Nanomed. J., 4(1): 55-60, Winter 2017

ORIGINAL RESEARCH PAPER

Synthesis, Characterization and renal toxicity of ZnO and polyethylene glycol Coated ZnO nanoparticles

Banafsheh Raisi Dehkourdi [1]; Soheil Fatahian [2]; Kahin Shahanipoor [1]*

[1]*Department of Biochemistry, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*
[2]*Department of Biosciences, Falavarjan Branch, Islamic Azad University, Isfahan, Iran*

ABSTRACT

Objective(s): The wide scale use of Zinc oxide nanoparticles (ZnO NPs) in the consumer market world makes human beings more prone to the exposure to ZnO nanoparticles and its adverse effects. Therefore, the aim of the present study is to assess renal toxicity potential of ZnO and Polyethylene glycol Coated ZnO Nanoparticles in rat.

***Materials and Methods*:** Co-precipitation chemical method was used in order to synthesize ZnO nanoparticles. The synthesized nanoparticles were coated with PEG (Polyethylene glycol) and the coating interactions were investigated by FTIR (Fourier Transform Infrared Spectroscopy). Structural properties of ZnO NPs were evaluated by TEM (Transmission Electron Microscope) and XRD (X Ray Diffraction). Toxicity assessment of ZnO and PEG coated ZnO nanoparticles were studied in rat by intra peritoneal injections during a one-month. Renal factors (Creatinine, Uric acid and Blood Urea Nitrogen) were measured 15 and 30 days post injection.

***Results*:** The synthesized nanoparticles were single phase and have spinel structure. Their size distribution was around 18 nm. Some kidney factors were changed due to the injection of both uncoated and coated nanoparticles (especially in groups received concentrations of more than 100 mg per kg of body weight). Renal factors changes were more considerable in groups received ZnO NPs in comparison with those received PEG coated ZnO NPs. Chemical toxicity studies showed that there was no irreversible effect in the groups received concentrations less than 200 mg/kg (mg per kg of body weight).

***Conclusion*:** The results indicated that renal factors were changed during 15 days post injection, especially in groups received high doses (200 mg/kg). The results of measurements 30 days post injection showed less change in comparison with the control and this indicates that there was no irreversible effect on kidney. Moreover, PEG coated nanoparticles were less toxic in comparison with Uncoated ZnO NPs.

Keywords: *Nontoxicity, PEG coated, Renal factors, ZnO nanoparticle*

How to cite this article
Raisi Dehkourdi B, Fatahian S, Shahanipoor K. Synthesis, Characterization and renal toxicity of ZnO and polyethylene glycol coated ZnO nanoparticles. Nanomed J. 2017; 4(1): 55-60. DOI:10.22038/nmj.2017.8054

INTRODUCTION

"Nano" prefix in the nanoparticle word comes from the ancient Greek language and means "dwarf", much smaller than most particles, indicating particles their diameter is between 1 to 100 nanometers (10^{-9} m) [1-2]. NPs (Nanoparticles) are widely used in health and fitness fields such as cosmetics, clothing, personal care, sport goods, and sunscreen products. Moreover, NPs are expected to be applied in many fields of medicine such as diagnosis, imaging, and drug delivery [3-6]. One of the most commonly used types of NPs is ZnO (zinc oxide) NPs.

ZnO Nanoparticle powders are widely used in cosmetics (sunscreens, foot care, ointments, and over-the-counter topical products), pigments and coatings (ultraviolet protection, fungicide in paints), electronic

*Corresponding Author Email: *sfatahian@yahoo.com*
Tel: (+98) *9131162632*
Note. This manuscript was submitted on August 17, 2016; approved on October 5, 2016

devices and catalysts [7-8]. The most abundantly usage of ZnO NPs is in biomedical applications due to their specific properties such as transparency, high isoelectric point, biocompatibility, and photocatalytic efficiency. They are widely employed in a variety of devices including toothpaste, fillings in medical materials, textiles, wall paints, and other building materials. They can also be utilized in environmental remediation for elimination or degradation of pollutants in water or air [9]. Furthermore, ZnO NPs have promising applications in the medicine field since they have been proposed as a possible treatment for cancer, autoimmune diseases and nerve injury [10].

