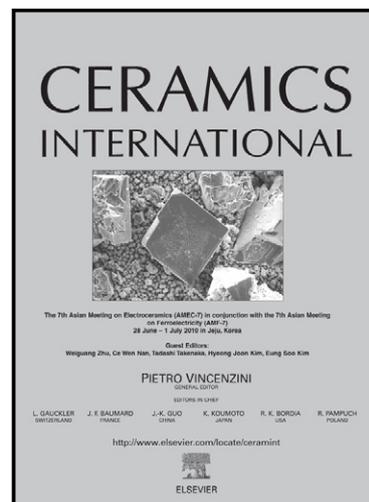


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Role of polyethyleneimine (PEI) in synthesis of zinc oxide nanoparticles and their cytotoxicity effects

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Abstract

Monodispersed zinc oxide nanoparticles (ZnO–NPs) were synthesized by a simple sol-gel method. In this process, polyethyleneimine (PEI) was used as a polymeric matrix and mild reaction conditions. The PEI acted as a stabilizing or capping agent and polymeric template for preparing of ZnO–NPs. The ZnO–NPs were successfully grown at different calcination temperatures, and their crystallite structures were characterized using various methods, including TEM, PXRD, FTIR, and TGA/DTA techniques. The PXRD analysis revealed wurtzite hexagonal ZnO with preferential orientation at (101) reflection plane. Spherical ZnO–NPs were synthesized and its TEM image showed the formation of nanopowders in size of about 25 nm, while dose dependent toxicity with non-toxic effect of concentration below 6.25 µg/mL was observed in the studies of *In vitro* cytotoxicity on neuro2A cells.

Keywords: ZnO; nanoparticles; polyethyleneimine; organic; cytotoxicity

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1. Introduction

Nanotechnology is now rapidly and extensively used throughout the medicine, electronics, chemical, and mechanical applications. The investigation of materials in nanoscale is a rapidly developing and broadening part of research. It presents a suitable area to develop and extend novel materials in which different properties can be formed at the sub-nano scale, and offer a remarkable potential medical and industrial applications. Among these nanomaterials, zinc oxide nanoparticles (ZnO-NPs) as an important rare-earth oxide material have attracted interest due to its unique physic-chemical properties such as luminescent properties, large band gap (3.37 eV), and large excitonic binding energy (60 meV) at ambient temperature [1-3]. It is widely used in many and various technological fields such as catalyst [4], UV lasers [5], piezoelectric devices [6], pigments [7], and medical usage in transparent UV protection [8]. In general, the fabrication on nanoparticles is a complicated process, and a wide variety of different variables may affect the properties of the final product [9]. In the case of fabrication of nanoparticles such as ZnO-NPs, it is very important to obtain monodispersed nanoparticles and to be able to control the morphology of the ZnO-NPs. The objectives can be achieved by using a suitable capping/stabilizing agent in the sol-gel process. Poly ethylene glycol (PEG) [10], triethanolamine (TEA) [11], gelatin [12], starch [13, 14], poly-vinyl alcohol (PVA) [15], diethanolamine (DEA) [16], polyvinylpyrrolidone (PVP) [17], and gum tragacanth [18] are some of these stabilizers that have been used to control the morphology and size of ZnO-NPs. Branched polyethyleneimine (PEI) has many primary amino groups which this molecular structure enables PEI to be used as a stabilizing agent to form various nanoparticles such as metal or metal oxide nanoparticles [19-21]. In this work, a simple sol-gel route was created to prepare ZnO-NPs in PEI media. PEI was used as a capping/stabilizing agent, and it serves as a

terminator for growing the ZnO-NPs because it expands during the calcination process and the particles cannot come together easily.

