

## Neuronal toxicity of biopolymer-template synthesized ZnO nanoparticles

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### Abstract

**Objective(s):** A simple and "green" method was developed for preparing zinc oxide nanoparticles (ZnO-NPs) in aqueous starch solutions. Starch was used as a stabilizer to control of the mobility of zinc cations and then control growth of ZnO-NPs prepared via a sol-gel method. Because of the special structure of the starch, it permits termination of the particle growth.

**Materials and Methods:** The dried gel was calcined at different temperatures of 400, 500, 600, and 700 °C. The prepared ZnO-NPs were characterized by different techniques such as X-ray diffraction analysis (XRD), transmittance electron microscopy (TEM), and UV-Vis absorption. **Results:** The XRD results displayed hexagonal (wurtzite) crystalline structure for prepared ZnO nanoparticles with mean sizes below than 50 nm. *In vitro* cytotoxicity studies on neuro2A cells showed a dose dependent toxicity with non-toxic effect of concentration below 6 µg/mL.

**Conclusion:** The results showed that starch is an eco-friendly material that can be used as a stabilizing agent in the sol-gel technique for preparing of ZnO-NPs in a large scale.

**Keywords:** Electron microscopy, ZnO, Sol-gel processes, Starch

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### Introduction

In recent years, the preparation of nanosized materials has garnered much more attention due to their different properties, such as industrial and medical properties compared to macro-sized materials (1). Zinc oxide nanoparticles (ZnO-NPs) are one of the most abundantly used nanomaterials in consumer products and biomedical applications due to their specific properties, e.g. transparency, high isoelectric point, biocompatibility, and photocatalytic efficiency. They are widely employed in a variety of devices including cosmetics, toothpaste, sunscreens, fillings in medical materials, textiles, wall paints, and other building materials (2). Metal oxide nanoparticles such as ZnO are very important; therefore, many synthetic routes have been explored for their preparation (3-7). Many techniques require special apparatuses for preparing of nanomaterials, which can be very difficult in several cases. Using new and easy methods to prepare nanomaterials is still actually possible. Recently, different methods have been applied to prepare nanomaterials, especially in size and shape, using natural materials as the stabilizing/capping agents such as proteins, peptides, biopolymers, oligonucleotides, carbohydrates, and plants extract (8-12). Starch has been used as a capping agent to the preparation of Ag nanoparticles for the first time (13) and it was suggested that the extensive network of hydrogen bonds in the templates provides surface helical organic as a passivation or protection against nanoparticle aggregation (14). Starch has a good chemical reactivity compared to other carbohydrates, by the reactivity of the hydroxyl groups of its  $\alpha$ -glucose units. These hydroxyl groups can react similarly to the hydroxyl groups of primary and secondary alcohols. Hence, they can be used as oxidation agents for metal salts (15) and for this reason; it can be a suitable capping agent.

In this work, a sol-gel method was applied for preparing ZnO nanoparticles using starch media. Starch was used as a capping agent, and it served as a terminator for growing the ZnO nanoparticles because of its long chain

made with glucose, which terminates the mobility of the cations. Therefore, the particles cannot come together easily and nanosized ZnO particles are obtained. The presence of starch in the reaction mixture avoids use of relatively toxic organic solvents.

### Materials and Methods

#### *Materials and reagents*

The  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  and starch were analytical grade and used as received without further purification.

#### *Synthesis of ZnO-NPs*

To prepare 1.5 g of ZnO-NPs, 4.5 g of  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  was dissolved in 10 ml of distilled water and then stirred for 30 min. Meanwhile, 0.2 g of starch was dissolved in 40 ml of distilled water and stirred for 90 min at 60°C to achieve a clear starch solution. After that, the zinc nitrate solution was added to the starch solution, and the container was moved to an oil bath. The temperature of the oil bath was fixed at 80°C. Stirring was continued for 12 h to obtain a white color resin. The final product was calcined at different temperatures (400, 500, 600 and 700 °C) in air for 1 h to obtain ZnO-NPs.

#### *Characterization of ZnO-NPs*

The prepared ZnO-NPs were characterized by powder X-ray diffraction (PXRD, Philips, X'pert,  $\text{Cu K}_\alpha$ ), thermogravimetry analysis (TGA, Q600), UV-vis spectrophotometry (UV-vis, Evolution 300<sup>®</sup> Thermo Fisher Scientific), and transmission electron microscopy (TEM, Hitachi H-7100).

