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Ultrasonic synthesis of Zn(II) methionine and ZnO nanostructures as a new precursor for ZnO nanoparticles and *in-vitro* study

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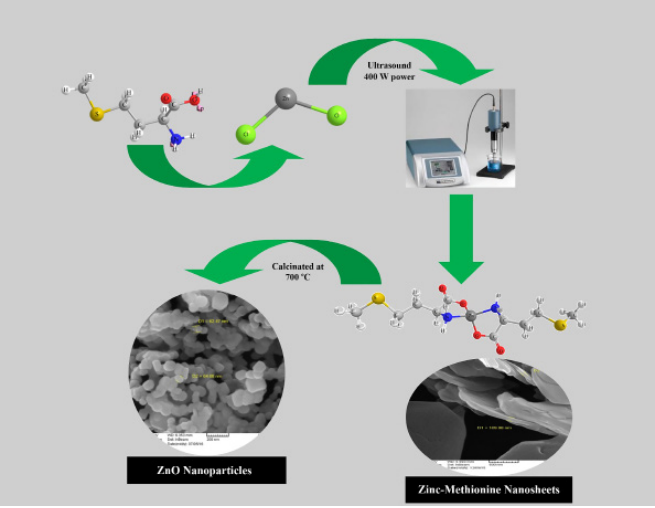
HIGHLIGHTS

- A new synthetic method for the nanostructures of Zn-methionine complex by ultrasonic irradiations in two different solvents has reported.
- ZnO nanoparticles were synthesized in 700 ºC for 2 hours under ambient atmospheric condition by using the nano Zn-methionine complex as precursor.
- Zn-methionine nano-complex were used as zinc source to measure the absorption of it in everted sac method.

GRAPHICAL ABSTRACT



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ABSTRACT

Zinc(II) ions play a special role in biological systems. Methionine is a sulphur containing amino acid with IUPAC name 2-amino-4-(methylthio)butanoic acid. In this study, ultrasonic synthesis and characterization of nanostructured Zn(II) methionine (Zn-Meth) in two different solvents were investigated. The reaction of $ZnCl_2$ and methionine ligand under ultrasonic irradiation in both methanol and DMSO leads to the formation of nano-sized Zn(II) methionine complexes. Characterization of the Zn(II) complex was performed using elemental analysis, FT-IR spectroscopy, X-ray powder diffraction (XRD), thermal gravimetry (TGA), field emission scanning electron microscopy (FE-SEM), and energy-dispersive X-ray spectroscopy (EDS). The nano Zn-Meth complex, $[Zn(CH_3SCH_2CH_2CHNH_2COOH)_2]_n$, was then used as a precursor to obtaining the nano ZnO particle. An *in-vitro* study of the everted gut sac was also done on this complex to measure the uptake amount of zinc. The results showed that the nano-sized Zn-Meth has a higher absorption compared to its commercial and inorganic forms.

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1. Introduction

Recognizing the benefit of metal elements such as iron, zinc, copper, etc. in human, animal, and plants health, opened a new horizon to study the bioavailability of their different forms. Working on mineral-amino acids complexes has been an interesting field for researchers because of their applicability in pharmaceutical, medicinal, agronomical and nutritional sciences [1]. Methionine is one of the sulfur-containing amino acids that are nutritionally essential for human, animals, and poultry; but one they cannot produce [2]. In nutrition, it acts as an antioxidant agent in biological systems [3].