2. Materials and methods

2.1. Materials and reagents

All the materials used were of analytical grade and were used without any purification. Zinc (II) nitrate hexahydrate was purchased from Fluka (Germany) and branched polyethyleneimine (PEI) with MWs of 1800 (99%) was purchased from Polysciences Inc. (Warrington, PA, USA). All glassware used in the laboratory experiments were cleaned with fresh solutions of HNO₃/HCl (3:1, v/v), washed thoroughly with doubly distilled water, and dried before use. Double distilled water was used in all experiments. For the evaluation of metabolic activity, Neuro2A murine neuroblastoma cells (ATCC CCL-131, Manassas, VA, USA) were grown in Dulbecco's modified Eagle's medium (1 g/L glucose, 2 mM glutamine), supplemented with 10% FBS, streptomycin at 100 mg/ml, and penicillin at 100 U/ml. All cells were incubated at 37 °C in a humidified 5% CO₂ atmosphere.

2.2. Synthesis of ZnO-NPs

To prepare ZnO-NPs, 50 ml of 1.50 M aqueous solution of Zn(NO₃)₂·6H₂O was prepared. Then, 10.0 g of PEI was dissolved in 150 ml of double distilled water and the solution was heated at 60 °C for 30 min to achieve a homogeneous and clear tannic acid solution. Subsequently, the zinc nitrate solution was added dropwise to the PEI solution and final solution container was kept to a water bath at 80 °C with continuous stirring for 12 h to obtain the brown resin. Finally, the obtained gel was divided to four parts and heated at different temperatures such as 400 (S1), 500 (S2), 600 (S3) and 700 °C (S4) in air for 8 h.

2.3. Characterization of ZnO-NPs

The phase evaluation and crystalline structure of the ZnO-NPs were investigated by powder X-ray diffraction (PXRD, Philips, X'pert, Cu K α). The prepared ZnO-NPs were also characterized by Fourier transform infrared (FTIR\ST-SIR spectrometer), thermogravimetric analysis and differential thermal analysis (TGA/DTA, TA Instruments, USA), and transmission electron microscopy (TEM, Leo 912 AB, Germany).

2.4. Evaluation of cytotoxicity effect

The cytotoxicity of obtained ZnO-NPs was evaluated by the method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [22]. Briefly, neuro2A cells were seeded at a density of 1×10^4 cells per well in 96-well plates and incubated for 24 h. Thereafter, the cells were treated with various concentrations of nanopowders in the presence of 10% FBS. The calcined ZnO-NPs (S₃) was suspended in a stock solution at 5 $\mu\text{g/ml}$ 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 $^{\circ}\text{C}$. 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 \pm SD of triplicate.

3. Results and discussion

This section reports the results and discussion of the prepared ZnO-NPs in PEI mediated solutions. As shown in Fig. 1, the color of sol-gel derived ZnO-NPs due to the increased calcination temperature changed from brown (S1) to white (S4).



Fig. 1. Lab photograph of prepared ZnO-NPs at different calcination temperatures.

The thermogravimetric analysis and differential thermal analysis (TGA/DTA) curves of the as-prepared PEI-based gel containing zinc nitrate by the sol-gel method is presented in Fig. 2. The heating treatment was started at about 20°C, and then increased up to 950°C along with a temperature rate change of 10°C/min. The TGA curve descends until it becomes horizontal around 495°C, and about 75% weight loss was observed during the heating treatment. The TGA/DTA traces show three main regions having an initial loss of water in the first weight loss between 20 and 185°C (6.7%; Ed1). Bend Ed1 is related to the evaporation of the initial water and decomposition of the organic bonds of PEI. The second weight loss from 185°C to 345°C (40.1%) is attributed to the decomposition of chemically bound groups, which corresponds to

bend Ed2. The third step from 345 to 495°C (28.2%) is related to the formation and decomposition of the pyrochlore phases along with the formation of ZnO pure phases indicated by bend Ed3. No weight loss between 495 and 950°C was detected on the TGA curve, which indicates the formation of nanocrystalline ZnO as the decomposition product [12, 23].

