

# Synthesis of ZnO Nanoparticles from Azo Complexes and Study their Biological Activity

Sanbul H. Jerwan<sup>1</sup>, Yousif H. Khalaf<sup>2</sup>, Ahmed Mishaal Mohammed<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, College of Science, University Of Anbar, Ramadi, Iraq

<sup>2</sup>Department of Clinical Lab. Sciences, College of Pharmacy, University Of Anbar, Ramadi, Iraq

\*Corresponding author email: sc.dr.ahmedm.mohammed@uoanbar.edu.iq

## ABSTRACT

An azo complex was prepared from the reaction of Zn(II) with the ligand 8- [(4-hydroxyphenyl) azo] guanine. A reaction with Zn(II) gave a new complex Bis(8- [(4-hydroxyphenyl) azo] guanine) zinc (II) chloride at pH = 8. Spectrophotometry of the prepared complex was performed using UV-Visible and infrared spectra and micro-elemental analysis. Zinc oxide nanoparticles were then prepared from the prepared complex by thermal decomposition method. The nano particles characteristic to know its structural properties was conducted by using a scanning electron microscope, atomic force microscopy and X-ray diffraction. The biological activity of the azo-zinc complex and the zinc oxide nanoparticles was studied against *E. coli*, *Ps. aeruginosa* and *S. aureus*. The iso-zinc oxide nanocomplex was more effective than the iso-zinc complex against these genera of bacteria.

**Keywords:** ZnO , Nanoparticles , Complex , Biological , guanine

## Correspondence:

Ahmed Mishaal Mohammed

Department of Chemistry, College of Science, University Of Anbar, Ramadi, Iraq

Email: sc.dr.ahmedm.mohammed@uoanbar.edu.iq

## INTRODUCTION

Nano is the science and engineering of small bodies less than 100 nm. The term nanoscale was defined in the Greek language 'Nanos', which means dwarf. To be precise, one nanometer equates to 10 hydrogen atoms in one extension [1].

Nanotechnology is the design and fabrication of structures at the atomic and molecular level that have at least one dimension measured between 1-100 nm whose particles are exploitable all physical and chemical properties that can be manipulated and controlled [2]. Methods of synthesis of nanomaterials are divided into two methods: Top-Down or Bottom-Up [3].

Multifunctional materials that are manufactured on the nanoscale represent a huge evolutionary advance for existing technology and are promising technologies for application in many industrial fields and in medicine, chemistry, or physics medicine, where the use of nanomaterials is being developed in medicine in order to improve social welfare [4]. The use of nanomaterials in the field of delivery of oncology drugs within the body is a major advance because this new treatment method can provide safe and effective treatment by using various nanopharmaceuticals in treating cancer cells without the application of harmful and destructive treatments. Applications of nanomaterials in the field of nanomedicine are to carry the anti-tumor reagent into the blood vessels of the tumor [5].

Zinc oxide nanoscale has anti-microbial activity when the particle size is reduced to the nanometer. Thus, the nanoscale of Zinc oxide can attack the surface of bacteria, enter the cell's interior, and act to kill them. Nano zinc oxide has the potential to stimulate plant growth as well as protect it from diseases due to its antimicrobial activity [6].

Azo compounds are organic compounds of widespread characterized by their multiple uses [7]. These compounds consist of two organic groups, homogeneous or heterogeneous, which are related to the bridging azo group (-N = N-). They are related to the symmetric groups aliphatic or aromatic [8]. Azo is more commonly used in

many areas due to its high stability due to the double bond between nitrogen in the azo group, and most of the complexes that form them with metallic ions are also stable [9-11].

This stability is also due to the resonance of the aromatic rings associated with the azo bridge [12]. Guanine is one of the purines that form one of the four foundations in the formation of deoxyribonucleic acid (DNA) [13,14]. Azo ligands of heterocyclic compounds are characterized by their great importance in the biological field due to the vital importance in bacterial research and oncology [15]. These compounds are used as drugs, anti-microorganisms and anti-bacterial [16, 17]. It was found that azo compounds and their complexes with metals such as zinc have biological efficacy against microorganisms.

These complexes have spread widely for their many uses of heterogeneous azo dyes, which is due to the strong correlation of metal ions with compounds due to their containing nitrogen atom as a heterogeneous atom in the heterogeneous ring [18]. Therefore, these complexes with metallic elements are effective compounds as anti-mold and anti-bacterial, where the effectiveness has explained the interaction of zinc metal with the guanine complexes as an oncological drug, anti-fungal and anti-bacterial, however it was found in the absence of metals, the complexes have no effect on microorganisms [19].

## MATERIALS AND METHODS

### Materials

All the chemicals used were supplied by Sigma-Aldrich and Santacruz Biotech (USA). The antibacterial impact study was done using three types of bacteria, Gram Positive Bacteria, *S. aureus*, and Gram Negative Bacteria, *E. coli* and *Ps. aeruginosa*. Pathogenic bacteria were diagnosed and cultured on Moller Hinton Agar medium. Disc diffusion method was utilized to investigate the deactivating capacity of the prepared compounds against the tested pathogenic bacteria.

### Methods

#### Synthesis of 8 - [(4-hydroxyphenyl) azo] guanine

## Synthesis of ZnO Nanoparticles from Azo Complexes and Study their Biological Activity

4 m.mol (0.415 gm) of para hydroxy aniline was dissolved in a mixture of 1 mL of concentrated hydrochloric acid and 3mL of distilled water. The mixture was cooled and then a solution of sodium nitrite (0.32gm) dissolved in 4 mL of distilled water was added drop by drop with continuous stirring and noticing that the temperature did not rise above 5 °C. The solution was left to settle for 15 minutes. Followed by adding the diazonium salt slowly with continuous stirring to a 4m.mol (0.5 gm) of guanine solution dissolved with 15 mL of HCl (%50) . A brick red color was formed. The mixture was cooled in an ice bath until the product is

settled down. The crystals were filtered, dried, and re-crystallized from ethanol.