Following iron, zinc is the most prevalent trace mineral in animals. Cell growth and division as well as protein metabolism require sufficient availability of zinc [4]. Previous research has shown the bioavailability of metal complex is higher than metal salts [5]. A study on zinc broiler chicks showed that the bioavailability of zinc complex is higher than zinc salt [6]. The metal ions in an amino acid complex are chemically inert due to coordinated amino acid ligands and do not precipitate as a result of interfering ions, while metal salt does precipitated via these agents [7]. Various methods have been used for the synthesis of metal amino acid complexes such as adjustment of pH [8], reflux [9] and microwave assistant [10]. In recent years many scientists have focused on the application and fundamental technology of nano-structure compounds [11]. Various methods for nanoscale synthesis of metal-organic compound and metal-organic frameworks are currently used. A recent research on nanoscale synthesis of nano zinc-methionine complex [12] used a method in patent US8288587 B2 for nanometal chelate synthesis with EDTA and EDDHA [13]. Used in recent years, ultrasound is one of the most powerful and efficient methods for synthesizing nano material [14]. Ultrasound waves, unlike other methods, creates an extraordinary, unusual condition in a solution [15]. The complex of zinc and cysteine has been previously synthesis utilizing the ultrasonic method and then used as a precursor for obtaining nano zinc [16], but no biological assessment has been done on this complex. Nano scaled material is different physically, chemically, and biologically compared to its macro scale. The consequence of these differences, directly effects the usage quantity [17]. Nano mineral nutrition has a better ability to be absorbed in the small intestine and circulates in different body parts such as the blood, liver, kidney, etc. [18]. Another study on piglets showed average feed daily intake of nano zinc oxide in a smaller dosage is similar to a larger dosage of zinc oxide [19]. In-vitro study of the effect of zinc oxide nanoparticles on ruminal microbial protein synthesis showed an increased number of this microorganisms and better efficiency in energy utilization [20]. Using nano zinc oxide as nutrition improved the production of a broiler [21]. Metal oxide nanostructures have developed greatly in recent decades and are applied in various fields, such as electronic, optic, medicine, sensors, etc., due to their extraordinary behavior. Transitional metal oxides have exclusive characteristic, magnetic properties [22], are dialectic constant [23], and have a wide band gap [24]. Several methods have been developed for assessing *in-vitro* intestinal absorption, including the everted gut sac isolated epithelial cells or brush border and the basolateral membranes separated from enterocytes methods [25]. The everted gut sac technique is an efficient and widely accepted tool for detection of the uptake rate of many nutrients [26]. This method helps scientists to better understand the absorption, transportation, and interaction mechanism [25]. In 1994 the uptake of zinc L-carnosine was measured by the everted gut sac method [27]. Nutrition compounds as nanostructures have been less studied. In this study, a Zn-methionine nanostructured compound has been synthesized by the sonochemical method for the first time, and was then used in an "*in-vitro*" analysis to determine its absorption compared to ordinary zinc salt and the commercial product, Zinpro® (Zinpro Animal Nutrition Company).

2. Experimental

All reagents and solvents for the synthesis and analysis were commercially available and were used as received. A multi-wave ultrasonic generator (Sonicator-4000; Misonix, USA), equipped with a converter/transducer and titanium oscillator (horn), 12.5 mm in diameter, operating at 20 kHz with a maximum power output of 600 W, was used for ultrasonic irradiation. FT-IR spectra (400-4000 cm^{-1}) were recorded by using TENSOR 27 Bruker in KBr matrix, and elemental analyses were performed with a FlashEA 1112 Analyzers by Thermo Fisher Scientific. Varian AA240FS atomic absorption was used to determine the percentage of Zn(II) in the complex. X-ray powder diffraction (XRD)

measurements were performed using an INEL Equinox 3000 diffractometer with monochromator Cu-K_α (λ = 1.5418 Å) radiation at room temperature in the 2θ range of 10-90°. The crystallite sizes of selected samples were estimated using the Scherrer formula. The simulated XRD powder pattern based on single crystal data were prepared using Mercury software 1.4.2 version. Thermogravimetric analysis was carried out using a STA 504 instrument. The compound was heated in a static atmosphere of air from 50-800 ºC, with a heating rate of 10 ºC.min^{-1}.

2.1. Synthesis of nanostructured Zn(II) methionine 1:2 complex

An amount of (0.2 mmol) methionine and (0.1 mmol) $ZnCl_2$ were suspended in 50 ml methanol/DMSO. An ultrasound probe was placed in the vessel with the rated output power of 400 W. White precipitate was obtained after 15 min. of programmed radiation, the precipitate was washed with cold acetone and methanol, respectively. The resulting white powder was (Zn-Meth) 81% yield. Decomd. at: upper than 200 ºC. Anal calcd. for $Zn(C_5H_{10}NO_2S)_2$: Zn, 18.18%; C, 33.38%; H, 5.04%; N, 7.79%. Found: $Zn(C_5H_{10}NO_2S)_2$: Zn, 15.02%; C, 34.63%; H, 5.23%; N, 8.09%. FT-IR (KBr, cm^{-1}): 439 (s), 553 (s), 1340 (s), 1413 (s), 1581 (s), 2914 (b). ^{1}HNMR (250 MHz, D_2O, ppm) : Hδ 3.69 (t, 1H, H_8), 3.67 (t, 1H, H_8), 3.64 (t, 1H, H_8), 2.48 (2H, dt, $H_{6,7}$) 2.43 (dt, 2H, $H_{6,7}$), 2.42 (dt, 2H, $H_{6,7}$), 2.03 (t, 2H, $H_{4,5}$), 1.95 (t, 2H, $H_{4,5}$) 1.94 (t, 2H, $H_{4,5}$) 1.19 (s, 3H, $H_{1,2,3}$).