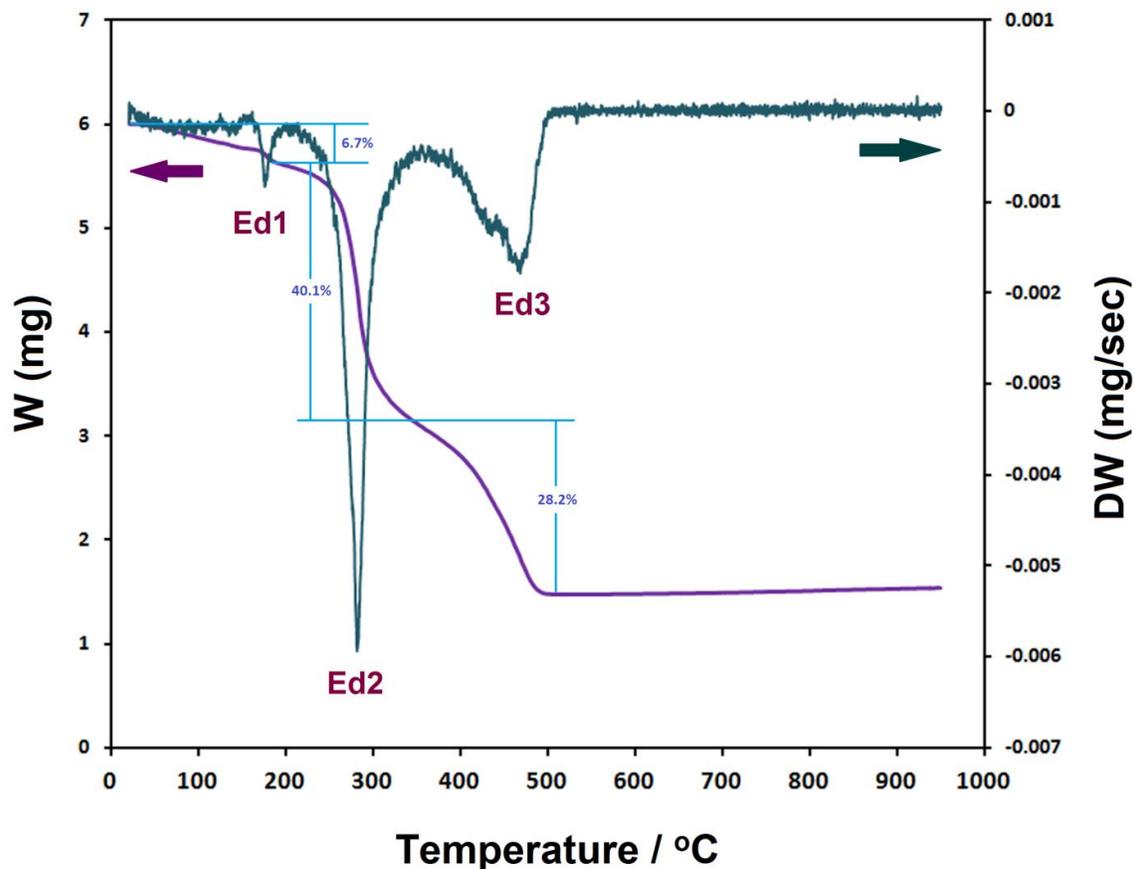


Fig. 2. The TGA/DTA curves of initial gel (Zinc nitrate+PEI) from 20 to 950°C.

The PXRD pattern of S1 is shown in Fig. 3. All of the detectable peaks with Miller indices (100), (002), (101), (102), (110), (103), (200), (112), (201), (202), and (203) can be indexed to the ZnO wurtzite hexagonal structure (JCPDS # 36-1451) with preferential orientation at (101) reflection plane [24]. The PXRD data of prepared ZnO-NPs illustrated the absence of any impurities,

which attested its high quality. The average crystallite size (D) of ZnO–NPs was determined by the Debye–Scherrer formula (Eq. 1) [25]:

$$D = 0.9\lambda / \beta \cos\theta \quad (1)$$

where constant 0.9 is the shape factor, λ is the x-ray wavelength of CuK_α radiation (0.15406 nm), θ is the Bragg diffraction angle, and β is the full-width at half-maximum (FWHM) of the (101) plane diffraction peak. The calculated average particle size was found to be around 16 nm.