### Synthesis of bis(8- [(4-hydroxyphenyl) azo] guanine)zinc(II) chloride

A solution of mineral salt ZnCl<sub>2</sub> (0.05 m.mole) dissolved in the optimized buffer solution (75 mL) was added gradually with continuous stirring to a solution (1 m.mole) of ligand dissolved in DMSO. A light purple precipitate was obtained. The crystals were filtered, dried, and re-crystallized from ethanol: water (1: 1) [20] as shown in Table (1).

Table 1: Describes the materials required to prepare of bis(8- [(4-hydroxyphenyl) azo] guanine) zinc(II) chloride

Complex	M:L ratio	gm of ligand	gm of metal salt	pH	Stirring time (min.)	mL. DMSO
[Zn(L) <sub>2</sub> ]Cl <sub>2</sub> DMSO	1:2	0.3942	1.701 ZnCl <sub>2</sub>	8	15	75

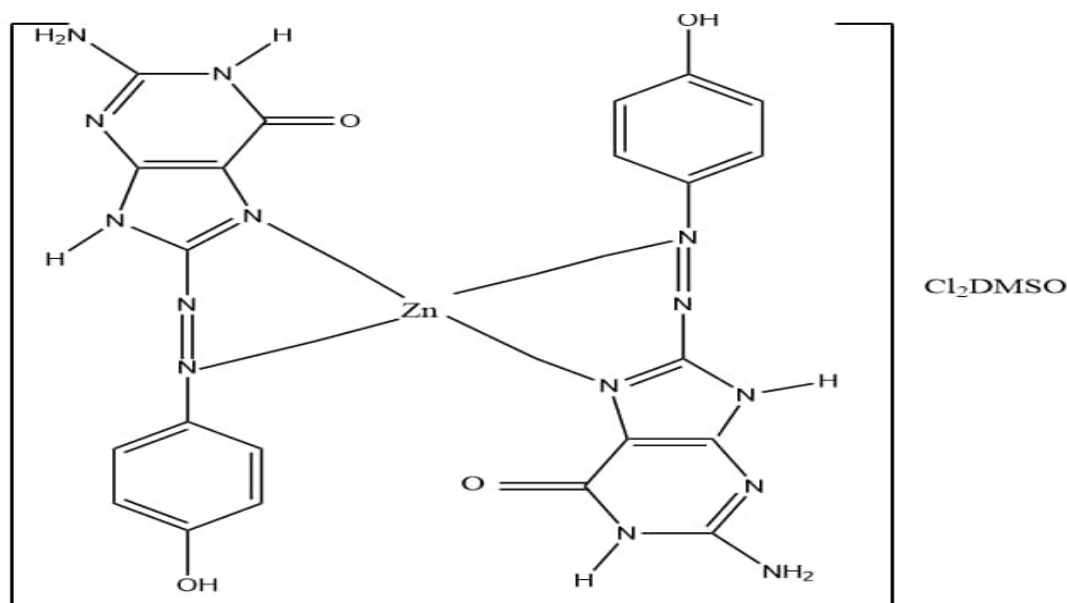


Figure 1: Shows the geometric composition of bis(8- [(4-hydroxyphenyl) azo] guanine)zinc(II) chloride

### Zinc oxide nanostructures

76.2 mg of Zn (L<sub>2</sub>) complex was dissolved 1.4 mL of oleic acid, a greenish-black solution was formed. Then, this solution was heated to 260 ° C for two hours, a black precipitate was formed. Next, 9 mL of toluene and methanol (2: 1) were added to the precipitate, dark solid of ZnO NPs was then obtained by the centrifugation, washed by ethanol, dried, and then it was diagnosed by using SEM, AFM, XRD [21].

### Biological study

Three genera of pathogenic bacteria were used; *E. coli*, *Ps. aeruginosa* (Cram-negative), and *S. aureus* (Gram-positive). These bacteria were isolated from patients and identified by the central Health Laboratory – Baghdad.

### Bacterial sensitivity test toward the prepared complexes

Disc diffusion method was used to test the effect of complexes against bacteria, four concentrations in DMSO were prepared, (10, 25, 50 and 100) mg/mL, for each

prepared substance, 10 filter paper discs (6 mm) were prepared for each concentration in clean glass tubes. Sterilized with an autoclave for 15 minutes and then added, then 1 mL of the prepared solutions were added and tubes were shaken, and the tablets were dried by placing them in the incubator at 40 °C for 48 hours. A control sample of DMSO solvent was used by adding 1 mL to 10 sterilised discs.

### Test method

The culture medium (Muller Hinton agar) was prepared by dissolving (38 g) of agar per liter of distilled water. The culture medium was sterilized and poured into the dishes, and after it cooled, a semi-solid gelatinous layer was formed. Then, the species used for the study were grown on nutrient broth for 24 hours at 37 °C. 0.1 mL of the bacterial suspension was transferred to the nutrient pellet plate and spread over the entire center of the surface by the diffuser and left for half an hour. In each dish, a tablet was placed for each concentration beside

the control (DMSO) sample. The dishes were incubated at 37 °C for 24 hours. The diameter of inhibition was measured using a millimeter ruler.

## RESULTS AND DISCUSSION

### Ultraviolet-visible spectra of 8 - [(4-hydroxyphenyl) azo] guanine

The UV-Visible spectra 8 - [(4-hydroxyphenyl) azo] guanine (Figure 2) was studied. (n-  $\pi^*$ ) transfer group at 361 nm was recorded due to (ph - N=N-), in addition to the emergence of an absorption beam at 241 nm, due to the electron transitions of the type ( $\pi \rightarrow \pi^*$ ) of the group (C = N) which belonging to the absorption of purine [22, 23].