2.2. Calcination method

To prepare the ZnO nanoparticles, the powders from the obtained Zn-Meth complex were calcinated at 700 ºC for 2 hours under ambient atmospheric condition. The product was then washed first with double distilled water and then acetone. The product was finally characterized by scanning electron microscopy (SEM), energy dispersive X-ray (EDAX), and X-ray powder diffraction (XRD).

3. Results and Discussion

The reaction of zinc chloride and methionine ligand under separate ultrasonic irradiation in methanol and DMSO, led to the formation of a nanostructured zinc methionine complex. Fig. 1 shows the method used in the synthesis of the Zn-Meth complexes.

Elemental analysis showed that the metal to ligand ratio is 1:2 and the corresponding chemical formula, $Zn(CH_3SCH_2CH_2CHNH_2COOH)_2$, was confirmed. Table 1 shows the FT-IR bands of Zn-Meth and the comparison between FT-IR spectrum of free methionine. The Zn-Meth complex revealed weak bands in 465 and 520 cm^{-1}, which indicated zinc to oxygen and nitrogen bands. A short band in 1324 cm^{-1} shows S-CH. A sharp band in 1426 cm^{-1} belongs to symmetric stretching of COO- and another sharp band in 1625 cm^{-1} which is attributed to the C=O stretching band. The band in 2920 cm^{-1} is assigned to C-H stretching of the CH_2-SCOO- group and broad weak bands in 3441 and 3619 cm^{-1} indicated amine groups. The disappearance of a band in 2100 cm^{-1} in comparison of free methionine means that NH^{3+} no longer exists and the amine group is involved in the coordination system.

$$ZnCl_2 + 2C_5H_{11}NO_2 \xrightarrow[\text{in MeOH or DMSO}]{\text{Ultrasonic irradiation, 400 W}} Zn(C_5H_{11}NO_2)_2 + 2Cl^-$$

Fig. 1. Synthesis method of Zn-Meth nanostructures.

Suggested structure for Zn-Meth due to FT-IR spectrum is depicted in Fig. 2.

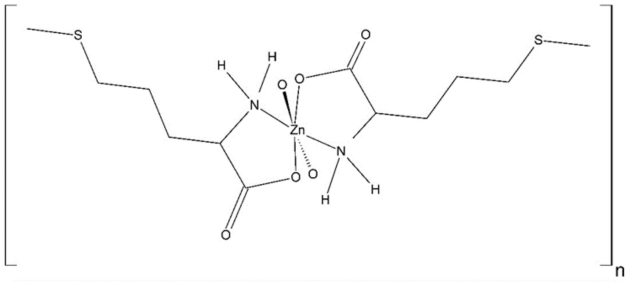


Fig. 2. The molecular structure of Zn-Meth complexes.

Table 1. Significant FT-IR bands (cm^{-1}) of the Zn-Meth complex.

Compound	ν(N–H) (str.)	ν (C–H) str. of (CH_2S)	ν (COO) (asym str.)	ν (COO) (sym str.)	ν (S–CH) (bend.)	Metal to organic bands
$Zn(C_5H_9NO_2S)_2$	3441-3619	2920	1625	1426	1324	465-520

Formation of a five-member ring is possible with FT-IR data and elemental analysis, and the same structure was reported for an iron complex. Microwave assisted synthesis of iron and methionine complexes had been done before [10]. The reaction between zinc chloride and methionine ligand in water produced a colorless crystalline compound. The geometry around each Zn(II) is a slightly distorted octahedral. Zn-Meth is a coordination polymer through the carbonyl group with another zinc atom, as depicted in Fig. 3 [28].