Because ZnO NPs are the most commonly utilized nanomaterials in various consumer products, assessment of their toxicity is important. Many studies have shown the toxic effects of ZnO NPs in several experimental models, including cell lines, bacteria, nematodes, algae, plants, and fish. Despite the widespread use of ZnO NPs, the safety of this compound for humans is still unclear [11]. In order to achieve the most effectiveness in biological systems, nanoparticles are coated by different biocompatible materials such as albumin, dextran [12], PEG (polyethylene glycol, polyethylene oxide [13-14], aspartic acid and DMSA (Dimercapto succinic Acid) [16-18]. Presence of such coatings help the stability of nanoparticles in biological solutions, blood circulation and tissue distribution as well as entrance to cells and also decrease nanoparticles toxic effects. In our study PEG is selected as shell of ZnO NPs and investigated their toxicity. Briefly the objectives of this study are to prepare PEG-modified ZnO core/shell Nano composites and assess their toxicity (*in vitro*) and also explore their potential application in the biomedical fields. Renal factors were selected while less attention has been paid to renal toxicity in the previous studies.

MATERIALS AND METHODS

Synthesis of ZnO nanoparticles coated with PEG

PEG-coated ZnO nanoparticles (ZnO@PEG) were synthesized by co-precipitation method. For this purpose, tow solutions of $ZnCl_2$ (0.01 M =1.36 g) and NaOH (0.02 M =0.8 g) (all from Merck Company) were prepared in the distilled deionized water (all of the concentration were selected according to the formula 1) under vigorous stirring. At first, $ZnCl_2$ solution was poured into a beaker container. After reaching to the boiling point, the NaOH were added to the $ZnCl_2$ solution. After several minutes the ZnO NPs were produced in the solution and should be washed with deionized water. In order to coat the ZnO NPs, 0.01 M PEG (MW=6000) solution was prepared in deoxygenated deionized water and added dropwise to the nanoparticle solution while stirring. After that the mixture solution was continuously stirred for 24 h at room temperature. Then they were again washed with deionized water several times. XRD (X Ray Diffraction, Philips pw3040 λ=0.154 nm Cu Kα radiation, Netherlands), TEM (Transmission Electron Microscope, Philips 208 S 100 kV, Netherlands) and FTIR (Fourier Transform Infrared Spectroscopy 6300, JASCO, Japan) techniques were used in order to characterization of synthesized NPs. It is noteworthy that all steps did under Nitrogen atmosphere for deoxygenation of reaction medium [16].

$$ZnCl_2 + 2NaOH\text{--------} \ ZnO + 2NaCl + H_2O \quad (1)$$

Experimental animals and study of renal toxicity of ZnO and PEG coated ZnO nanoparticles

For this purpose, 84 male rats of *wistar* strain were obtained (3 months old, 250-300 g) from the Razi Vaccine and Serum Research Institute. They were kept in natural light and humidity at 22-24%C. They were divided into 7 equal groups (each group contained 12 rats). One group was injected with normal saline and served as the control group and the 6 remaining groups received ZnO and ZnO@PEG. Different concentrations of 50, 100 and 200 mg/kg (mg per kg of rat weight) intra-peritoneally injected into rats. All processes of this study were performed in accordance with regulatory guidance on the care and use of experimental animals. Rats' weights were measured and recorded at the time of injection and every week during a month.

Blood samples were taken directly from the rat heart under mild anesthesia with ketamine at time intervals of 15 and 30 days post injection. Blood samples were poured into pipe. Then, kidney factors such as Cr (Creatinine), U.Ac (Uric acid) and BUN (Blood Urea Nitrogen) were measured by an automatic analyzer (Cobas C311 analyzer series/ Roche Diagnostics USA).