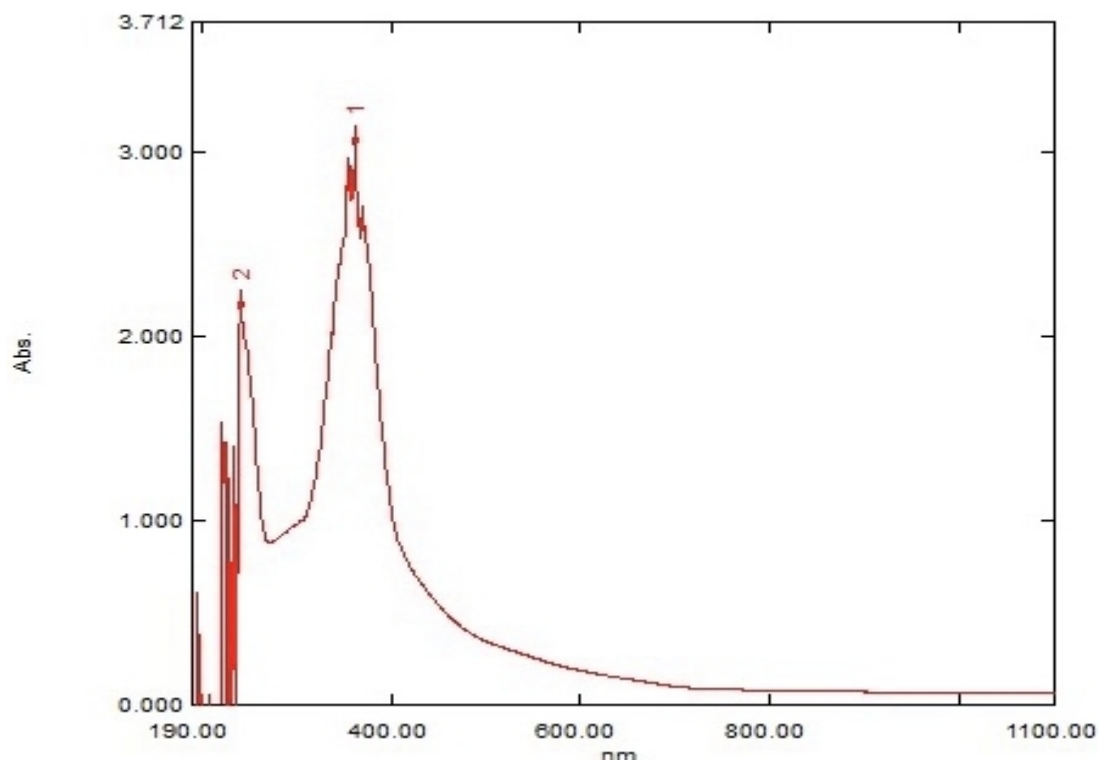


Figure 2: Ultraviolet-Visible spectra of 8 - [(4-hydroxyphenyl) azo] guanine

### FT-IR Spectra and mode of bonding

The difficulty of interpreting the infrared spectra of azo-ligands, in particular the heterocyclic ring, lies in the many interferences, Therefore, much of the literature lacks an accurate explanation of the spectra of these types of organic compounds [24].

FT-IR spectra of free ligand Figure 3 showed strong band at frequency 3170  $\text{cm}^{-1}$  attributable to the stretchy vibrations of (N-H) bond of the amidazole ring. Whereas, wide strong bands between (3336-2700)  $\text{cm}^{-1}$  resulting from overlap of the stretching vibration (OH) and (NH). This is consistent with other studies [25]. Infrared spectra also showed vibrational frequencies of the active groups, including (N = N), (C = N), (C = C), and (CN = NC). The absorption of medium intensity at a frequency (1672-1512)  $\text{cm}^{-1}$  refers to frequency (C = N) of the amidazole ring.

The spectrum of ligand showed two absorption bands at the frequency 1473  $\text{cm}^{-1}$  and 1373  $\text{cm}^{-1}$  attributable to  $\nu(\text{C}=\text{C})$  and  $\nu(\text{N}=\text{N})$ , respectively. The position  $\nu(\text{C}=\text{C})$  and  $\nu(\text{N}=\text{N})$  absorption bands was pointed in the literature [26]. A vibrational frequencies also was observed at the frequency 1259-1219  $\text{cm}^{-1}$  due to the stretching frequency of the bond (CN = NC).

FT-IR spectra of prepared complex with zinc were recorded and compared to free ligand spectra. Figure (4) shows Bis(8- [(4-hydroxyphenyl) azo] guanine) zinc(II) chloride spectra. It was observed that the stretching

vibration in the complex spectra had undergone some changes, as well as the occurrence of small or large displacements for most of stretching vibration. These changes are a clear indication of the occurrence of the symmetry process and the formation of the new complex. The spectrum of the binary zinc complex showed absorption bands at (3440-3330)  $\text{cm}^{-1}$  due to the stretchy vibrations (symmetric and asymmetric) of the amine group associated with the co-ordinate with the bilateral zinc.

These bands suffered from changes in symmetry as they shifted towards a higher and lower frequency by changing the shape and location of the absorption bands due to the amplitude-frequency of the amine group from evidence based on the symmetry between the nitrogen atom in the group and the metal ion [27] as the spectrum showed absorption vibrations at 1672  $\text{cm}^{-1}$  and 1633  $\text{cm}^{-1}$  of the complex belong to the group (C = C). The spectrum of the zinc complex showed an absorption vibrations at 1415  $\text{cm}^{-1}$  belonging to the azo group, which suffered from a change in the position of the band at a higher wavelength, which indicates its participation in the symmetry with the metal ion. Besides, the emergence of new vibrations at 470-443  $\text{cm}^{-1}$  of the zinc complex. These bands are due to the vibrations of the (MN) family. The reason for its appearance is attributed to the consistency of the metal with the nitrogen of the azo group and the nitrogen of the amine group [25].

