Comparative Characterization of Different Samples Containing Nano-ZnO Particles with Applicability in Topical Therapies

RAUL CHIOIBAS[1,#], FLORIN BORCAN[2,#], OVIDIU MEDERLE[3]*, DANA STOIAN[3]*, CODRUTA MARINELA SOICA[2]

[1] MEDCOM Clinic CBS Hospital, 12 Popa Sapca Str., 300057, Timisoara, Romania

[2] Victor Babes University of Medicine and Pharmacy Timisoara, Faculty of Pharmacy, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2] Victor Babes University of Medicine and Pharmacy Timisoara, Faculty of Medicine, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

Zinc oxide (ZnO) is an inorganic compound used for its antiseptic and skin healing properties. It is an excellent protective filter against UV radiation and it can be used as white pigment in pharmaceutical preparations. In this study, nano-ZnO particles were obtained by ultrasound treatment, and respectively by repeated freezing/heating process. The influence of synthesis method and of ultrasound generator parameters on the particles size and stability was observed. The results reveal that were obtained samples with a very good stability and sizes between 15 and 96 nm. It was found that synthesis based on ultrasound treatment lead to the formation of nanoparticles with lower sizes.

Keywords: DSC, inorganic particles, nanosize, ultrasound, Zeta potential

The *dwarf-technology*, known as nanotechnology (*nano* means *dwarf* in Greek), is the science working on an extremely small scale (a nanometer is 10^{-9} m). This very young science is based on the idea that it is possible to create *machines* by manipulating molecules (R. Feynman, 1959). But the applications of nanotechnology announce revolutionary changes in many areas, including medical, only after 30 years. Although a delayed onset, the applications of nanotechnologies in medicine are so numerous that this part of nanotechnology becomes an individual science called nanomedicine [1]. A.B. Chirila and R.T. Cristina described some of the nanomateriales used in medicine: nanoemulsions, nanofibres, nanoparticles for MRI, carbon nanotubes, nanopores, biochips, nano-sensors, drug delivery systems, polymer micelles etc. [2].

Zinc oxide is a white powder, with a very low solubility in water (solubility product constant, Ksp = $3.86 \cdot 10^{-10}$). Many scientists have reported significant increases in solubility of metal oxides with decreasing sizes of particles [3-6]. Nano-metal oxides are probably the most widely used particles having applications in cosmetics, medicine, and textile [7].

The synthesis of nanosized metal oxides includes different methods based on the liquid-solid and gas-solid nature of the transformations [8]. The most common methods, the liquid-solid transformations, cover the following specific procedures: co-precipitations, sol-gel processes, microemulsion techniques, solvothermal and template/surface derivatized methods [9]. On the other hand, many induced effects (such as generation, growth and collapse of microstructures) appear when ultrasound waves are applied inside the synthesis reactors [10].

The aim of this research was to compare the sizes and the stability of nano-ZnO particles obtained through different synthesis procedures and using different parameters of an ultrasound generator.

Experimental part

All chemicals were of the analytical reagent grade; anhydrous $Zn(NO_3)_2$ hexahydrate from Sigma-Aldrich, ethanol, HCl 1M and NaOH 1 M from Chimopar (Romania) and distilled water were used in all preparations.

The raw materials were used without any previous purification. The obtained samples were kept in sealed tubes inside of a dessicator in order to avoid the humidity retention.

Synthesis of nano-ZnO particles

Two different procedures were used to synthesize nano-ZnO particles: (1) an intense ultrasound treatment and (2) a repeated freezing/heating process as a cryochemical method applied to convert ZnO micrometer to nanosized powder.

Ultrasound treatment: 0.5M ethanol solution of $Zn(NO_3)_2 \cdot 6H_2O$ was stirred using a magnetic stirrer to completely dissolve the salt (400 rpm, 1 hour). After complete dissolution, 1M NaOH aqueous solution was added under stirring (650 rpm), drop by drop (slowly for 30 min) touching the walls of the beaker. Different samples were obtained based on different parameters (Table 1) of an ultrasound generator model UP200S from Hielscher (Germany) with the S3 (micro tip 3) standard sonotrode.

