International Journal of Biological Macromolecules 101 (2017) 729–735

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Facile synthesis of bovine serum albumin conjugated low-dimensional ZnS nanocrystals

Bhanu Mullamuri[a], Subrahmanya Diwakar Bhagavathula[b], Krishna C.S.B. Kasturi[a], Venu Reddy[a,*]

[a] School of Engineering Science and Technology, University of Hyderabad, Gachibowli, Hyderabad, 500046, India
[b] Department of Engineering Chemistry, SRKR Engineering College, China Amiram, Andhra Pradesh, 534204, India

ARTICLE INFO

Article history:
Received 27 December 2016
Received in revised form 27 March 2017
Accepted 28 March 2017
Available online 30 March 2017

Keywords:
Bovine serum albumin
ZnS nanoparticles
Bioconjugation

ABSTRACT

We present a facile synthesis of bovine serum albumin (BSA) conjugated low-dimensional ZnS nanocrystals. The experimental parameters such as effects of BSA concentration and precursor vol ratios of Zn:S on the formation of ZnS nanoparticles in BSA matrix were investigated. The ZnS crystalline sizes of 1.9, 1.8 and 1.6 nm were obtained by using the BSA concentrations of 1×10^{-4}, 5×10^{-4} and 10×10^{-4} g/mL, respectively, with a fixed Zn:S vol ratio of 1:1. The ZnS samples prepared from 1:10 and 10:1 vol ratios of Zn:S at BSA concentration of 5×10^{-4} g/mL shows the crystalline sizes of ZnS are 2.1 and 1.5 nm, respectively. FT−IR analysis suggests that the prepared ZnS nanoparticles might be conjugated through the interactions of hydroxyl and amine groups present in BSA. We evaluate the cytotoxicity of the prepared ZnS nanoparticles, the THP-1 cells showed a good viability (>88%) for all the prepared ZnS samples. The plausible mechanism for the formation of ZnS-BSA composite has also been discussed.

1. Introduction

Chalcogenide semiconductors have attracted much attention due its potential applications in electro-luminescence devices (e.g. light emitting diodes and lasers), nanoelectronics, sensors and actuators [1–5]. Among, the zinc sulfide (ZnS) is an important II-IV semiconductor material with a broad rand range of band gap (3.72 and 3.77 eV for cubic zinc blende and hexagonal wurtzite phases at 300 K, respectively) and large exciton binding energy (40 meV). It has a wide range of applications such as flat-panel displays, electroluminescent devices, infrared windows, sensors and lasers [6–10].

Recently, the low dimensional (0D) ZnS nanocrystals (nanoparticles) have aroused considerable interest from the biomedical field due to their unique optical properties [11]. Thus far, different methods have been used to prepare ZnS nanoparticles such as ion-exchange method, microwave, photochemical, hydrothermal, solvothermal, coprecipitation, electrochemical deposition and mechanochemical methods [12–18]. Nevertheless, all of these methods involved stringent conditions such as high temperature, high pressure, inert gas environment, special instruments, and long reaction time. Therefore, the development of facile synthetic routes for ZnS nanoparticles is highly desirable.

In this context, a very few methods are reported to fabricate ZnS nanoparticles using different host matrixs and stabilizing agents [19–22]. However, most of these ZnS products are limited to use in the biomedical applications because of their cytotoxicity. Therefore, exploring a novel approaches for preparing the low cytotoxicity ZnS nanoparticles with facile conditions is a great challenge. Recently, there is an increasing interest in the preparation of bioconjugated nanoparticles due to its unique properties like aqueous solubility, compatible for biosystems (low cytotoxicity) and used as labels in bioassays [23,24]. Typically, the bovine serum albumin (BSA) is an extensively used as biocompatible template for the synthesis of nanoparticles due to presence of abundant functional groups in BSA, such as, hydroxyl, amine, carboxyl and thiol [25,26]. It facilitate the synthesis of nanoparticles through its microcavity and able to catalyze the process in an aqueous media [24]. BSA is also used as a drug carrier for administering chemotherapeutic drugs, which is approved by U.S [27].