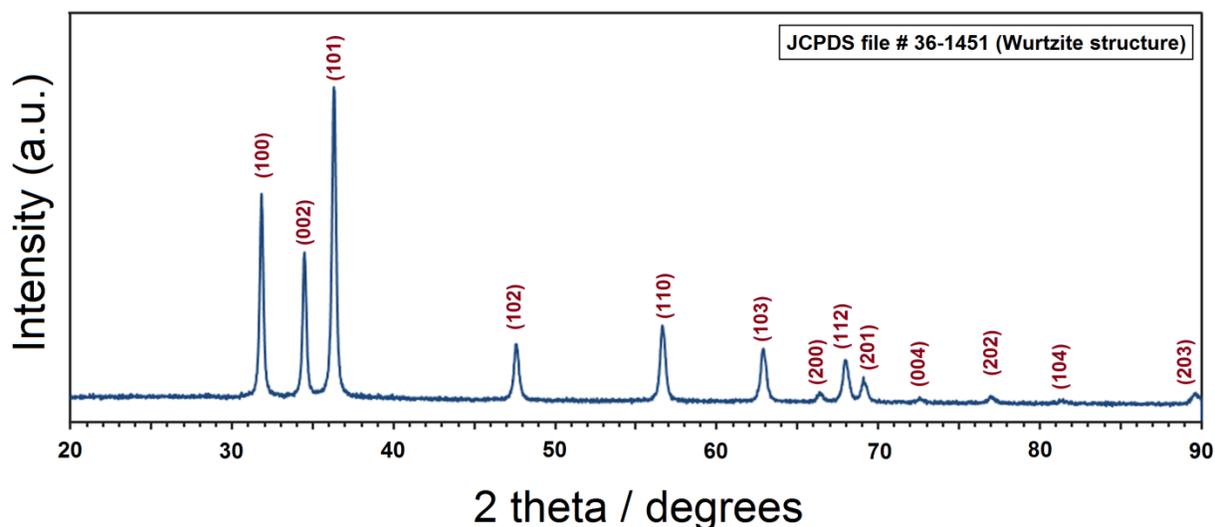


Fig. 3. The PXRD pattern of prepared ZnO–NPs (S3).

The PXRD patterns of S1 to S4 which heated at different temperatures are shown in Fig. 4, respectively. The as-prepared ZnO–NPs were calcined at different temperatures for 2 h, PXRD peaks became sharper with increasing calcination temperatures and FWHM decreased, indicating that the crystallinity of ZnO–NPs is accelerated by the calcination process. Moreover, no other

peaks related to an impurity regarding the prepared ZnO-NPs at different calcination temperatures, indicating that the final nano powders were high pure.