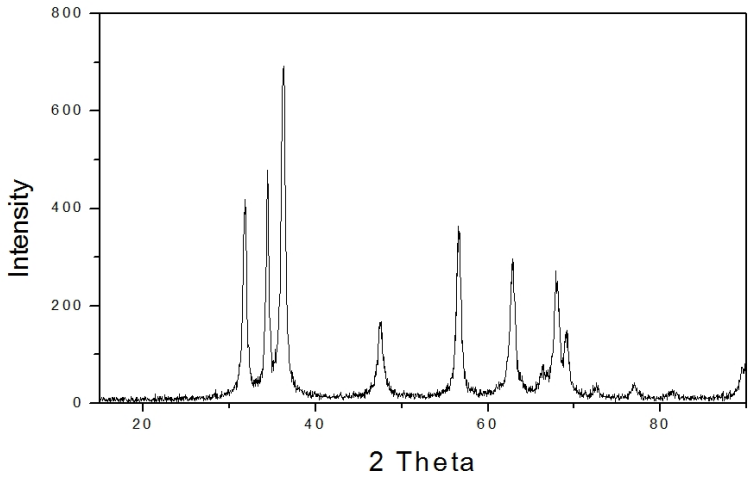

Fig. 1. XRD Pattern of ZnO NPs

Statistical analysis

For all groups, the mean values of renal factors (with treatment dose segregation) were compared by the ANOVA test (analysis of variance) and t-test using the Statistical Package for Social Sciences (SPSS) (version 21) computer program. Results are the mean values of five separate experiments for each group. Moreover, the data was demonstrated as Mean ± SD and a P-value of less than 0.05 that was considered significant.

RESULTS

Physical properties and quality assurance of ZnO and PEG coated ZnO nanoparticles

The structure of all samples was assessed by XRD. Fig. 1 indicates the XRD pattern of uncoated ZnO and PEG coated ZnO NPs. As presented by the Figure, all samples were single phase and have ferrite spinel structure. The mean size of the particles was determined by the Debye-Scherer formula [20]. It was calculated 16 nm.

TEM photograph of the uncoated ZnO NPs is shown in Fig. 2. This photograph indicates that the sizes of the particles are around 18 nm with approximately uniform size distribution.

This is compatible with the results of the XRD patterns (Fig. 1) because the XRD pattern is generally used in order to assessment of the crystal structure and it is an approximate method to determine the particle size.

FTIR curves of the ZnO, PEG and PEG coated ZnO are demonstrated in Fig. 3.

It can be observed that 3455 cm^{-1} peak in the PEG curve and ZnO@PEG curve, are related to OH group of PEG. The 2850 cm^{-1} peak in both PEG and ZnO@PEG curves are related to CH group of PEG and it is a reason of correct coating of PEG at the ZnO surface.

Renal factors measurement

BUN, Cr and U.Ac factors were measured 15 and 30 days post injection in all 7 groups and the results of 15 and 30 days were not very similar. Table 1 shows the BUN, Cr and U.Ac measurement results 15 and 30 days post injection. Figs. 4, 5 and 6 also show the comparison of BUN, Cr and U.

Ac measurement result levels between 30 and 15 days post injection. As indicated, in comparison with

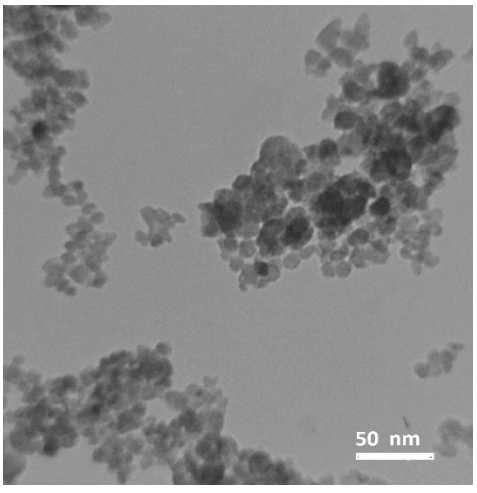

Fig. 2. TEM Photograph of the Uncoated ZnO NPs

the control, although there was significant change in ZnO NPs treated groups (P value<0.05) but in the groups received ZnO@PEG there were less significant change compared with control group (P value>0.05). As indicated by the results there were more differences between the ZnO treated and ZnO@PEG groups for all renal factors.The results of 30 days post injection for Cr, U.Ac and BUN in the groups received ZnO@PEG NPs reduced in comparison with that of 15 days post injection. It seems that renal

