (AuNCs) by green chemical approach. Optical properties of the AuNCs were studied using UV–vis and luminescence spectroscopy. Lifetime of the fluorescent AuNCs was measured using time correlated single photon counting technique (TCSPC). High-resolution transmission electron microscopy (HR-TEM) and dynamic light scattering (DLS) were used to measure the sizes of the clusters. *In-vivo* toxicity and bioimaging studies of sodium cholate (NaC) templated AuNCs were carried out at different developmental stages of zebrafish embryos. The survival rate, hatching rate, heart rate, malformation and apoptotic gene expression experiments shows no significant toxicity in developing embryos up to 100 μL/mL of AuNCs concentration and the AuNCs stained embryos exhibited green fluorescence with high intensity over the period from 4 to 96 hpf (hours post fertilization) which shows that AuNCs were stable in living organisms.

1. Introduction

Noble metal nanoclusters consist of few to tens of atoms with size <2 nm (relative to the Fermi wavelength of electrons) have attracted much attention in the past few years owing to their interesting optical and electronic properties [1]. The properties of metal nanoclusters are distinct from their larger nanoparticle, with the spatial confinement of free electrons in discrete energy level leading to the molecular-like properties such as step like feature in the absorption profile [2], unusual intrinsic magnetism [3] and possess size dependent fluorescence ranging from UV to near-IR depending on the number of atoms in the cluster [4]. Therefore, these NCs are now being extensively studied for potential applications including bio-chemical sensing, bio-imaging, optoelectronics, catalysis and single molecule studies [5–11]. In particular, Au nanoclusters have received considerable research interest due to their chemical stability, size-tunable light emission, high brightness, resistance against photo-bleaching and low toxicity [12]. A number of strategies have been reported in the literatures to synthesize atomically precise monolayer protected and template assisted AuNCs in solution phase using various thiols (Au_{10}, Au_{15}, Au_{18}, Au_{20}, Au_{22}, Au_{25}, Au_{29}, Au_{33}, Au_{38}, Au_{39}, Au_{102}, Au_{130}, Au_{144} and Au_{187}) [10,13–15], phosphines (Au_7, Au_8, Au_{20}, Au_{11}, Au_{13}, Au_{39} and Au_{55}) [10,16], dendrimers (Au_5, Au_8, Au_{13}, Au_{23} and Au_{31}) [4,17,18], polymers [19–22], proteins (Au_5, Au_8, Au_{13} and Au_{23} and Au_{25}) [12,23,24] and DNA [25,26]. Most of the reported clusters emit wavelength ranging from red to NIR region [4–11,18–25]. However, there have been only limited reports documented on the synthesis of green and blue light-emitting AuNCs [27]. Dickson and co-workers have reported on the water soluble, high quantum yield AuNCs with UV (Au_5), blue (Au_8), green (Au_{13}), red (Au_{23}), and near-IR (Au_{31}) emission using poly(amido amine) dendrimer (PAMAM) [1,4,17]. Bao et al. have reported the nanoparticle free synthesis of blue, green and red emitting AuNCs at physiological temperature using PAMAM and ascorbic acid as capping and reducing agent respectively [18]. Kawasaki et al. had reported on the pH-dependent synthesis of pepsin mediated AuNCs with blue (Au_5 and Au_8), green (Au_{13}) and red (Au_{23}) emission [24]. Wang et al. have reported the blue luminescent Au_{11} NCs superlattices with high thermal stability [28]. Huang et al. have reported the synthesis of blue light emitting AuNCs using $NaBH_4$ as the reductant and thioether polymer as stabilizing ligand [29]. One-pot synthesis of blue light-emitting AuNCs and formation of photo-patternable composite films using amino-terminated poly(1,2-butadiene) has

* Corresponding author.
E-mail address: nrajendiar@yahoo.com (R. N.).

http://dx.doi.org/10.1016/j.colsurfb.2016.03.067
0927-7765/



Scheme 1. Schematic representation of the NaC-templated AuNCs formation.

also been documented [30]. Duan and Nie demonstrated ligand-induced etching process for the synthesis of green fluorescent and water soluble AuNCs using hyperbranched polyethylenimine [21]. Venkatesh et al. demonstrated water soluble green fluorescent 8-mercapto-9-propyladenine capped AuNCs for cell nuclei imaging applications [31]. Very few reports are available for the synthesis of bluish-green emitting AuNCs. Muhammed et al. reported the pH-dependent synthesis of bluish-green emitting mercaptosuccinic acid capped Au_8 NCs by ligand etching method [32]. Water soluble bluish-green emitting AuNCs using histidine as both reductant and stabilizing ligand had been extensively studied [33–35].