The chemical reaction was allowed to proceed for approx. three hours after the complete addition of NaOH. The beaker was sealed and the solution was maintained at room temperature for one night and then, the supernatant was separated carefully. The precipitated nano-ZnO particles were washed for three times with a distilled water and ethanol mixture to remove the byproducts and then heated in air atmosphere above 250°C for 1 hour in order to convert $Zn(OH)_2$ in to ZnO.

Repeated freezing/heating process: 0.5M ethanol solution of $Zn(NO_3)_2 \cdot 6H_2O$ was stirred using a magnetic stirrer to completely dissolve the salt (400 rpm, 1 h). After complete dissolution, the zinc nitrate solution was cooled at 0-3°C and 1M NaOH aqueous solution was added under stirring (650 rpm), drop by drop on the walls of the beaker. The reaction was allowed to proceed for 2 hours after complete addition of NaOH.

*email: ovidiu.mederle@gmail.com; stoian.dana@umft.ro

[#] Authors with equal contribution

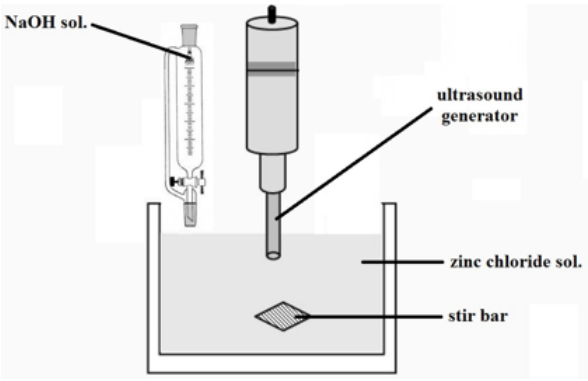

Fig. 1. Scheme of instalation for the synthesis based on ultrasound treatment

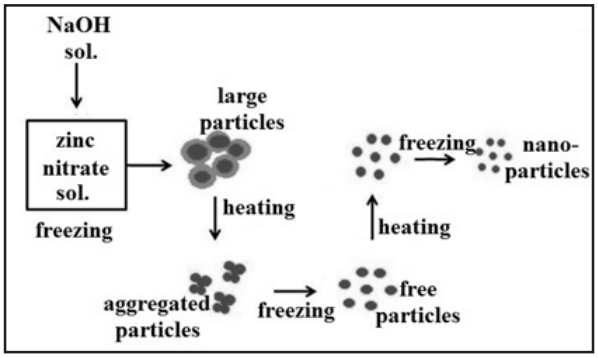

Fig. 2. Modifications of sample during the experiment

Sample code	Parameters of ultrasound generator	
	Pulse-pulse mode factor	Amplitude, %
US_1	0.5 (Power discharge 0.5 sec., pause 0.5 sec.)	20
US_2	0.5 (Power discharge 0.5 sec., pause 0.5 sec.)	60
US_3	0.5 (Power discharge 0.5 sec., pause 0.5 sec.)	100
US_4	1.0 (Power discharge continuously)	20
US_5	1.0 (Power discharge continuously)	60
US_6	1.0 (Power discharge continuously)	100

Table 1
THE CHARACTERISTICS OF ULTRASONATED SAMPLES

The beaker was then heated up to 300°C for 30 minutes and it was cooled again. These freezing/heating processes were repeated 2-3 times and samples were collected in every step in order to evaluate the modifications of particles' sizes; every sample was washed for three times with a distilled water and ethanol mixture prior any investigation in order to remove the byproducts.

Samples were labelled as follow: FH_1 (sample obtained before first heating); FH_2 (sample obtained after the first heating); FH_3 (sample obtained before the second heating); FH_4 (sample obtained after the second heating); FH_5 (sample obtained after final freezing).