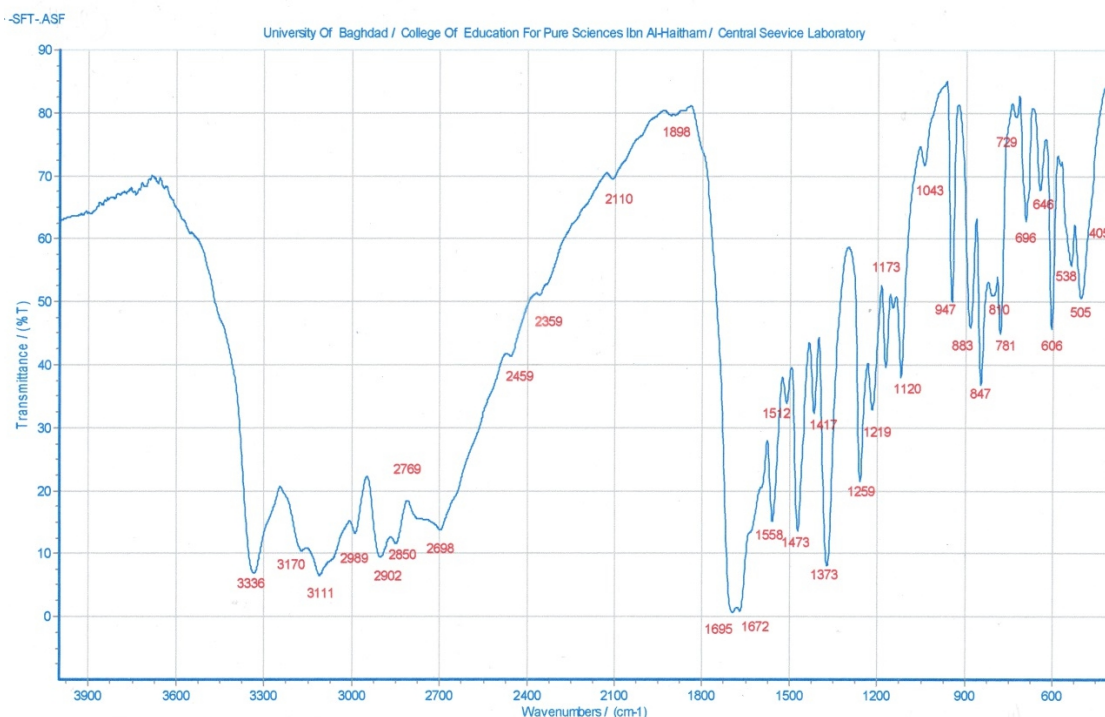


Figure 3: Shows FT-IR spectrum of 8 - [(4-hydroxyphenyl) azo] guanine

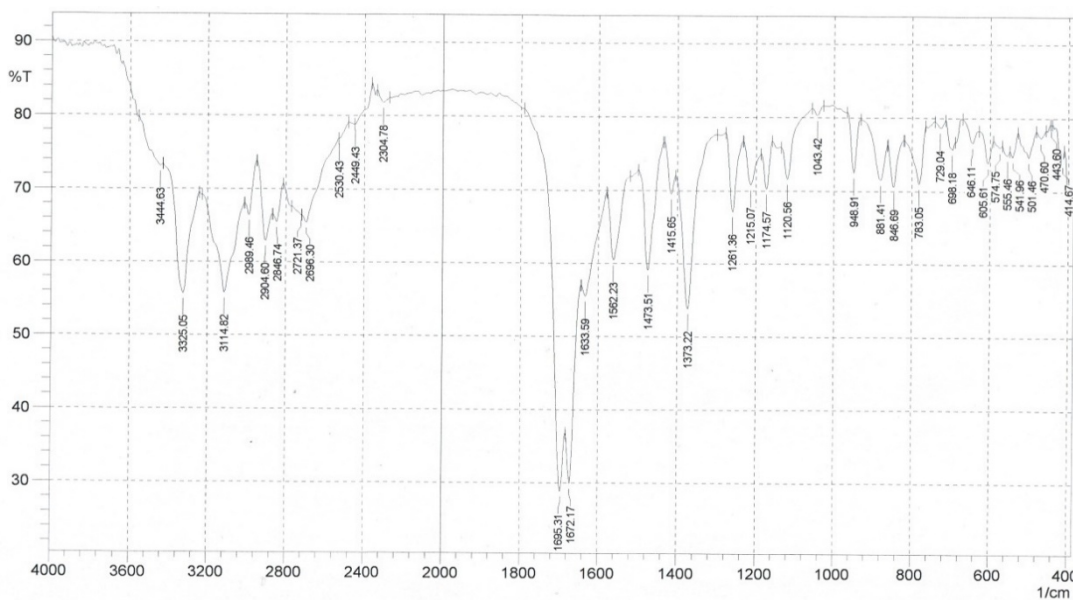


Figure 4: Shows FT-IR spectrum bis(8- [(4-hydroxyphenyl) azo]guanine)zinc(II) chloride

**Critical Elemental Analysis (C.H.N)**

The precise analysis of each of the elements of carbon, hydrogen, and nitrogen was measured for the bis(8- [(4-hydroxyphenyl) azo] guanine)zinc(II) chloride. Close

results were obtained between the theoretical and practical ratios of these elements and the molecular weights as shown in Tables (2 and 3).

Table 2: Accurate analysis of carbon, hydrogen, and nitrogen elements bis(8- [(4-hydroxyphenyl) azo] guanine)zinc(II) chloride the computed theoretical ratio

Theoretical ratio	C%	H%	N%
Zn	43.59	2.97	32.36

Table 3: Shows accurate analysis of carbon, hydrogen, and nitrogen elements for bis(8- [(4-hydroxyphenyl) azo] guanine)zinc(II) chloride