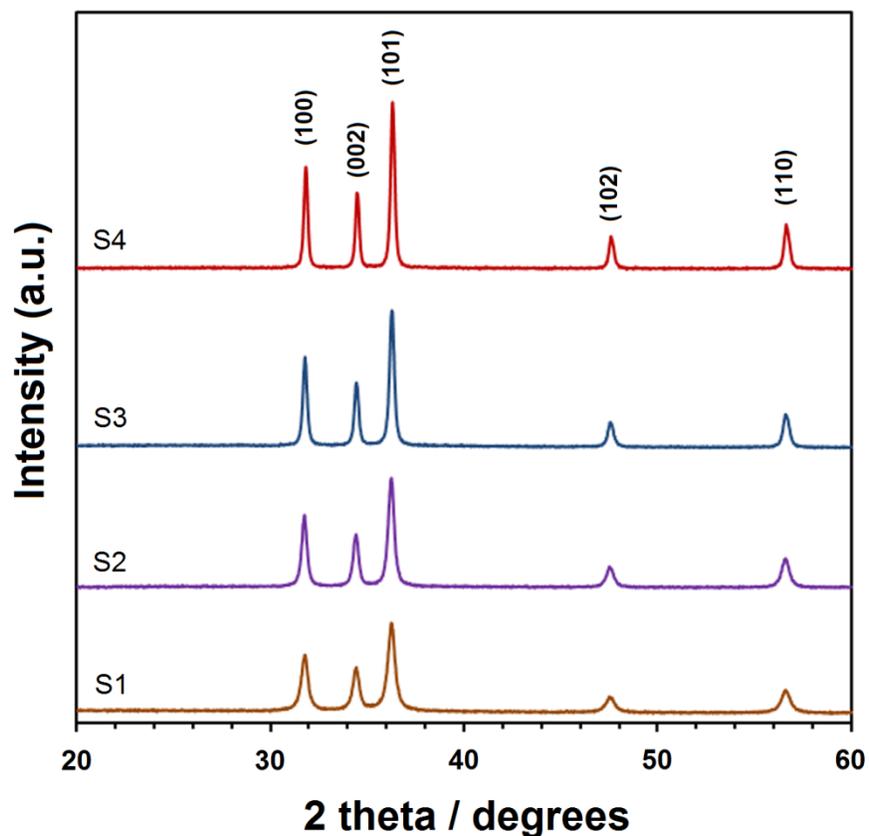


Fig. 4. PXRD patterns of prepared ZnO-NPs at different calcination temperatures.

As it is confirmed by the TEM image of ZnO-NPs (S3) (Fig. 5), the particle size of the sample is in nanoscale which was implied by the broadening of the peaks, and through achieving such fine and small sizes, a satisfying result was gained. As shown in Fig. 5, the nanopowders possess a spherical and uniform shape in size about 25 nm.

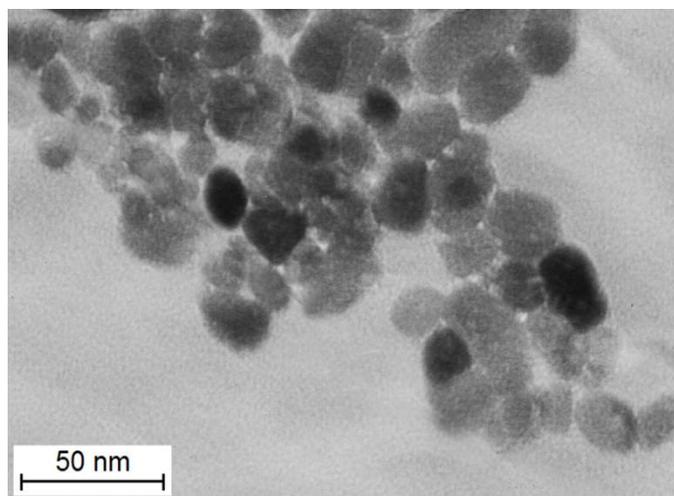


Fig. 5. The TEM image of prepared ZnO-NPs (S3).

The FTIR spectra of the ZnO-NPs (S1 to S4) which heated at different temperatures are illustrated in Fig. 6. Pellet made using KBr powder with prepared ZnO-NPs was employed for the FTIR absorption studies. For all as-prepared samples at different temperatures a strong absorption peak in the range of 400-700 cm^{-1} could be attributed to ZnO stretching modes [12, 26]. This stretching mode peak is indicated of prepared of ZnO-NPs as already confirmed by PXRD patterns. For the FTIR spectra of the calcined samples a series of absorption peaks from 1000 to 4000 cm^{-1} can be found which correspond to the carboxylate and hydroxyl impurities in the samples. A broad absorption peak in the range of 2900-3700 cm^{-1} can be attributed as overlapping of physically absorbed molecules of water and stretching modes of O-H and C-H groups. The peaks observed at about 1600, 1550, 1400, and 880 cm^{-1} are due to the asymmetrical and symmetrical stretching of the zinc carboxylate [27]. As displayed in the FTIR spectra, the spectral signatures of carboxylate impurities disappear in higher calcination temperatures (600 and 700°C).

The results of *in vitro* cytotoxicity effects of ZnO-NPs after 24 h of incubation with different concentrations of nanoparticles, in a range of 0-100 $\mu\text{g/mL}$, are shown in Fig. 7. As the results

illustrated, for concentration above 6.25 $\mu\text{g/mL}$ the metabolic activity was decreased in a concentration dependent manner meaning that metabolic activity started to decrease from 6.25 $\mu\text{g/mL}$ and at 100 $\mu\text{g/mL}$ maximal decreases was observed.