Analysis

The size and the charge of the ZnO particles were measured using weak acidic solutions (1:500 w/v) of every sample and a Cordouan system including a Vasco Size Analyzer and a Wallis Zeta Potential Analyzer (Cordouan Technol, France). There were set the following Vasco Particle Size Analyzer parameters: temperature (25°C), time interval (30μs), and number of channels (~400), laser power (35%), acquisition mode (continuous), and analysis mode (Pade-Laplace). The following Wallis Zetapotential Analyzer parameters were chosen: plastic cuvette, temperature (25°C), laser power (30%), applied field (automatic), resolution (medium, 0.8 Hz), 3 measures/sequence, and Henry function (Smoluchowski) and the measurements were carried out three times for each sample.

The thermal analysis of samples was performed using a Mettler-Toledo DSC1 (Switzerland) and aluminium crucibles (samples' weights between 3.17-3.49 mg) in an inert atmosphere (100 mL/min Ar) between 75 and 350°C with a 5 degree/min heating speed.

The size of nanoparticles influences the entire properties of materials. UV-Vis spectroscopy is used as a technique to examine the optical properties of nanosized particles. Samples' UV-Vis Absorption Spectra were recorded using a SI Analytics UViLine 9400 spectrophotometer, plastic cuvettes and weak acidic solutions (1:500 w/v) of every sample.

Results and discussions

The obtaining of nanostructured materials is important for many research fields because it was found that these materials are capable to interact faster with other substances from medium. O. Cadar *et al.* [11] reported that nano-scale materials have different chemical, electrical, optical and mechanical properties than their corresponding bulk solid. Nano-ZnO presents an improved antimicrobial and antibacterial activity and it can be used as a coating material for different applications.

The nano-ZnO powders were dissolved in a weak acidic solution to evaluate the particles' size and stability (Table 2 and 3).

The isothermal oxidation behavior of nano-ZnO particles have been investigated using the DSC technique over a temperature range of 75-350°C in an inert atmosphere. Figure 3 shows the DSC curves of zinc oxide particles obtained by ultrasonated synthesis. The large endothermic peaks between 100 and 160°C is due to the loss of solvents' molecules adsorbed on the surface of nano-ZnO particles during the synthesis.

Similar DSC curves were obtained in the case of samples synthesized by repeated freezing/heating processes (Figure 4). In this experiment it was observed that repeated heating determines the obtaining of samples without traces of solvents (the large endothermic peak between 100 and 160°C disappear in the case of sample FH_4 and FH_5).

Important endothermic peaks around 260°C can be assigned to the conversion of zinc hydroxide to zinc oxide [12].

Sample code	Particle size (nm)		Zeta Potential (mV)
	Mean ± SD	Polydispersity index	Mean ± SD
US_1	51 ± 17	0.3	74.2 ± 9.2
US_2	42 ± 8	0.2	76.1 ± 6.4
US_3	44 ± 11	0.2	74.8 ± 8.1
US_4	24 ± 4	0.2	88.1 ± 7.6
US_5	23 ± 7	0.3	81.5± 9.2
US_6	15 ± 3	0.1	86.3 ± 7.9