Sample name	C%	H%	N%
S-Zn	43.5	2.96	32.2



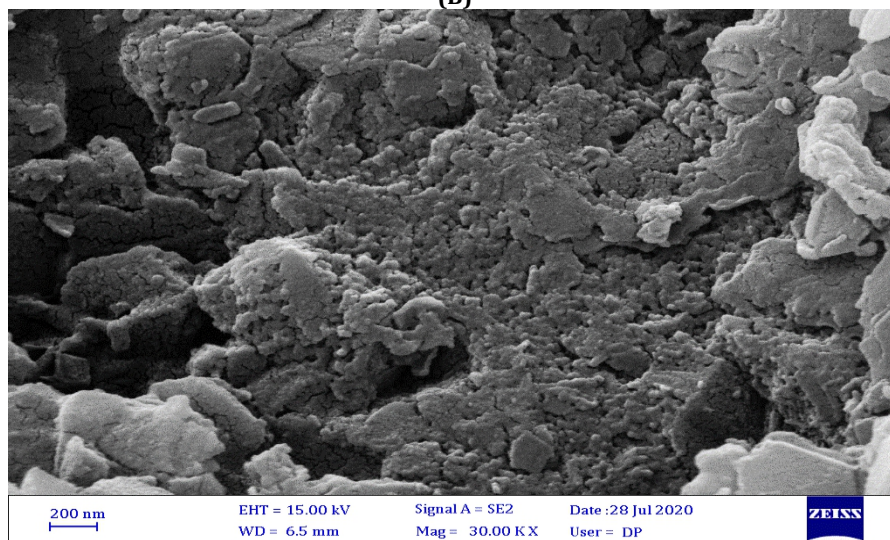
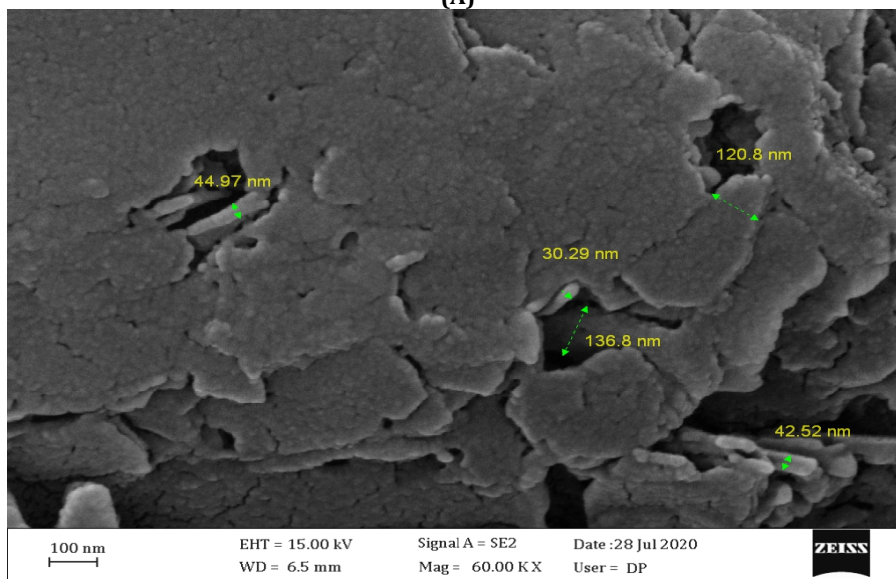
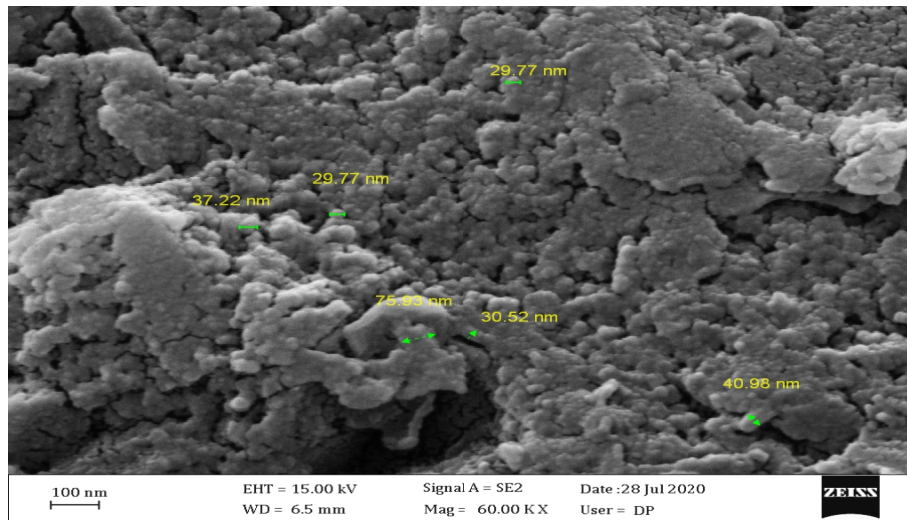
# Synthesis of ZnO Nanoparticles from Azo Complexes and Study their Biological Activity

## Structural properties of ZnO nanostructures

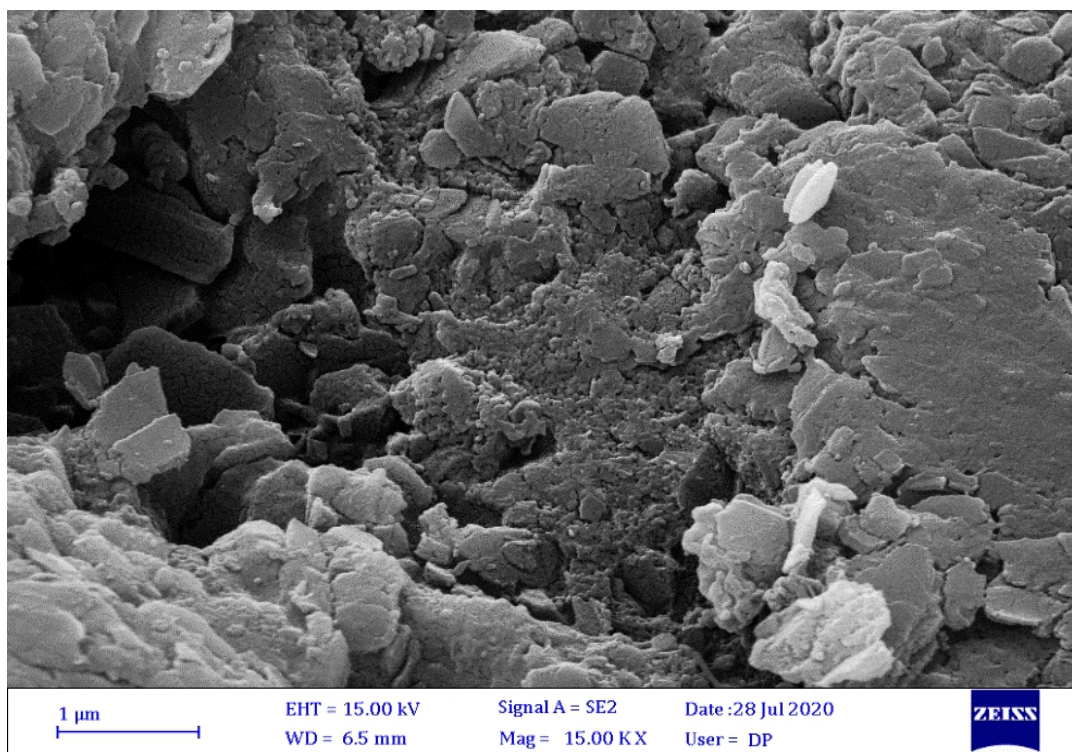
### Scanning electron microscope

SEM analysis was conducted by scanning the surface of the samples producing various signals that contain