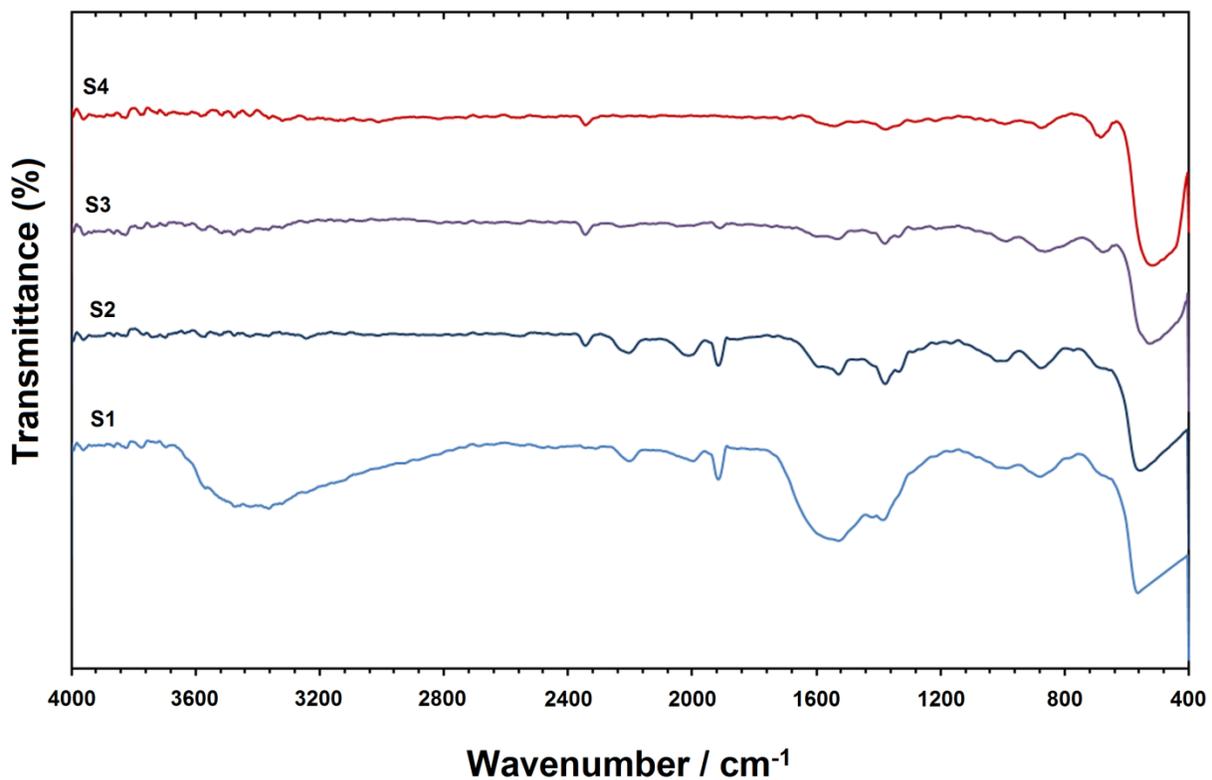


Fig. 6. FTIR spectra of the ZnO-NPs prepared at different calcination temperatures; 400 (S1), 500 (S2), 600 (S3), and 700°C (S4).