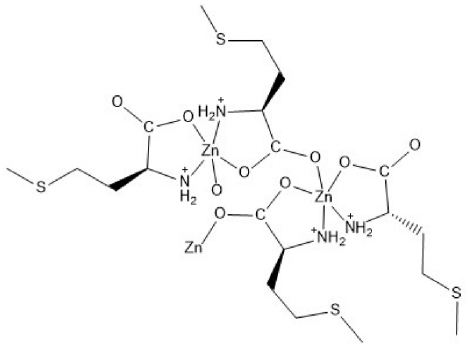


Fig. 3. Polymeric view of the Zn-Meth complex.

The XRD analysis of zinc-methionine 1:2 (zinc to methionine) has been shown in Fig. 4. Peaks in 4.307 2θ have the highest count, followed by 21.367° and 20.898° 2θ. Using Scherer's equation, crystal size has been calculated at 21.90 nm. The crystal structure of synthesized complexes has been characterize in 1977. According to the CIF of this complex depicted in the Cambridge crystallographic data center (CCDC), the simulated XRD pattern s compromised of a nano Zn(II)-Meth 1:2 complex, as seen in Fig. 5, with characteristic peaks in 2θ of 4.307°, 11.449°, 17.025°, and 20.369°.

A ^{1}HNMR study on the zinc complex shows the singular peak for the methyl group that appears in 2.125 ppm for free methionine shifted to 1.943 ppm (Fig. 6). This shows that the electron density increases around the complex, resulting in negative shift of hydrogens peak. For H:1,2,3 a single peak appears in 1.194 ppm and a H:6,7 doublet triplet in the 1.910, 1.953, 2.033 ppm range as well as a H:4,5 triplet peak in 2.423, 2.434, 2.462 ppm and a H:8 triplet peak in 3.649, 3.676, 3.697 ppm. The integration of ^{1}HNMR spectra shows the number of hydrogens in this complex. Due to proton exchange, the nitrogen's proton can't be determinde in D_2O solvent. Accordingly, the ^{1}HNMR spectrum shows trans conformation. Trans conformation was found in 1969 with crystallographic data [29]. The ^{1}HNMR spectra of Zn-Meth is depicted in Fig. 6.

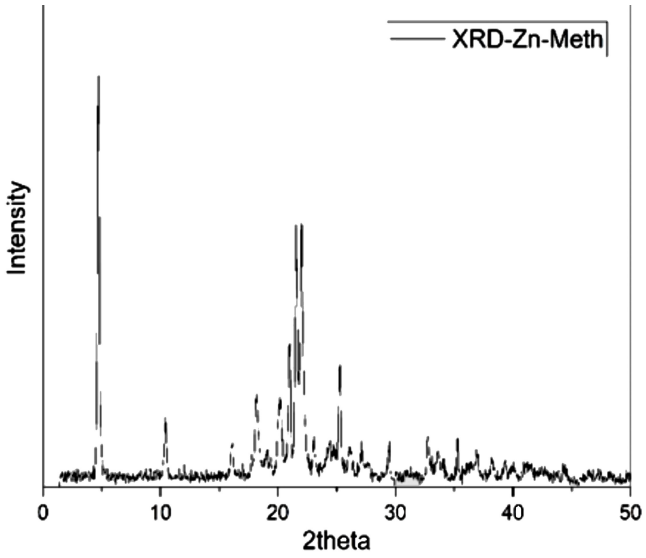


Fig. 4. XRD pattern of nano Zn-Meth.

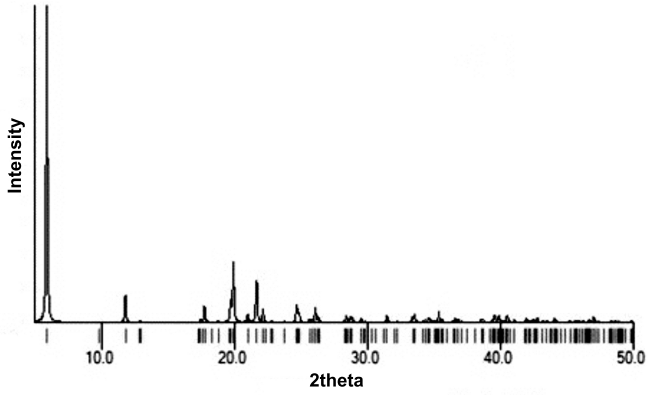


Fig. 5. XRD pattern of crystal Zn-Meth.