information about the surface topography and composition of the sample, which were shown through the images (A, B, C, D) in the form of nanoparticles shown in Figure. (5) with different enlargement strengths.







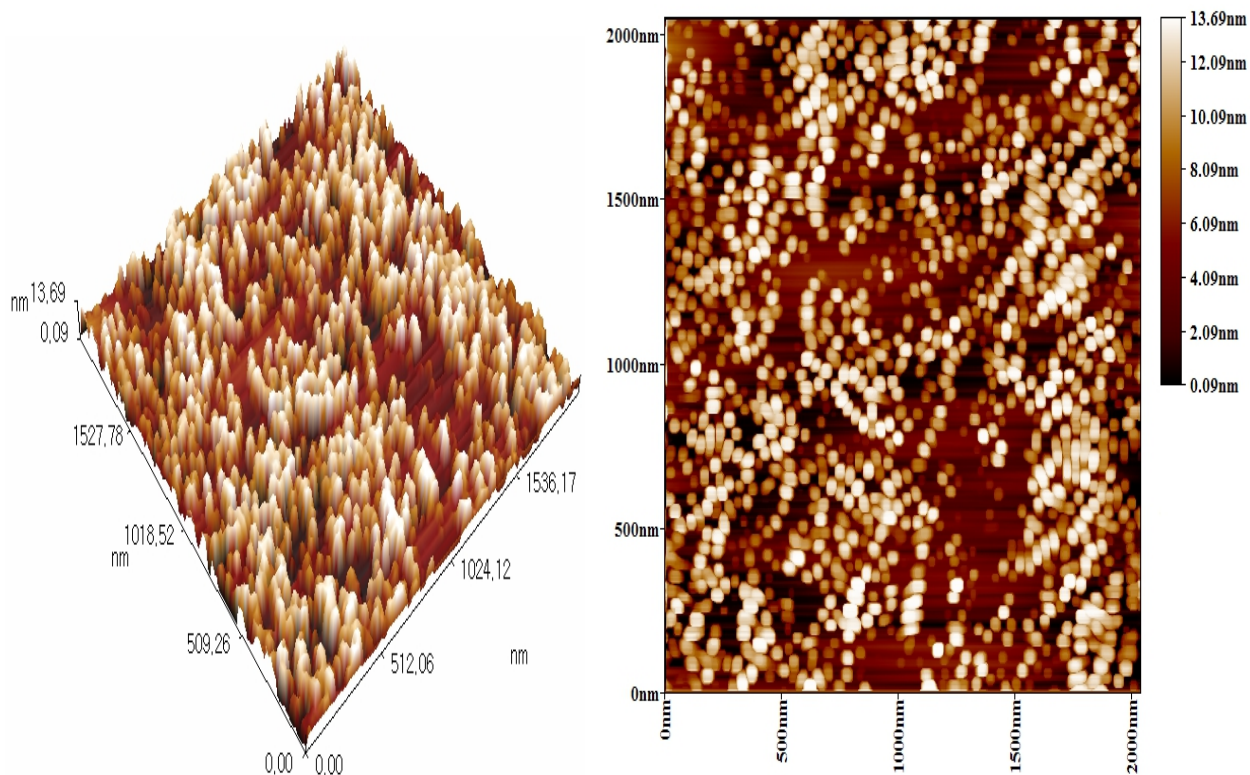
(D)

Figure 5: A scanning electron microscope demonstrates nanoscale zinc oxide prepared by the thermal decomposition method with different enlargement power for images A, B, C, D.

**Atomic force microscopy**

Atomic force microscope is used to determine the surface of morphology, topography, thickness, and toughness of

substances. Figure (6) shows the two-dimensional and three-dimensional AFM images of the prepared zinc oxidesurface



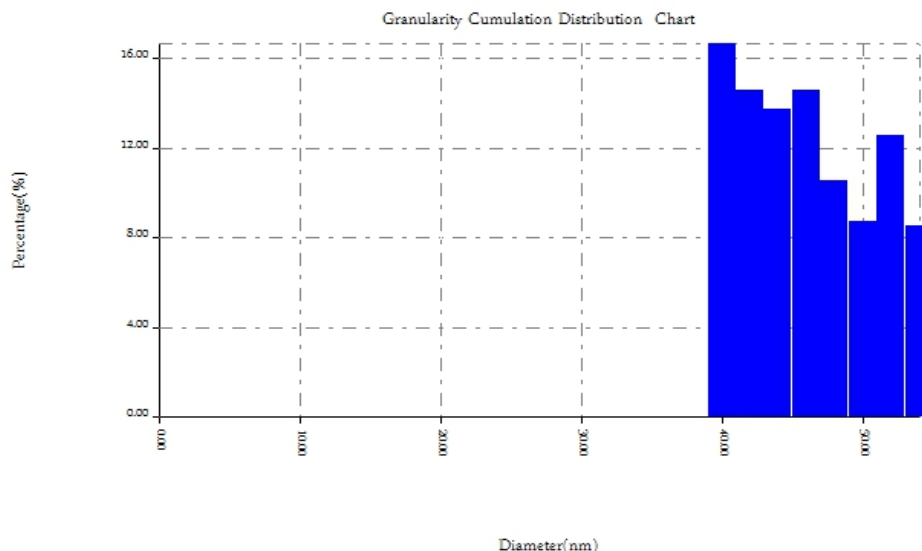


Figure 7: Shows the rate of distribution for prepared zinc oxide nanoparticles with a diameter of 45.18 nm

**X-ray diffraction**

Figure (8) shows the X-ray diffraction results of zinc oxide nanoparticles in comparison with the standard reference for zinc oxide JCPDS 01-076-0205. Diffraction peaks (100), (002), (101), (102), (110), (103), and (112) were found, which are confirmed by the hexagonal zinc

oxide (Wurtzite), according to the standard reference and through the figure, three very clear zinc oxide peaks were noted at the angles 31.96°, 34.84°, and 36.42° which attributed to (100), (002), and (101) respectively, compared to the International Center for Diffraction Data (JCPDS) card [28].