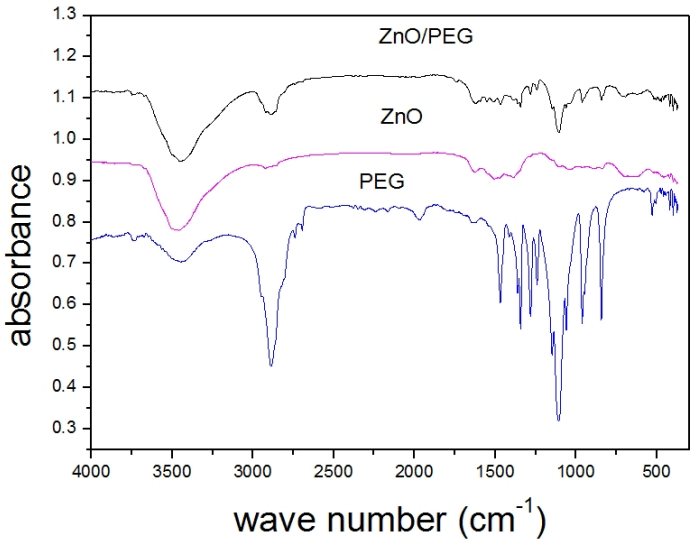

Fig. 3. FTIR Curve of ZnO, PEG and ZnO@PEG NPs

Table 1. The renal factors results from 15 and 30days

Renal Factors	Dose mg/kg	Groups	Mean±SD (mg/dl)15 dayes	Mean±SD (mg/dl)30 dayes
Cratinine (Cr)		Control	0.556 ± 0.023	0.54 ± 0.087
	50	ZnO	0.79 ± 0.031	0.62 ± 0.13
	100	ZnO	0.87 ± 0.054	0.72 ± 0.027
		ZnO	0.95 ± 0.087	0.7 ± 0.063
	200	ZnO@PEG	0.5 ± 0.021	0.6 ± 0.042
		ZnO@PEG	0.53 ± 0.032	0.63 ± 0.26
	50	ZnO@PEG	0.54 ± 0.013	0.66 ± 0.082
	100			
	200			
Uric acid (U.Ac)		Control	1.17 ± 0.098	1.07 ± 0.21
	50	ZnO	1.77 ± 0.063	0.95 ± 0.097
	100	ZnO	1.63 ± 0.074	0.91 ± 0.054
		ZnO	2.3 ± 0.058	0.8 ± 0.047
	200	ZnO@PEG	1.07 ± 0.076	1.3 ± 0.056
		ZnO@PEG	1.27 ± 0.81	1.13 ± 0.13
	50	ZnO@PEG	1.37 ± 0.43	1.33 ±0.59
	100			
	200			
Blood Urea Nitrogen (BUN)		Control	42 ± 1.23	49 ± 2.07
	50	ZnO	52 ± 1.87	50 ± 1.11
	100	ZnO	59 ± 1.69	58.33 ± 2.01
		ZnO	68 ± 2.09	59 ± 1.90
	200	ZnO@PEG	58 ± 1.52	50.33 ± 2.33
		ZnO@PEG	62.33 ± 2.04	51 ± 1.42
	50	ZnO@PEG	68.33 ± 1.076	49 ± 1.09
	100			
	200			

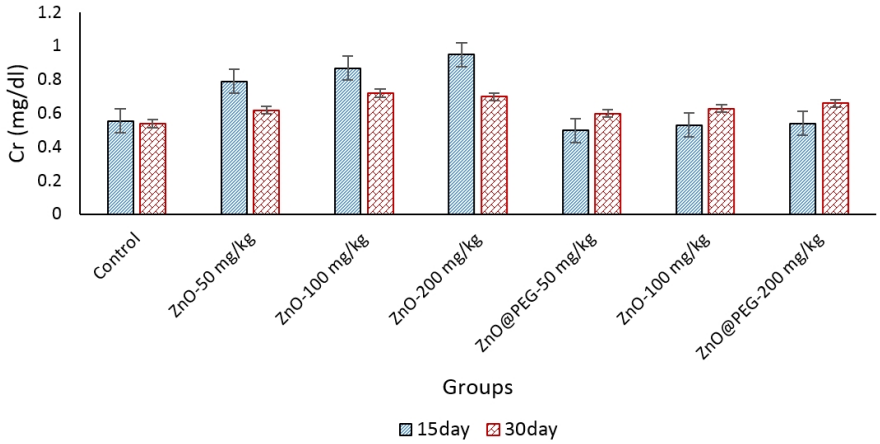

Fig. 4. Mean value of Cr measurement 15 and 30 days post injection in all rat groups received ZnO nanoparticles