In view of these facts, herein, we report a facile synthesis of low toxicity ZnS nanoparticles (BSA conjugated ZnS nanoparticles). The synthesized nanoparticles were systematically characterized with transmission electron microscopy (TEM), X–ray diffraction (XRD) and fourier transform infrared spectroscopy (FT–IR) techniques. We investigate the production of ZnS nanoparticles in BSA matrix

* Corresponding author.
E-mail address: rvenu8@uohyd.ac.in (V. Reddy).

http://dx.doi.org/10.1016/j.ijbiomac.2017.03.164
0141-8130/

as a function of the concentration of BSA and vol ratios of Zn:S. The prepared ZnS nanoparticles were demonstrated to have no considerable toxicity for the treatment of THP-1 cells. The possible mechanism of as prepared bioconjugated ZnS nanoparticles was also discussed.

2. Experimental

2.1. Chemicals and instruments

Zinc acetate dihydrate, sodium sulfide and acetic acid were procured from Merck, India. Bovine serum albumin was purchased from Sigma-Aldrich, USA. Roswell park memorial institute medium (RPMI) 1640 medium and penicillin–streptomycin were obtained from Thermo Fisher Scientific, USA. Fetal bovine serum was purchased from American Type Culture Collection (ATCC), USA. Cell counting kit-8 (CCK-8) was procured from ENZO life technologies, USA.

The obtained ZnS nanoparticles were characterized by means of XRD, TEM and FT–IR. XRD measurements were performed on a Bruker's AXS Model D8 Advance powder X–ray diffractometer instrument operated with Cu K_α radiation as the X–ray source (λ = 1.54 Å). TEM images, selected area electron diffraction pattern (SAED) and energy dispersive X–ray (EDX) data were obtained on an FEI Technai G2 S–Twin. For TEM analysis, a few drops of the as-prepared ZnS samples dispersion onto the carbon coated copper TEM grid and dried overnight. The FT–IR spectra were collected on the Nicolet 5700 Thermo Scientific instrument with the samples as KBr pellets. The culture plate absorbance was measured on Thermo Scientific Multiskan GO microplate spectrophotometer.

2.2. Methods

2.2.1. Synthesis of BSA stabilized ZnS nanoparticles

BSA conjugated ZnS nanoparticles were prepared at room temperature. A 20 mL of BSA aqueous solution with concentrations of 1×10^{-4}, 5×10^{-4} and 10×10^{-4} g/mL were taken into three beakers. Under constant stirring, a 2 mL of 0.5 M acetic acid was slowly added into each beaker to attain slightly acidic condition. Then, 5 mL of 0.1 M zinc acetate aqueous solution and 5 mL of 0.1 M sodium sulfide aqueous solution were added into the each beaker. The resultant mixtures were allowed to stand for 1 h and then centrifuged at 1100 rpm for 15 min. The prepared ZnS nanoparticles at various concentrations of BSA were washed with water for 5 times. The washed ZnS nanoparticles were redispersed into 10 mL of water. In other experiments, 10:1 and 1:10 vol ratios (mL) of Zn:S were used with a fixed BSA concentration of 5×10^{-4} g/mL.

2.2.2. In vitro *cytotoxicity studies*

The THP-1 cells were cultured in RPMI 1640 medium containing fetal bovine serum and penicillin–streptomycin solution. The cells were cultured in a 100 mm cell culture dish and maintained in a humidified incubator at 37 °C with 5% of CO_2 and 95% of air.