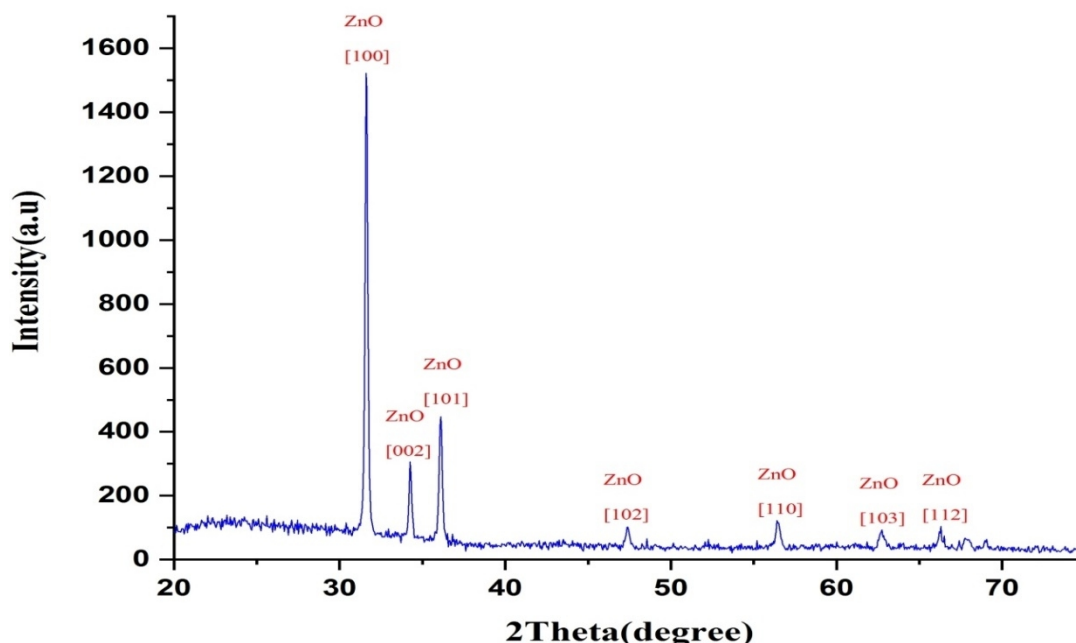


Figure 8: Shows X-ray diffraction of the prepared nanostructures of zinc oxide compared to standard reference for zinc oxide JCPDS (01-076-0205).

**Biological investigation**

The effect of the prepared complexes was studied against three genera of pathogenic bacteria, *E. coli*, *P. aeruginosa* and *S. aureus*. The sensitivity changes of bacteria toward the prepared complexes was tested by Kirby-Bauer technique. Three concentrations in DMSO of complexes have been tested.

The nano-zinc oxide azo complex showed positive results as antimicrobials against bacterial species used in the test more than the usual azo-zinc complexes. It looks from table (4) that azo-zinc oxide nano code and the normal zinc azo-zinc complex showed efficacy for all bacteria positive and negative for the Gram stain. They

gave the highest activity at the highest concentration of 100 mg / mL. with varying degrees of inhibitory effect against the growth of the tested bacteria species, and this is consistent with other studies [29]. Table (4) also shows that the azo-zinc oxide nanocomplex is more effective compared to azo-zinc complex in inhibiting genera of pathogenic bacteria used in the study. At the highest concentration (100 mg / mL), nanoparticles of the azo-zinc oxide complex showed an inhibition zones of (11.5, 18 and 13) mm against *E. coli*, *P. aeruginosa* and *S. aureus*, respectively. This is what was observed by Parsae *et al.* [30], who succeeded for using different concentrations of nanoparticles of zinc oxide and proved their anti-

bacterial efficacy against gram-positive and gram-negative bacteria in concentration-dependent manner.

Table 4: Diameters of inhibition zones for prepared complexes against some of bacteria genera

Bacteria	Conc. mg/mL	Bis (8- [(4-hydroxyphenyl) azo] guanine)zinc(II) chloride	Zn Nps	DMSO control
<i>Esherichia coli</i>	100	7.5 ± 0.1	11.5 ± 0.1	---
	50	7 ± 0.1	7.1 ± 0.1	---
	25	---	6 ± 0.5	---
	10	---	---	---
<i>Pseudomonase aeruginosa</i>	100	11 ± 0.5	18 ± 0.1	---
	50	9.5 ± 0.1	14 ± 0.2	---
	25	---	---	---
	10	---	---	---
<i>Staphylococcus aureus</i>	100	10.5 ± 0.5	13 ± 0.5	---
	50	9 ± 0.2	11 ± 0.1	---
	25	6.5 ± 0.0	8 ± 0.1	---
	10	---	---	---
Bacteria	Conc. mg/mL	Bis (8- [(4-hydroxyphenyl) azo] guanine) zinc (II) chloride	Zn Nps	DMSO control
<i>Esherichia coli</i>	100	7.5 ± 0.1	11.5 ± 0.1	---
	50	7 ± 0.1	7.1 ± 0.1	---
	25	---	6 ± 0.5	---
	10	---	---	---
<i>Pseudomonase aeruginosa</i>	100	11 ± 0.5	18 ± 0.1	---
	50	9.5 ± 0.1	14 ± 0.2	---
	25	---	---	---
	10	---	---	---
<i>Staphylococcus aureus</i>	100	10.5 ± 0.5	13 ± 0.5	---
	50	9 ± 0.2	11 ± 0.1	---
	25	6.5 ± 0.0	8 ± 0.1	---
	10	---	---	---

**CONCLUSION**

The prepared solid metallic complex was characterized by its high stability and not affected by heat. This study showed that the prepared nano-zinc oxide possesses a system identical to the standard references, pictures of the nanoparticles showed the homogeneity of their surfaces. The results of the biological study revealed that the nano-zinc oxide azo complex has a considerable activity against different genera of pathogenic bacteria, which may make it a potent antibacterial agent.