The morphology of Zn-Meth prepared by the ultrasonic technique is nanosheets, which was examined by FE-SEM (Fig. 7). The results showed the thickness of the planes in the methanol as solvent was

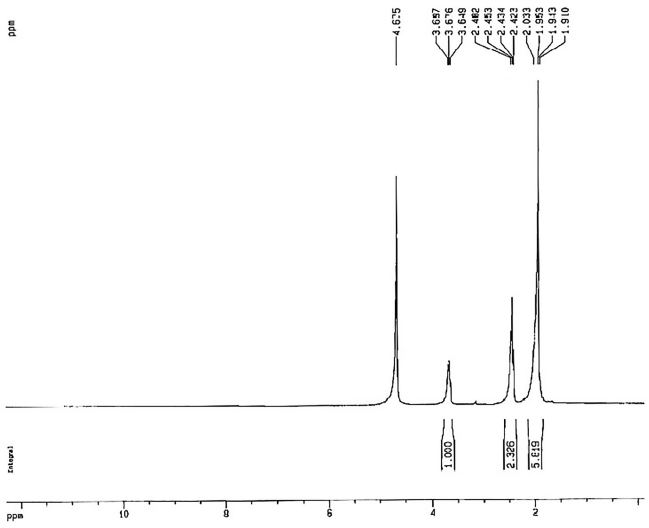
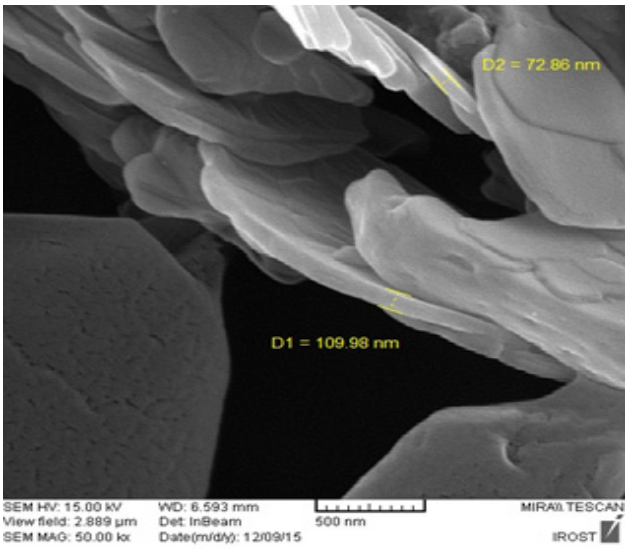


Fig. 6. ^{1}HNMR spectrum of zinc (II) methionine complex.



Fi.g 7. SEM photograph of Zn-Meth complex in methanol.

about 70 to 100 nm, while the thickness of these sheets in the DMSO was about 246 to 358 nm (Fig. 8).

In this study, the plane nanostructure of Zn-Meth was obtained by the ultrasonic method, however, the hydrothermal method was used in another study of complex of Zn(II)-cysteine and a spherical particle with the size of 24 nm was obtained [16]. The analysis results of TG/DTA shows the decomposition of Zn-Meth started at 242 °C (Fig. 9). Three different mass losses were observed in the TG/DTA diagram. At about 250 °C the complex started to decompose, the first step occurred in the range of about 250 to 300 °C, and may be related to losing the carboxyl group into CO_2. The second step from 320 to 400 °C is related to losing the NH_2 group, and the last step at 420 to 700 °C is due to the loss of sulphur moiety in the complex [8]. Step one,