The CCK-8 assay was used to perform *in vitro* cytotoxicity tests for the prepared ZnS samples. The cells with a density of ~2.5×10^4 cells/well were placed in 96-well tissue culture plates. The cell loaded 96-well tissue culture plates were kept in a humidified incubator at 37 °C with 5% of CO_2 and 95% of air and allowed to grow for 8 h in the wells. Then, 10 μL of various BSA conjugated ZnS nanoparticles were added into 96-well tissue culture plates and incubated the culture plates for testing times of 12, 24 and 48 h. The cell suspension without ZnS nanoparticles was considered as a control. After completion of specific times (12, 24 and 48 h), 10 μL of (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H tetrazolium, monosodium salt (WST-8) in CCK-8 kit was placed into culture plates and incubated for 3 h at 37 °C according to the manufacturer's instructions. The culture plate absorbance at 450 nm was measured with microplate reader. The toxicity of the treated samples was expressed as a percentage of control cell samples (without nanoparticles treatment), assumed to be 100%.

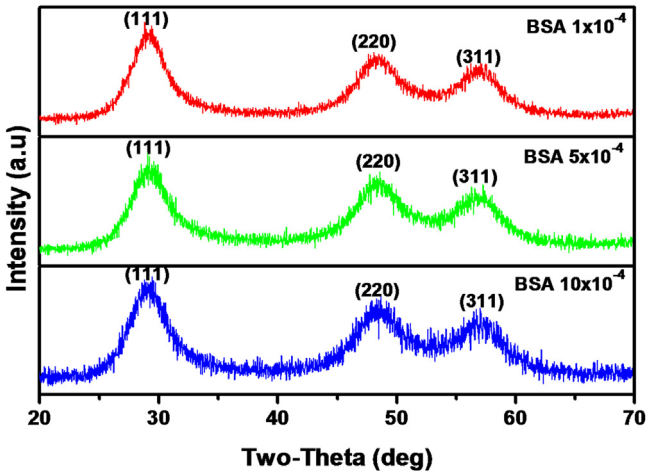

Fig. 1. XRD patterns of ZnS nanoparticles with BSA concentrations of 1×10^{-4}, 5×10^{-4} and 10×10^{-4} g/mL at 1:1 vol ratio of Zn:S.

3. Results and discussion

3.1. Characterization of ZnS nanoparticles

Fig. 1 displayed the XRD patterns of ZnS nanoparticles prepared from equal volumes (5 mL) of 0.1 M zinc and 0.1 M sulfur precursors at BSA concentrations of 1×10^{-4}, 5×10^{-4} and 10×10^{-4} g/mL. The diffraction patterns at two theta = 29, 48 and 56° represents the (111), (220) and (311) planes of cubic zinc blende phase of ZnS, respectively [28].

Furthermore, the spacing d_{hkl} between adjacent lattice planes were measured from Bragg relation:

$$2d_{hkl}\sin\theta = n\lambda \quad (1)$$

where θ is the angle of incidence, λ is the wavelength of X-ray (λ = 1.54 Å) and the integer *n is* the order of the corresponding reflection.

The cell constant (a) of obtained ZnS crystallites were calculated from the XRD peaks by means of following formula:

$$d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}} \quad (2)$$

The spacing d_{hkl} between adjacent lattice planes and cell constant values of as-prepared ZnS nanoparticles were presented in Table 1. The cell constant of the obtained ZnS crystallites were almost similar to that of standard ZnS phase (a = 5.39 Å). The crystallize size information of the prepared ZnS were obtained from the Debye-Scherrer equation:

$$L = \frac{0.9\lambda}{\beta cos\theta} \quad (3)$$

where, β is the full width at half maximum of the XRD peaks. The estimated crystalline sizes of obtained ZnS nanoparticles were shown in Table 1. The ZnS samples with BSA concentrations 1×10^{-4}, 5×10^{-4} and 10×10^{-4} g/mL were found to have no considerable change in crystalline sizes of ZnS particles. Thus, in the present synthesis, the variation in BSA concentrations could not affect the crystalline sizes of ZnS particles.