Table 2
THE ZETASIZER CHARACTERIZATION OF ULTRASONATED SAMPLES

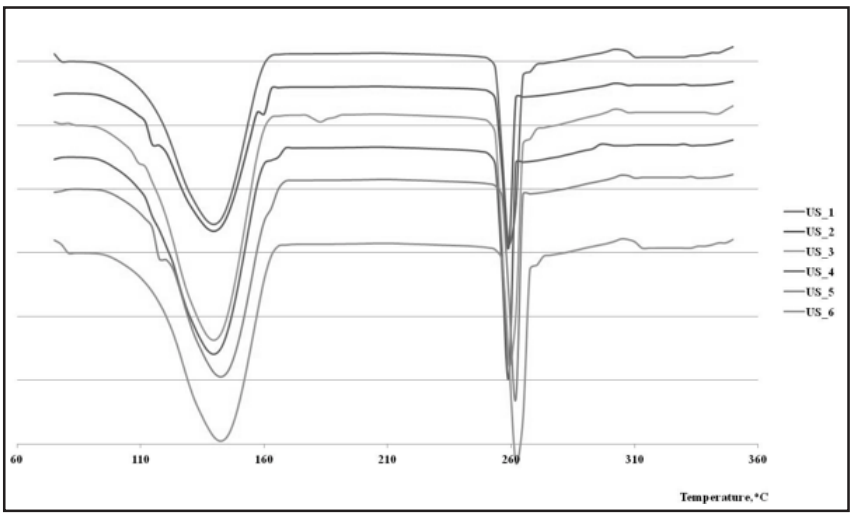

Fig. 3. Thermograms of ultrasonated samples

Sample code	Particle size (nm)		Zeta Potential (mV)
	Mean ± SD	Polydispersity index	Mean ± SD
FH_1	96 ± 19	0.5	71.3 ± 6.1
FH_2	84 ± 13	0.4	73.6 ± 5.9
FH_3	81 ± 13	0.4	74.9 ± 7.3
FH_4	67 ± 9	0.3	78.9 ± 3.2
FH_5	53 ± 11	0.3	70.4 ± 4.7

Table 3
THE ZETASIZER CHARACTERIZATION OF SAMPLES OBTAINED BY REPEATED FREEZING / HEATING PROCESSES

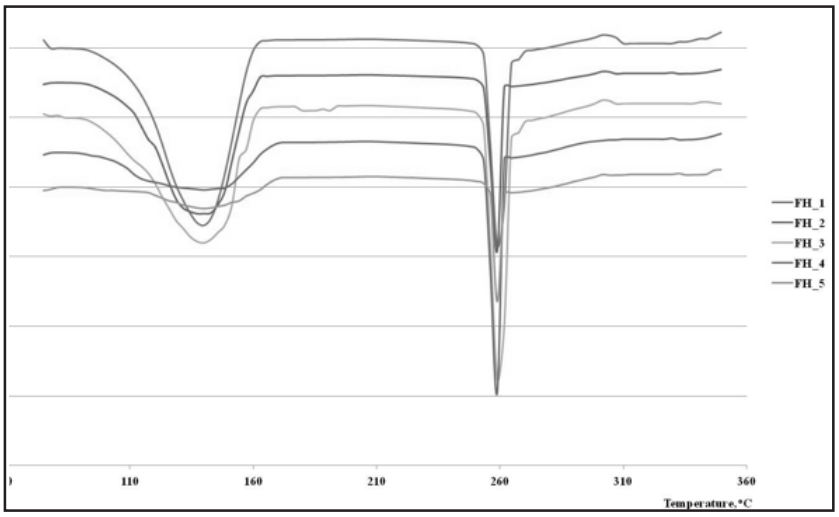

Fig. 4. Thermograms of samples obtained by repreated freezing / heating processes

On correlating the size of nano-ZnO particles with the parameters of ultrasonic generator (pulse-pulse mode factor and amplitude), a significant negative correlation was revealed with respect to the pulse-pulse mode factor (Table 4 and Figure 5).

Parameter of ultrasonic generator	Correlation with particles' sizes	Pearson correlation coefficient, r	p
Pulse-pulse mode factor	Negative	-0.954**	0.003
Amplitude	-	-0.249	0.634