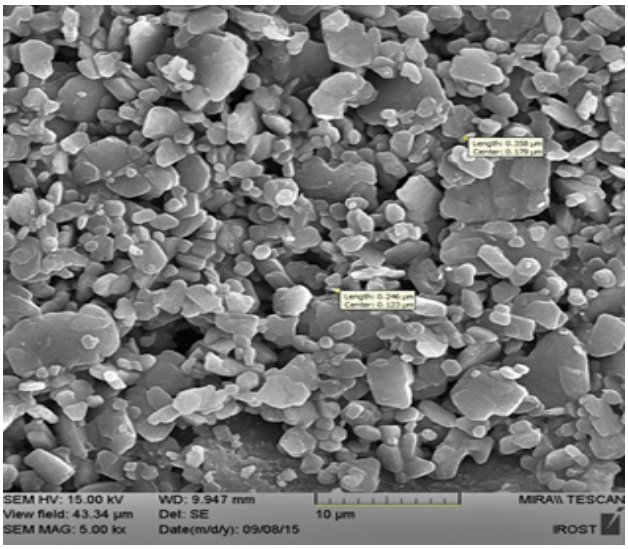


Fig. 8. SEM photograph of Zn(II)-Meth complex in DMSO.

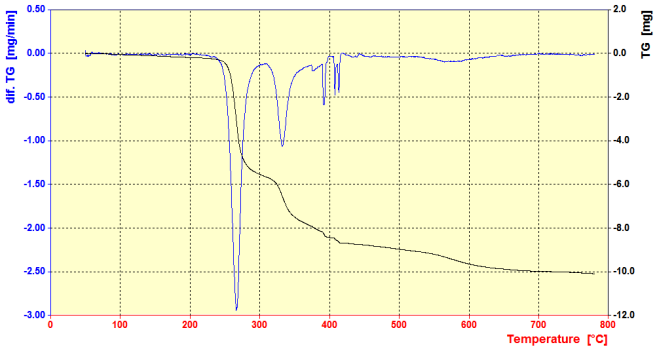


Fig. 9. TG/TDA of Zn-Meth.

two, and three mass losses were about 50, 30, and 20%, respectively.

The XRD pattern of the prepared ZnO nano powder, using Cu-K_α radiation source of $\lambda = 1.5406$ Å, is shown in Fig. 10. The pattern shows a sharp peak at 31.442T, 34.9T and 35.982T. The obtained pattern is similar to ZnO with ICSD code (hexagonal, space group $P6_3mc$, cell constants a = b = 3.2648 Å and c = 5.2194 Å).

EDS analysis of calcinated products from Zn-Meth confirmed the presence of zinc and oxygen (Fig. 11).

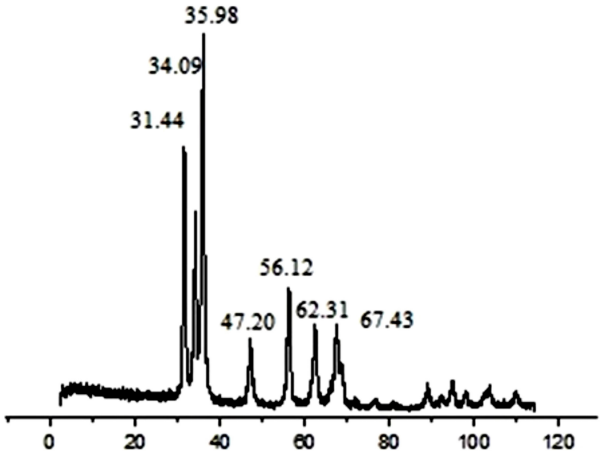


Fig. 10. XRD pattern of nano ZnO.

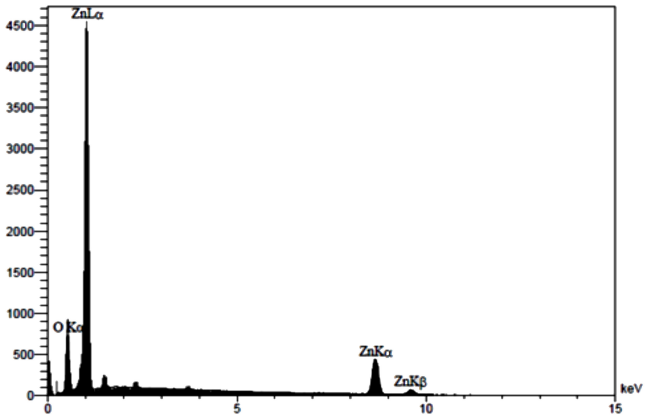


Fig. 11. Energy-dispersive X-ray analysis of hexagonal ZnO particles.