Although, these synthetic strategies offer good control over the cluster size and stabilities, in some cases it involves non-aqueous media, toxic reducing agent, costly chemicals and separation in the mixture of clusters, which may have limitations on the use of certain applications. Therefore, in the present investigation, we report a simple green chemical approach for the synthesis of bluish-green emitting AuNCs using NaC as a template and reducing agent. Since, NaC is a naturally occurring steroidal detergent in mammals which are amphiphatic in nature, possess chemically different functional groups, enantiomeric purity, easy availability, and low cost, making them as an ideal template for the preparation of metal nanoclusters [36]. The prepared Au clusters had an ultra-small size of <1.4 nm, high luminescence, large stoke shift and were photostable. In recent years AuNCs have been extensively used as a potential fluorescent probe for bioimaging applications. Due to their ultra-small size, bright luminescence, good biocompatibility, high stability under physiological conditions and low toxicity make them potentially more promising substituents for organic dyes, fluorescent proteins and semiconductor quantum dots [12]. However, though many reports are available for *in vitro* toxicity studies and bioimaging of these NCs, *in vivo* studies are limited [37–41]. Although toxicological evaluation of higher order organisms is desirable, such models are often expensive, time consuming and difficult to handle [42]. The more accessible and reliable *in vivo* models are required to evaluate the bio-distribution and complete toxicological assessment of metal nanoparticles (MNPs), therapeutic effects of drug molecules, and environmental effects of toxic chemicals and thereby bridging the *in vitro* cell models and small mammalian models [43–45]. Zebrafish (Danio rerio) is considered as an emerging vertebrate model system for *in vivo* studies because of the close homology shared with the human genome and good correlation with *in vitro* models [43,45]. In addition, early embryonic development of zebrafish is completed within 120 h with well-defined developmental stages, transparent embryonic and larval stages facilitate the direct optical observation of the toxic effects of nanoparticles *in vivo*, with inexpensive experimental setup and smaller size of the larvae saves space, time and resources [43–47]. Zebrafish embryos possess two distinct membranes i.e., the outer chorion and the inner vitelline membrane and a viscous fluid present between two membranes. The chorion possesses pores (0.5–0.7 μm in diameter) which allow the metal NPs to pass through the embryo via passive diffusion [43–45]. In the past few years, zebrafish model has been used extensively for toxicological studies of metal oxides, hydroxyapatite nanoparticles, dendrimers, carbon nanotubes, fullerenes, graphene based nano systems, copper, nickel, silver and gold nanoparticles [42,43,48–54]. Also, there are several reports available for the bioimaging studies of zebrafish embryos using different fluorescent probes [55,56]. Recently we have reported sodium cholate templated blue light emitting Ag subnanoclusters for *in vivo* bioimaging studies in zebrafish embryos [36]. The present study describes the synthesis and characterization of NaC templated AuNCs and their evaluation of *in vivo* toxicity and bioimaging studies on zebrafish embryos.

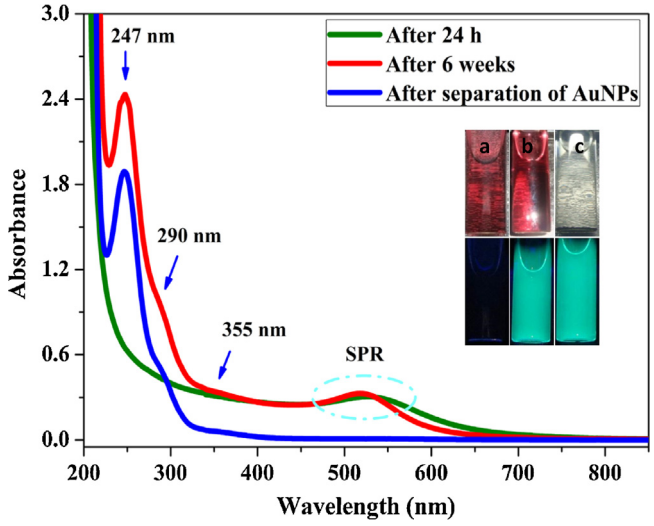


Fig. 1. UV–vis spectra of NaC templated AuNCs. Inset shows the corresponding photographs under visible (top) and UV (down) light: (a) after 24 h, (b) after 7 weeks and (d) after separation of AuNPs.

2. Experimental section

2.1. Synthesis of AuNCs

The fluorescent NaC templated AuNCs was synthesized by mixing about 3.4 mg of $HAuCl_4$ (0.1 mM in 100 mL reaction mixture) with aqueous solution of 0.1 M NaC (In 2nd CMC) in 100 mL under constant stirring for 10 min and the resulting solution was stored at room temperature. The color of the solution changed from pale yellow to red in 24 h due to the formation of AuNPs. For all the experiments, the reaction mixture was maintained at neutral pH.