#### *Evaluation of neurotoxicity effect*

The cytotoxicity of nanoparticles was evaluated by the method using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (16). Briefly, neuro2A cells were seeded at a density of  $1 \times 10^4$  cells per well in 96-well plates and incubated for 24 h. Thereafter, the cells were treated with various concentrations of nanoparticles in the presence of 10% FBS. The calcined ZnO-NPs at 600°C were suspended in a stock solution

at 5 µg/ml in a solution of dimethyl sulfoxide (DMSO)/double distilled water. After 24 h of incubation, 20 µl of 5 mg/ml MTT in the PBS buffer was added to each well, and the cells were further incubated for 4 h at 37°C. The medium containing unreacted dye was discarded, and 100 µl of DMSO was added to dissolve the formazan crystal formed by live cells. Optical absorbance was measured at 590 nm (reference wavelength 630 nm) using a microplate reader (Statfax-2100, Awareness Technology, USA), and cell viability was expressed as a percent relative to untreated control cells. Values of metabolic activity are presented as mean±SD of triplicates.

## Results and Discussion

The thermogravimetric and derivative analysis (TGA/DTA) curves of the prepared gel by the sol-gel method in a starch environment are presented in Figure 1.

The heating process was started at 20°C, and then increased up to 1000°C with a temperature rate change of 10°C/min. The TGA curve descends until it becomes horizontal around 500°C, and about 79% weight loss was observed during the heating process. The TGA/DTA traces show three main regions. The first weight loss between 20 and 110°C (8%) is an initial loss of water. The second weight loss from 110°C to 150°C (49%) is attributed to the decomposition of chemically bound groups and the formation of the pyrochlore phases.

The third step from 150 to 500°C (22%) is related to the decomposition of the pyrochlore phases and the formation of ZnO pure phases. No weight loss between 500 and 1000°C was detected on the TGA curve, which indicates the formation of nanocrystalline ZnO as the decomposition product (7).

The room temperature UV-vis absorption spectrum of the ZnO-NPs is shown in Figure 2. The ZnO-NPs were dispersed in water with concentration of 0.1% wt and then the solution was used to perform the UV-vis measurement. The spectrum reveals a characteristic absorption peak of ZnO at wavelength of 370-375 nm which can be assigned to the intrinsic band-gap absorption of ZnO due to

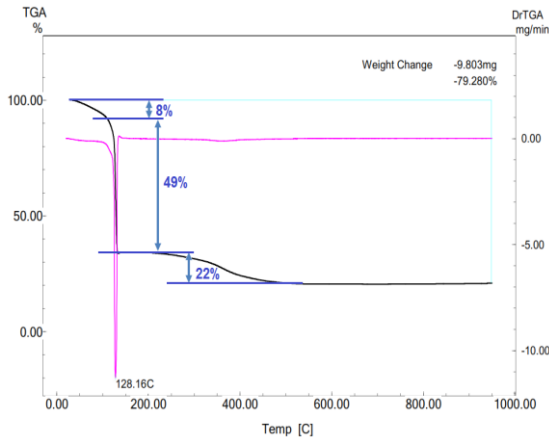
the electron transitions from the valence band to the conduction band ( $O_{2p} \rightarrow Zn_{3d}$ ) (17, 18). In addition, this sharp peak shows that the particles are in nano-size, and the particle size distribution is narrow. It is clearly shown that the maximum peak in the absorbance spectrum does not correspond to the true optical band gap of the ZnO-NPs. A common way to obtain the band gap from absorbance spectra is to get the first derivative of the absorbance with respect to photon energy and find the maximum in the derivative spectrum at the lower energy sides (5, 9). The derivative of the absorbance of the ZnO-NPs indicates a band gap of 3.3 eV for the ZnO-NPs. The good absorption of the ZnO-NPs in the UV region proves the applicability of this product in such medical application such as sun-screen protectors or as antiseptic in ointments (19). Typical PXRD pattern of the ZnO-NPs calcined at 600°C is shown in Figure 3. All of the detectable peaks with Miller indices [100], [002], [101], [102], [110], [103], [200], [112], [201], [004], and [202] can be indexed to the ZnO wurtzite structure (20).

The broadening of the peak indicates that the crystalline size are in nanoscale (21) and this result indicates that the size of the obtained samples is fine and small (below 50 nm), as confirmed by the TEM image (Figure 4) of prepared ZnO-NPs at 600°C. Furthermore, no other peaks related to an impurity for prepared ZnO-NPs in this work, indicating that the final nano powders were relatively pure. The TEM image (Figure 4) confirms that a high homogeneity of prepared ZnO-NPs which can be obtained in starch media. Starch occurs in a variety of botanical sources including potato, wheat and corn.

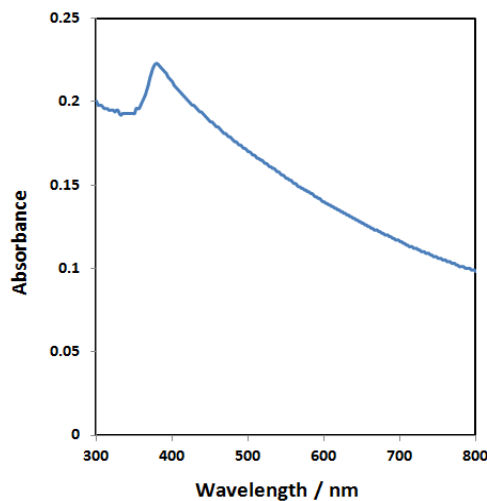
Starches from different botanical sources vary widely in structure and composition, but all granules consist of two major molecular components, amylose and amylopectin, both of which are polymers of  $\alpha$ -glucose units in the  ${}^4C_1$  conformation. Starch becomes soluble in water when the temperature of the cloudy solution reaches 75 °C and the semi-crystalline structure is lost.