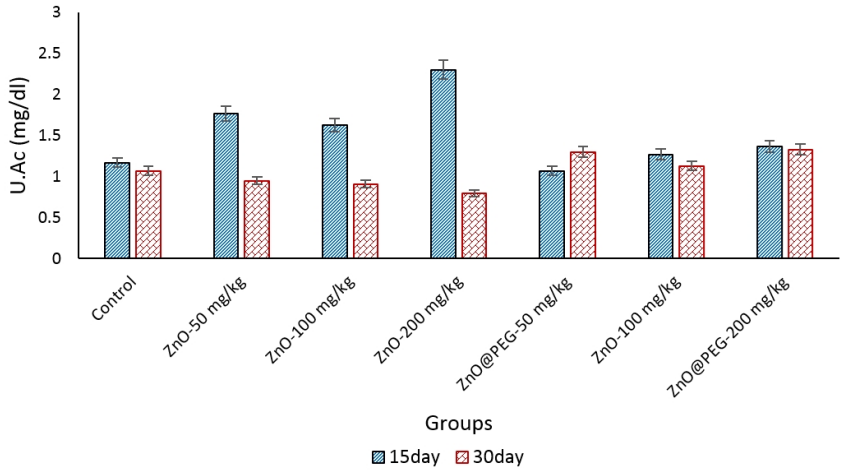

Fig. 5. Mean value of uric acid measurement 15 and 30 days post injection in all rat groups received ZnO nanoparticles

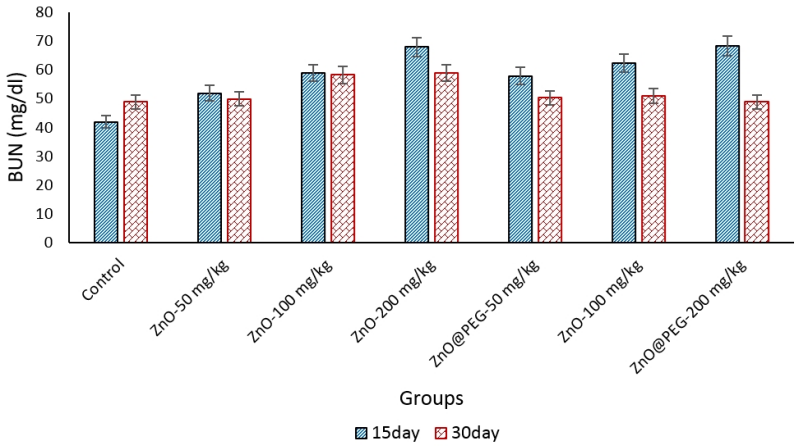

Fig. 6. Mean value of BUN measurement 15 and 30 days post injection in all rat groups received ZnO nanoparticles

damages by ZnO (200 mg/kg) NPs are more serious than other groups. Comparison of the 15 and 30-days post injection results for creatinine demonstrated that amount of Cr was reduced in ZnO NPs treated groups but increased in ZnO@PEG NPs treated groups and there was same result for U.Ac but for BUN the results were some different. It seems that kidney damages affected by ZnO NPs were more serious than ZnO@PEG NPs.

DISCUSSION

The findings suggest that uncoated ZnO nanoparticles has greater effects on Kidney function in comparison with coated iron oxide nanoparticles. By surveying the results from 30 days post injection for ZnO NPs, it can be concluded that most values were returning to the normal levels and it is expected that all measured factors would return to normal values in the near future, but for ZnO@PEG NPs the mean value of Cr and BUN were increased 30 days post injection. This probably occurred, due to their greater stability in blood circulation and consequently better penetration in different organs and cells. Therefore, it seems that the use of coating materials such as PEG on the surface of the ZnO NPs increases their stability. However, according to the results it seems that ZnO@PEG was less toxic in comparison with ZnO NPs. Therefore PEG can be a suggestion as a good compound for coating of ZnO and other nanoparticles.

CONFLICT OF INTEREST

The authors confirm that this article content has not any conflicts of interest.

ACKNOWLEDGEMENTS

The authors are grateful from Faculty of Basic Sciences, Falavarjan Islamic Azad University for their cooperation and supplying the experimental equipment.