SEM photography shows that nano ZnO was obtained from this method (Fig. 12). The range of ZnO particles size is between 40-140 nm. The particles distributions diagram is shown in Fig. 13 and demonstrates the majority of ZnO particles are about 80 nm long.

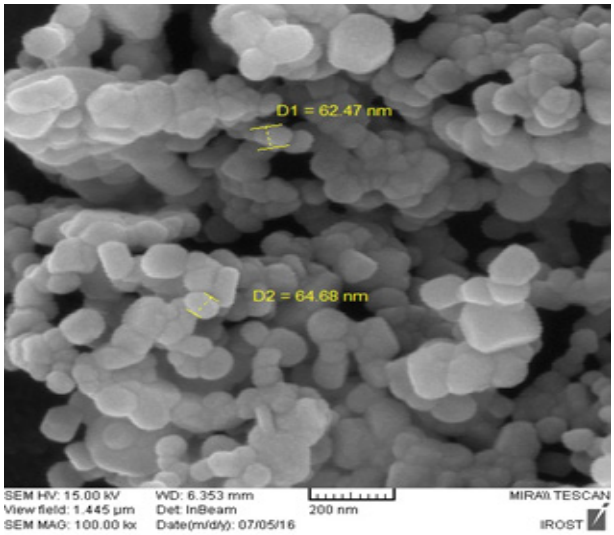


Fig. 12. SEM photograph of ZnO Nanoparticles.

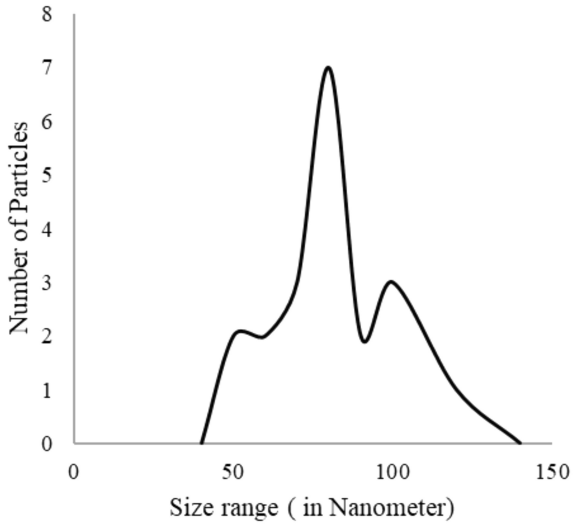


Fig. 13. ZnO particle distribution curve.

The amount of zinc in the serosal liquid obtained from each sample was measured by atomic absorption. The results are shown in Table 2.

Using reaction in Fig. 1, the uptake percentage was calculated twice for Zn chloride at 3.19 and 3.261%. Results for the same calculation for commercial "Zinpro® 120" compound uptake percentages were 4.34 and 4.142% and for zinc methionine nano sheets were 5.525 and 5.430%. As expected, zinc inorganic salt had the lowest amount of uptake with an average uptake of 3.20% and the value for Zinpro® complex was 4.24%. The highest amount of uptake found for zinc methionine nano sheets was 5.47%. Our findings are in agreement with a study by Ji *et al.* [30] who reported better manganese source absorbance rate for intestinal uptake of amino acid-chelated Mn in comparison to inorganic Mn salts. As enormous numbers of biological process occur in nanoscale [31], when the particle sizes decreased, the active sites will increased and be more available.

4. Conclusion

According to findings from this study, using methanol as solvent resulted in a smaller particle size than using DMSO. Results of this study also showed using ultrasonic waves is a simple and fast method for the synthesis of nanostructures of Zn-Meth. Calcination of this complex under ambient atmospheric conditions leads to nano ZnO powder. In-vitro intestinal absorption rate evaluation with different Zn sources by the everted gut sac method showed the highest absorption rate for nanostructures of Zn-Meth complex as compared to $ZnCl_2$ and the commercial complex Zinpro®.

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Table 2. Absorption of Zn(II) Compounds

Zinc source	Initial value (ppm) in incubation solution		Final value (ppm) in serosal solution		Average percentage of absorption (%)
	replicate 1	replicate 2	replicate 1	replicate 2	
Zinc chloride	80	80	4.79	5.05	3.20
Zinpro® 120	80	80	5.98	5.82	4.24
Nano Zn-Meth	80	80	7.76	6.26	5.47

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