** Correlation is significant at the 0.01 level (2-tailed)

Table 4
CORRELATION OF PARTICLES' SIZES WITH PARAMETERS OF ULTRASONIC GENERATOR

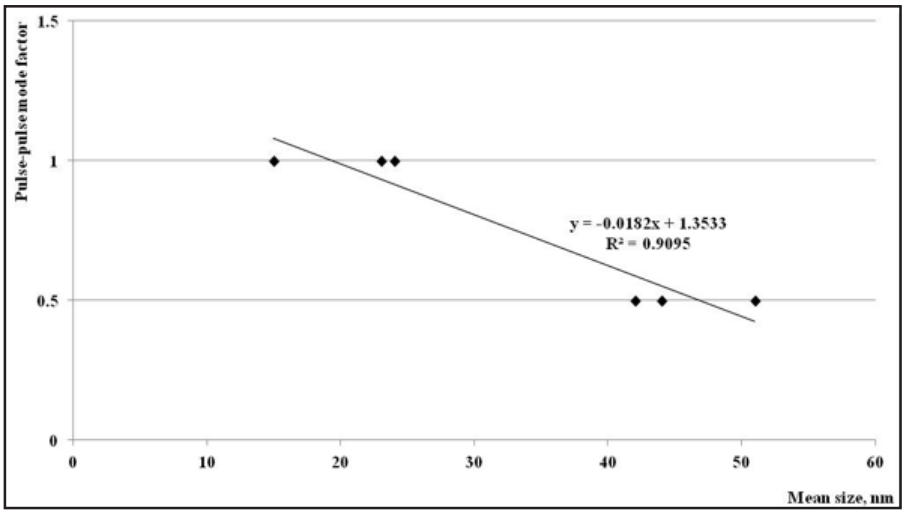

Fig. 5. Dependence between particles size and pulse-pulse mode factor

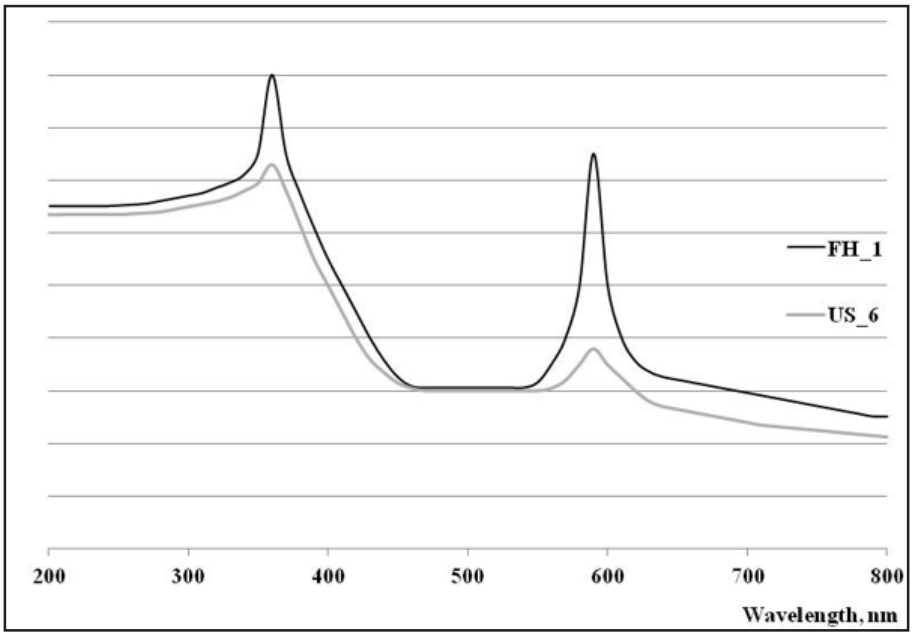

Fig. 6. Comparative UV-Vis spectra of samples US_6 VS. FH_1

There are no important differences between the UV-Vis spectra of samples. Figure 6 reveals the differences between sample US_6 synthesized by ultrasonated synthesis, chosen for its decreased size (15 nm), and sample FH_1 without ultrasound treatment, chosen for its increased size (96 nm). Two main peaks at 360 and 590 nm were observed in both cases.

According to M. Wang *et al.* [13] a better absorption is observed in the case of sample with an increased amount of $Zn(OH)_2$; thus, it can be appreciated that samples obtained through a repeated freezing/heating process contain a mixture of zinc oxide and hydroxide. No important modifications of UV-Vis spectra were observed inside every group of samples (ultrasonated samples and samples based on a repeated freezing/heating process).

