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Zinc selenide nanoparticles (ZnSe-NPs): Green synthesis and investigation of their cytotoxicity effects

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ABSTRACT

Mono-dispersed crystalline Zinc selenide nanoparticles (ZnSe-NPs) have been synthesized at ambient temperature conditions via a "***green***" chemistry method. The nanoparticles were characterized by Transmission electron microscopy (TEM) and UV–vis spectrophotometry. *In vitro* cytotoxicity studies on Neuro2A and HeLa cell lines illustrated a dose dependent toxicity with non-toxic effect of ZnSe-NPs up to a concentration of 3.9 and 7.8 μg/mL, respectively. The influencing reaction time was also investigated, and the possible formation mechanism was also proposed. The suggested method is facile, green, easy, and it can be employed to large-scale fabrication.

1. Introduction

In recent years, preparation of different nanomaterials has attracted the attention of a large number of research studies due to their unique physical and chemical properties [1,2]. Among the various type of semiconductors (i.e. CdS, CdSe, CdTe), there are relatively few reports on ZnSe-NPs nanostructures. ZnSe is a n-type semiconductor with a wide band gap of ~2.7 eV at 25 °C [3]. As a member of selenide nanoparticles family (CdSe, FeSe, etc.), ZnSe-NPs can be utilized in different fields, such as in flat panel displays, photo detectors, light-emitting diodes, biosensors and biomedical applications [4–10].

There are wide studies on the synthesis of ZnSe–NPs such as wet chemical [11], sol–gel [12], hydrothermal [13], solvothermal [14], microemulsion [15], reverse micelles [16], and spray pyrolysis [17]. However, these methods suffer from several drawbacks, like, use of high pressure and temperature, hazardous materials/chemicals and specific stabilizers as additives [18], which limits the purity of final product. Therefore, it is necessary to prepare the ZnSe-NPs via a facile, green, economical and eco-friendly method. In this study, we report a facile, low cost and reproducible synthesis of ZnSe-NPs at ambient temperature by an ecofriendly benign "green" method.

2. Materials and methods

2.1. Materials and reagents

Zinc nitrate [$Zn(NO_3)_2 \cdot 6H_2O$], ascorbic acid, sodium hydroxide and sodium selenide (Na_2SeO_3) were purchased from Merck. Ultrapure water employed for the experiments. The Neuro2A neuroblastoma and HeLa cell lines were purchased from Iranian Academic Center for Education, Culture and Research (ACECR), Mashhad.

2.2. Synthesis of ZnSe-NPs

This preparation method involves addition of the selenide ions (via dissolving) to the aqueous solution of zinc nitrate and ascorbic acid at ambient temperature. In this method, the aqueous solution

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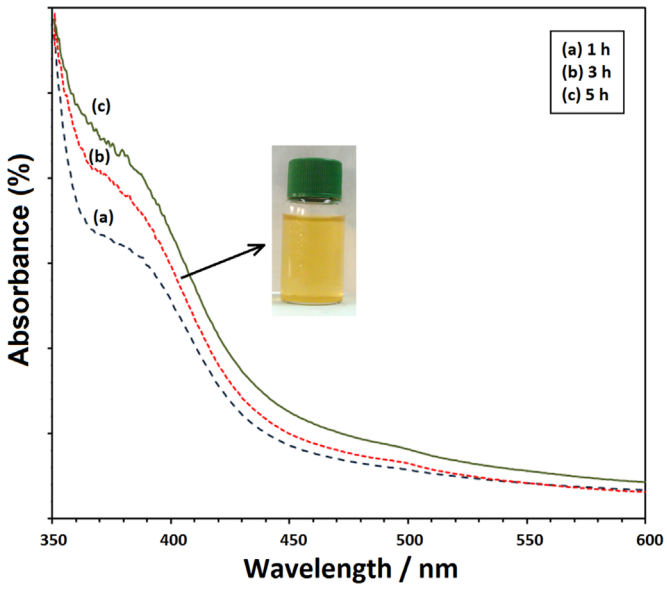

Fig. 1. The UV–vis spectra for synthesized ZnSe-NPs at different reaction times (pH=12).

of $Zn(NO_3)_2$ (16×10^{-3} M) was mixed to a solution of ascorbic acid (16×10^{-2} M), under constant stirring at ambient temperature. The pH of as-prepared solution was raised up to 12 by the dropwise addition of NaOH solution (1.0 M). This was followed by the slow addition of selenide solution (Na_2SeO_3, 16×10^{-3} M) at the same conditions. The molar ratio of Zinc, Selenide and Ascorbic acid was proposed 1, 1, and 10, respectively. In order to monitor the formation of ZnSe-NPs, the synthetic procedure was carried out at different reaction times i.e., 1, 3, 5 h.