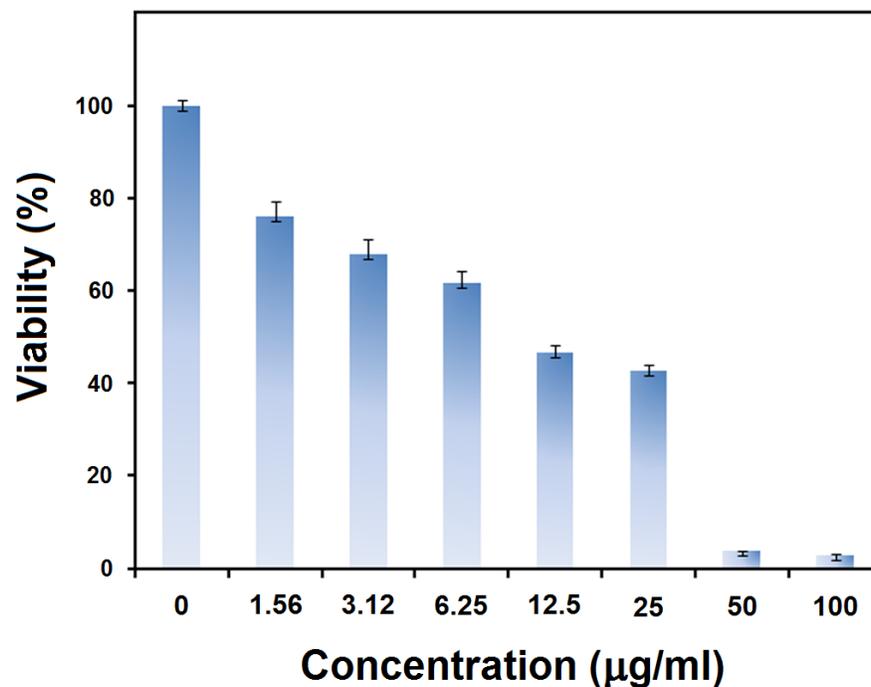


Fig. 7. Cell viability of neuro2A cells towards ZnO-NPs (S3) measured by the MTT assay.

4. Conclusion

In summary, ZnO-NPs have been successfully prepared via a simple sol-gel method in aqueous medium using PEI molecules as organic template. Such branched polymer PEI exhibited double roles as template and stabilizer in the preparation of ZnO-NPs with average size of about 25 nm. This facile way for the preparation of ZnO-NPs may make them have a wide range of applications in various fields such as cosmetics and optical/electrical devices as well as medical applications.

References

- [1] J. Zhong, A.H. Kitai, P. Mascher, W. Puff, The influence of processing conditions on point defects and luminescence centers in ZnO, *Journal of The Electrochemical Society* 140 (1993) 3644-3649.
- [2] Z.L. Wang, ZnO nanowire and nanobelt platform for nanotechnology, *Materials Science and Engineering: R: Reports* 64 (2009) 33-71.
- [3] Z. Deng, M. Chen, G. Gu, L. Wu, A facile method to fabricate ZnO hollow spheres and their photocatalytic property, *The Journal of Physical Chemistry B* 112 (2008) 16-22.
- [4] W.F. Elseviers, H. Verelst, Transition metal oxides for hot gas desulphurisation, *Fuel* 78 (1999) 601-612.
- [5] M.H. Huang, S. Mao, H. Feick, H. Yan, Y. Wu, H. Kind, E. Weber, R. Russo, P. Yang, Room-temperature ultraviolet nanowire nanolasers, *Science* 292 (2001) 1897-1899.
- [6] S. Fujihara, H. Naito, T. Kimura, Visible photoluminescence of ZnO nanoparticles dispersed in highly transparent MgF₂ thin-films via sol-gel process, *Thin Solid Films* 389 (2001) 227-232.
- [7] F.A. Sigoli, M.R. Davolos, M. Jafelicci Jr, Morphological evolution of zinc oxide originating from zinc hydroxide carbonate, *Journal of Alloys and Compounds* 262-263 (1997) 292-295.
- [8] H. Akiyama, O. Yamasaki, H. Kanzaki, J. Tada, J. Arata, Effects of zinc oxide on the attachment of *Staphylococcus aureus* strains, *Journal of Dermatological Science* 17 (1998) 67-74.
- [9] A.K. Zak, W. Majid, M. Darroudi, R. Yousefi, Synthesis and characterization of ZnO nanoparticles prepared in gelatin media, *Materials Letters* 65 (2011) 70-73.
- [10] P.V. Adhyapak, S.P. Meshram, D.P. Amalnerkar, I.S. Mulla, Structurally enhanced photocatalytic activity of flower-like ZnO synthesized by PEG-assited hydrothermal route, *Ceramics International* 40 (2014) 1951-1959.
- [11] A.K. Zak, R. Razali, W.A. Majid, M. Darroudi, Synthesis and characterization of a narrow size distribution of zinc oxide nanoparticles, *International Journal of Nanomedicine* 6 (2011) 1399.
- [12] M. Darroudi, Z. Sabouri, R. Kazemi Oskuee, A. Khorsand Zak, H. Kargar, M.H.N. Abd Hamid, Green chemistry approach for the synthesis of ZnO nanopowders and their cytotoxic effects, *Ceramics International* 40 (2014) 4827-4831.
- [13] A. Khorsand Zak, W.H. Abd. Majid, M.R. Mahmoudian, M. Darroudi, R. Yousefi, Starch-stabilized synthesis of ZnO nanopowders at low temperature and optical properties study, *Advanced Powder Technology* 24 (2013) 618-624.
- [14] R. Zamiri, A. Zakaria, H.A. Ahangar, M. Darroudi, A.K. Zak, G.P.C. Drummen, Aqueous starch as a stabilizer in zinc oxide nanoparticle synthesis via laser ablation, *Journal of Alloys and Compounds* 516 (2012) 41-48.