After adding the zinc nitrate solution to starch

## Neurotoxicity effect of sol-gel derived ZnO nanoparticles



**Figure 1.** The TGA/DTA curves of gels from 20°C to 1000°C. It shows about 79% loss weight in three steps to achieve ZnO-NPs.



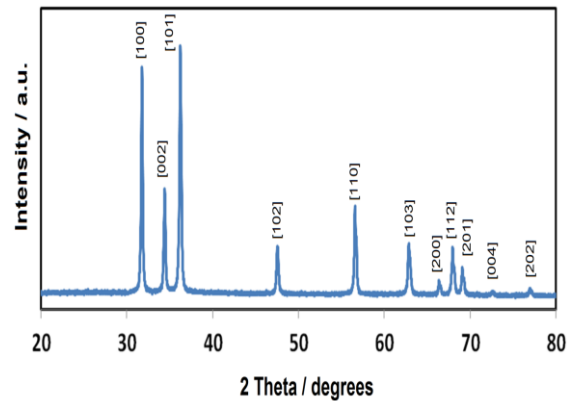
**Figure 2.** UV-vis spectrum for band gap estimating of prepared ZnO-NPs at 600°C.

solution, the metal cations are attracted by oxygen of the OH branches. By continuing the heating process to decrease the amount of water, the smaller amylose molecules start forming a network that holds water, and increases the mixture's viscosity. This process is called starch gelatinization. The nitrate decomposed to nitrogen dioxide and oxygen during the heating process, and will be removed from the compounds (17).

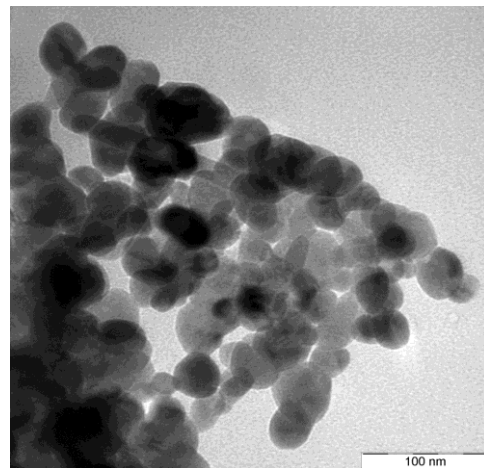
The results of *in vitro* cytotoxicity studies after 24 h of incubation with different concentrations of nanoparticles, ranging from 0 to 100 µg/mL, are shown in Figure 5.

As the results showed, for concentration above 6 µg/mL the metabolic activity was decreased in a concentration dependent manner meaning that metabolic activity was

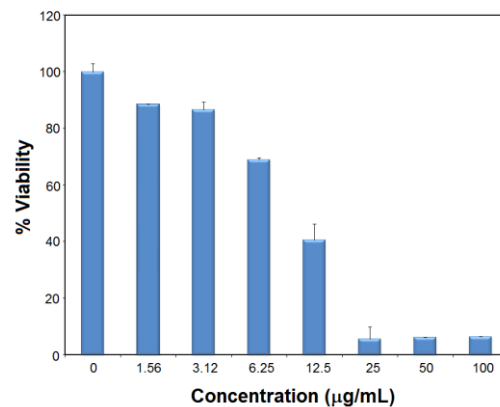
started to decrease from 6 µg/mL and in 100 µg/mL maximal decreasing was observed. One of the advantages of this method is being able to use renewable material like starch, which is eco-friendly agent without any additional physical conditions.



**Figure 3.** The PXRD pattern of synthesized ZnO-NPs in air at 600°C.



**Figure 4.** The TEM image of synthesized ZnO-NPs in air at 600°C.



**Figure 5.** Cell viability of neuro2A cells measured by the MTT assay. Cells were incubated for 24h with the indicated concentrations of the nanoparticles.

## Conclusion

ZnO-NPs were synthesized by the sol-gel method in starch media. From PXRD results, it was observed that prepared ZnO-NPs exhibited the high purity with the hexagonal, wurtzite structure. General toxicity of nanoparticles could be differ and dependent on many factors such as the types, size and method of preparation of nanoparticle. Even the cell line used in the experiment may have impacts on the results. This method is interesting for applying and extending the green chemistry rules in preparation of nanoparticles. Due to the reaction mechanism and the results, we observe that starch is a suitable stabilizer and capping agent to prepare ZnO-NPs on a large scale using the sol-gel method. It is expected that these nanoparticles can find potential applications in different fields such as cosmetics and optical/electrical devices as well as medicinal applications.

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