A PerkinElmer Lambda 25 UV–vis Spectrophotometer was employed to analyze the optical properties in the range of 350–600 nm. A Philips CM120 microscope was used for TEM measurements.

3. Results and discussion

3.1. MTT assay

The Neuro2A neuroblastoma and HeLa cell lines were cultivated in Dulbecco's Modified Eagle's medium (DMEM) with 2–10% Fetal Calf Serum (FCS) and incubated at 37 °C in 5% CO_2. After 48 h when the cells reached approximately 80% confluence, they were detached with trypsin (3–5 min) and centrifuged (1400 rpm, 5 min). Afterwards, the cells were counted and distributed in 96 well plates (ELISA), with 10,000 cells in each well. The plate was incubated for 24 h at 37 °C in a 5% CO_2 atmosphere to allow the cells to get attached to the bottom of the well. Then morphology of cells was observed in invert microscope before exposure to ZnSe-NPs. After 24 h, the supernatants were removed and the ZnSe-NPs were diluted (triplicates) and were added to the wells. Spent medium was replaced with 100 µL of fresh medium, and 10 µL of MTT solution (5 mg/mL PBS, sterile) were added to each well and incubated for 4 h at 37 °C in a 5% CO_2. The MTT crystals were dissolved by adding 100 µL of DMSO to each well. The plates were placed on a shaker (15 min) for complete removal of crystals and then the optical density of each well was determined using a plate reader at 545 nm. The absorbance of different concentration directly indicates the number of living cells. The relative cell viability (%) related to control wells containing cell culture medium without nanoparticles was also calculated.

3.2. Mechanism

Initially formation of zinc complex was carried out in the presence of ammonia as the complexing agent [19]. A green reducing agent (like glucose and ascorbic acid) was required to be introduced in the chemical reaction. Ascorbic acid reduces Na_2SeO_3 and HSe^- to an extent to have enough Se^{2-} ions available for ZnSe formation (Eqs. (1) and (2)). In this work, addition of Se precursor solution (in presence of ascorbic acid) resulted in a gradual change in the color of the final solution from colorless to yellowish, indicating the formation of ZnSe-NPs. Therefore, following chemical

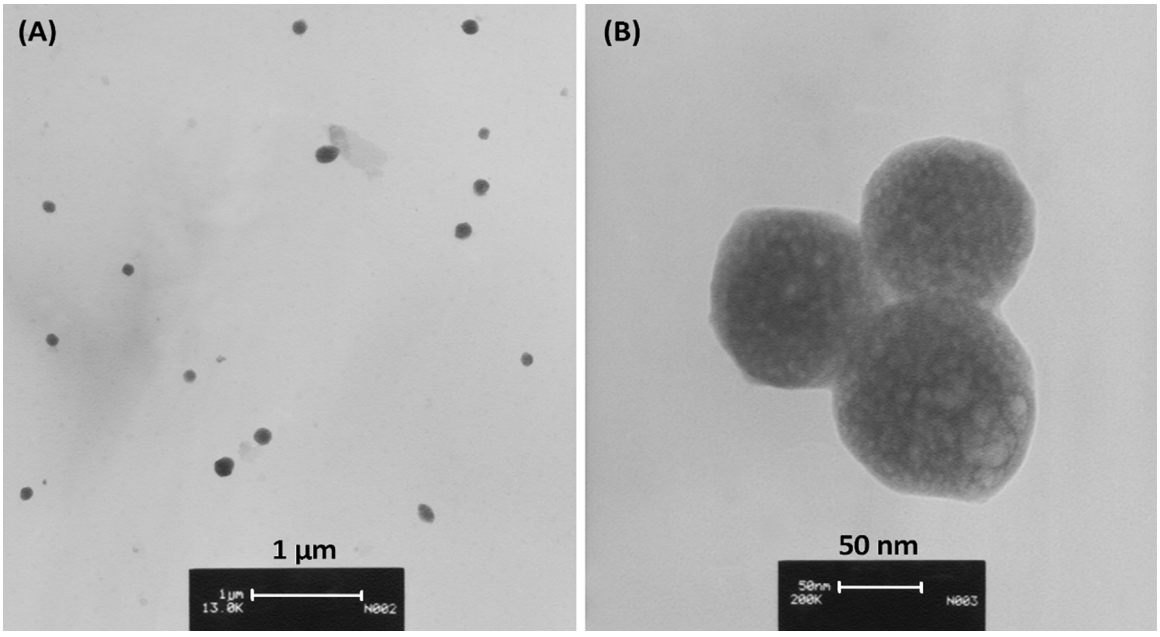

Fig. 2. The TEM images for synthesized ZnSe-NPs (pH=12 after 3 h) at different magnifications (× 13,000 (A), and × 200,000 (B)].