REFERENCES

1. Hatchett DW, Josowicz M. Composites of intrinsically conducting polymers as sensing nanomaterials. J Chem Rev. 2008; 108: 746-769.
2. Kumar A, Jakhmola A. RNA-mediated fluorescent Q-Pb nanoparticles. Langmuir. 2007; 23: 2915-2918.
3. Hardman RA. Toxicological review of quantum dots:Toxicity depends on physic-chemical and environmental factors.J Environ Health Perspect. 2006; 114: 165-172.
4. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP: Semiconductor nanocrystals as fluorescent biological labels. J Science. 1998, 281: 2013-2016.
5. Chan WCW, Nie SM: Quantum dot bioconjugates for ultrasensitive nonisotopic detection. J Science. 1998, 281.
6.Wang S, Mamedova N, Kotov NA, Chen W, Studer J: Antigen/ antibody immunocomplex from CdTe nanoparticle bioconjugates.Nano Letters. 2002; 2: 817-822.
7. Jule LT, Dejene FB, Roro KT, Urgessa ZN, Botha JR. Rapid synthesis of blue emitting ZnO nanoparticles for fluorescent applications. Physica B Condens Matter. 2016; 497: 71-7.
8. Salata OV. Applications of nanoparticles in biology and medicine. J Nanobiotechnology. 2004; 2: 3-8.
9. Akan JC, Abdulrahman FI, Sodipo OA. Chiroma, YA. Distribution of Heavy Metal in the Liver, Kidney and Meat of Beef, Mutton, Caprine and Chicken From Kaasuwan Shanu Market in Maiduguri Metropolis, J B State Nigeria. Research J Applied S, E & T. 2010; 2: 743-748.
10. Kotharangannagari VK, Krishnan K. Biodegradable hybrid nanocomposites of starch/lysine and ZnO nanoparticles with shape memory properties. Materials & Design. 2016; 109: 590-595.
11. Kuang H, Yang P, Yang L, Aguilar ZP, Xu H. Size dependent effect of ZnO nanoparticles on endoplasmic reticulum stress signaling pathway in murine liver. J Hazard Mater. 2016; 317: 119-126.
12. Patel PC, Ghosh S, Srivastava PC. Structural, magnetic and optical properties of ZnO nanostructures converted from ZnS nanoparticles. Mater Res Bull. 2016; 81: 85-92.
13. Shahmohammadi Jebel F, Almasi H. Morphological, physical, antimicrobial and release properties of ZnO nanoparticles-loaded bacterial cellulose films. J Carbohydr Polym. 2016; 149: 8-19.
14. Wang S, Gao M, She Z, Zheng D, Jin C, Guo L, Zhao Y, Li Z, Wang X. Long-term effects of ZnO nanoparticles on nitrogen and phosphorus removal, microbial activity and microbial community of a sequencing batch reactor. J Bioresour Technol. 2016; 216: 428-36.
15. Amanullah M, Javed Q-u-A, Rizwan S. Surfactant-assisted carbon doping in ZnO nanowires using Poly Ethylene Glycol (PEG). Mater Chem Phys. 2016; 180: 128-34.
16. Fatahian S, Shahbazi D, Pouladian M, Yousefi MH, Amiri GhR, Shahi Z. Preparation and magnetic properties investigation of Fe_3O_4 nanoparticles ^{99m}Tc labeled and Fe_3O_4 nanoparticles DMSA coated. Digest J Nanomaterials Biostructures. 2011; 6(3):1161-65.
17. Fatahian S, Shahbazi-Gahrouei D, Pouladian M, Yousefi MH, Amiri GhR, Noori A. Biodistribution and Toxicity Assessment of Radiolabeled and DMSA Coated Ferrite nanoparticles in mice. J Radioanal Nucl Chem. 2012; 293: 915-921.
18. Hajshafiei P, Fatahian S, Shahanipoor K. In Vivo toxicity assessment of bovine serum albumin and dimercaptosuccinic acid coated Fe_3O_4 nanoparticles. Iranian J Biotech. 2014; 12(2): 63-68.
19. Hariharan R, Senthilkumar S, Suganthi A, Rajarajan M. Synthesis and characterization of doxorubicin modified ZnO/PEG nanomaterials and its photodynamic action. J P & PB: Biology. 2012; 116: 56-65.
20. Amiri GhR, Yousefi MH, Aboulhassani MR, Keshavarz MH, Manouchehri S, Fatahian S. Magnetic properties and microwave absorption in Ni–Zn and Mn–Zn ferrite nanoparticles synthesized by low-temperature solid-state reaction. J Magn Magn Mater. 2011; 323: 730-734.