- [15] A. Oudhia, A. Choudhary, S. Sharma, S. Aggrawal, S.J. Dhoble, Study of defect generated visible photoluminescence in zinc oxide nano-particles prepared using PVA templates, *Journal of Luminescence* 154 (2014) 211-217.
- [16] R. Razali, A.K. Zak, W.H.A. Majid, M. Darroudi, Solvothermal synthesis of microsphere ZnO nanostructures in DEA media, *Ceramics International* 37 (2011) 3657-3663.
- [17] A. Aimable, M.T. Buscaglia, V. Buscaglia, P. Bowen, Polymer-assisted precipitation of ZnO nanoparticles with narrow particle size distribution, *Journal of the European Ceramic Society* 30 (2010) 591-598.
- [18] M. Darroudi, Z. Sabouri, R. Kazemi Oskuee, A. Khorsand Zak, H. Kargar, M.H.N.A. Hamid, Sol-gel synthesis, characterization, and neurotoxicity effect of zinc oxide nanoparticles using gum tragacanth, *Ceramics International* 39 (2013) 9195-9199.
- [19] S. Wen, F. Zheng, M. Shen, X. Shi, Synthesis of polyethyleneimine-stabilized gold nanoparticles for colorimetric sensing of heparin, *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 419 (2013) 80-86.
- [20] H.J. Lee, S.G. Lee, E.J. Oh, H.Y. Chung, S.I. Han, E.J. Kim, S.Y. Seo, H.D. Ghim, J.H. Yeum, J.H. Choi, Antimicrobial polyethyleneimine-silver nanoparticles in a stable colloidal dispersion, *Colloids and Surfaces B: Biointerfaces* 88 (2011) 505-511.
- [21] M.A. Do, G.J. Yoon, J.H. Yeum, M. Han, Y. Chang, J.H. Choi, Polyethyleneimine-mediated synthesis of superparamagnetic iron oxide nanoparticles with enhanced sensitivity in T2 magnetic resonance imaging, *Colloids and Surfaces B: Biointerfaces* 122 (2014) 752-759.
- [22] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *Journal of Immunological Methods* 65 (1983) 55-63.
- [23] Q. Zheng, G. Fang, F. Cheng, H. Lei, W. Wang, P. Qin, H. Zhou, Hybrid graphene-ZnO nanocomposites as electron acceptor in polymer-based bulk-heterojunction organic photovoltaics, *Journal of Physics D: Applied Physics* 45 (2012) 455103.
- [24] S. Kumar, P. Vats, S. Gautam, V.P. Gupta, K.D. Verma, K.H. Chae, M. Hashim, H.K. Choi, Electronic structure, magnetic and structural properties of Ni doped ZnO nanoparticles, *Materials Research Bulletin* 59 (2014) 377-381.
- [25] M. Darroudi, M.B. Ahmad, A.K. Zak, R. Zamiri, M. Hakimi, Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-light, *International Journal of Molecular Sciences* 12 (2011) 6346-6356.
- [26] D. Zhang, G. Zhang, Y. Liao, C. Wang, Y. Chen, H. Lin, H. Morikawa, Synthesis of ZnO nanoparticles in aqueous solution by hyperbranched polymer, *Materials Letters* 102-103 (2013) 98-101.

[27] G. Xiong, U. Pal, J.G. Serrano, K.B. Ucer, R.T. Williams, Photoluminescence and FTIR study of ZnO nanoparticles: the impurity and defect perspective, *Physica Status Solidi C* 3 (2006) 3577-3581.

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