D. Bombos *et al.* [14] presents the catalytic activity of zinc oxide in the esterification of glycerol with technical olein. This procedure is very important due to the glycerol excess resulted in biodiesel fabrication. On the other hand, it is important to mention the efficacy of ZnO and TiO_2 nanoparticles as coatings for medical devices. M. Glod *et al.* [15] studied the genotoxicity of these particles on neoplastic HeLa and normal Vero cells and they found minor effects of the tested compounds on the cells viability, that suggesting a reduced cytotoxicity on both cell lines, but a better tolerability was observed in the case of the normal cells.

Conclusions

Literature describes various methods used for the preparation of nano-ZnO powders, which are not widely used on a large scale, but which are based on simple and less expensive procedures, such as: chemical vapor deposition, spray pyrolysis, microemulsion, thermal decomposition of organic precursor, ultrasonic, microwave-assisted techniques, hydrothermal and precipitation methods etc. In this study, nano-ZnO particles were synthesized using zinc nitrate and sodium hydroxide as precursors with and without an ultrasonic treatment. The results reveal the obtaining of nano-ZnO particles with

decreased sizes and an increased content in zinc oxide in the case of ultrasonated samples. All samples present two main peaks around 360 and 590 nm in UV-Vis spectra and it was observed a negative correlation between the pulse-pulse mode factor of ultrasonic generator and the sizes of nano-ZnO particles.

References

1. MARTIN, M.S. Nanotehnologia ºi medicina. Hyperdia **27**, 2014, p. 1277.
2. CHIRILA, A.B., CRISTINA, R.T. Veterinary Drug, **7**, 2013, p. 19.
3. LIU, J., ARUGUETE, D.M., MURAYAMA, M., HOCHELLA, M.F. Environ. Sci. Technol., **43**, 2009, p. 8178.
4. BIAN, S.W., MUDUNKOTUWA, I.A., RUPASINGHE, T., GRASSIAN, V.H. Langmuir, **27**, 2011, p. 6059.
5. VAN EERDENBRUGH, B., VERMANT, J., MARTENS, J.A., FROYEN, L., HUMBEECK, J.V., VAN DEN MOOTER, G., AUGUSTIJNS, P. Mol. Pharm., **7**, 2010, p. 1858.
6. MEULENKAMP, E.A. J. Phys. Chem. B, **102**, 1998, p. 7764.
7. RUPASINGHE, R.A.T.P. Dissolution and aggregation of zinc oxide nanoparticles at circumneutral pH; a study of size effects in the presence and absence of citric acid. Master thesis, Univ. of Iowa, 2011.
8. RODRIGUEZ, J.A., FERNANDEZ-GARCIA, M. Synthesis, Properties and Applications of Oxide Nanoparticles. Whiley: New Jersey, 2007.
9. LUKEHART, C.M., Scott, R.A. Nanomaterials: Inorganic and Bioinorganic Perspectives. Whiley: New Jersey, 2008.
10. SAEZ, V., MASON, T.J. Molecules, **14**, 2009, p. 4284.
11. CADAR, O., ROMAN, C., GAGEA, L., CADAR, S., MICLEAN, M. Rom. J. Mater., **40**, 2010, p. 250
12. NAIN, R., SINGH, D., JASSAL, M., AGRAWAL, A.K. Nanoscale, **8**, 2016, p. 4360.
13. WANG, M., JIANG, L., KIM, E.J., HAHN, S.H. RSC Adv., **5**, 2015, p. 87496.
14. BOMBOS, D., BOMBOS, M., BOLOCAN, I., VASILIEVICI G., ZAHARIA, E. Rev. Chim. (Bucharest), **61**, 2010, p. 784.
15. GLOD, M., DAMIR, D., NICHITUS, S., CALIN, G., DUCEAC, L.D., GORGAN, D.L., TASCU, S., CIUHODARU, M.I. Rev. Chim. (Bucharest), **69**, 2018, p. 609.

Manuscript received: 6.07.2019