**REFERENCES**

- Sharma, N., Ojha, H., Bharadwaj, A., Pathak, D., and Sharma, R. K., "Preparation and catalytic applications of nanomaterials: A review", *RSC Advances*, Vol. 5(66), 53381-53403 (2015).
- Hazeem, L. J., Kuku, G., Dewailly, E., Slomianny, C., Barras, A., Hamdi, A., Boukherroub, R., Culha, M., and Bououdina, M., "Toxicity effect of silver nanoparticles on photosynthetic pigment content, growth, ROS production and ultrastructural changes of microalgae", *Nanomaterials*, Vol.9(7), 914-922 (2019).
- Abdullah, Z. W., Dong, Y., Davies, I. J., and Barbhuiya, S., "PVA, PVA blends, and their nanocomposites for biodegradable packaging application", *Polymer-Plastics Technology and Engineering*, Vol. 56(12), 1307-1344 (2017).
- Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H., Ann, L. C., Bakhori, S. K., and Mohamad, D., "Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism", *Nano-Micro Letters*, Vol. 7(3), 219-242 (2015).
- Singh, A., Singh, N. B., Afzal, S., Singh, T., and Hussain, I., "Zinc oxide nanoparticles: A review of their biological synthesis, antimicrobial activity, uptake, translocation and bio transformation in plants", *Journal of Materials Science*, Vol. 53(1), 185-201 (2018).
- Oh, J., and Han, D., "Based nanomaterials and nanostructures", *Nanomaterials*, Vol. 10(3), 567-572 (2020).
- Mohammed, I. A., and Mustapha, A., "Synthesis of new azo compounds based on N-(4-Hydroxyphenyl) maleimide and N-(4-Methylphenyl) maleimide", *Molecules*, Vol. 15, 7498-7508 (2010).
- Zarei, A., Hajipour, A. R., Khazdooz, L., Mirjalili, B. F., and Chermahini, A. N., "Rapid and efficient diazotization and diazo coupling reaction on silica sulfuric acid under solvent-free conditions", *Dyes and Pigments*, Vol. 81(3), 240-244 (2009).
- Blumel, S., Knackmuss, H., and Stolz, A., "Molecular cloning and characterization of the Gene coding for the aerobic azoreductase from xenophilusazovorans", *App. Env. Micr*, Vol. 68(8), 3948-3958 (2002).



10. Koh, J., and Greaves, A. J., "Synthesis and application of an alkali clearable azo disperse dye containing a flurosulfonyl group and analysis of its alkali hydrolysis kinetics", *Dyes and Pigments*, Vol. 50(2), 117-126 (2001).
11. Sabi, Y., Tamada, S., Iwamura, Oyamada, T., Bruder, M., F., Oser, R., Berneth, H., and Hassenruck, K., "Development of organic recording media for blue high numerical aperture optical disc system", *J. Appl. Phys*, Vol. 42, 1056-1058(2003).
12. Li, X., Wu, Y., Gu, D., and Gan, F., "Spectral, thermal and optical properties of metal(II)-azo complexes for optical recording media", *Dyes and Pigments*, Vol. 86(2), 182-189 (2010).
13. Mamdouh, S. M., Swasan, S. H., Alaa, E. A., and Nessma, M. N., "Synthesis and spectroscopic characterization of gallic acid and some of its azo complexes", *J. Mol. Struct*, Vol. 1014, 17-25 (2012).
14. Hemang, M., Yogesh, K. S., and Ashish R. S., "Azo group containing bis ligand and its coordination polymers", *Chemical Science Transactions*, Vol. 2(1), 301-307 (2013).
15. El-Sonbati, A., Diab, M., Belal, A. and Morgan, S., "Supramolecular structure, mixed ligand and substituents effect on the spectral studies of oxovanadium (IV) complexes of bioinorganic and medicinal relevance", *Spectrochim. Acta*, Vol. 95, 627-636 (2012).
16. El-Sonbati, A., Diab, M., Belal, A. and Morgan, S., "Coordination chemistry of supra molecular rhodanineazo-dye sulphadugs", *Review. Inorg. Chim*, Vol. 404, 175-187 (2013).
17. Khalaf, Y., "Synthesis a number of azo compounds derived from guanine and studying their biological activity on pathogenic bacteria", *J. of Al-Anbar University for Pure Science*, Vol. 2(3), 150-157 (2008).
18. Kumar, B., Kanchi, S., Bisetty, K., and Jyothi, N. V., "Analytical and biological evaluation of two Schiff's bases: Spectrophotometric analysis of copper (II) in water and soil samples", *J. Environ. Anal. Chem*, Vol. 1, 1-7 (2014).
19. Upadhyay, K., Kumar, A., Zhao J., and Mishra, R. K., "Naked-eye recognition of Cu(II), Zn(II) and acetate ion by the first guanine-based difunctional chromoinophore", *Talanta*, Vol. 8, 714-721 (2010).
20. Al-Hasani, T. J., and Almaliky, Z. S., "New Pd and Pt complexes of Guanine-Azo Dye: Structural, spectroscopic, dyeing performance and antibacterial activity studies", *Iraqi Journal of Science*, Vol. 56 (4A), 2718-2731 (2015).
21. Akgül, F. A., "Influence of Ti doping on ZnO nanocomposites: Synthesis and structural characterization", *Composites Part B: Engineering*, Vol. 91, 589-594 (2016).
22. Onda, K., Yamochi, H., and Koshihara, S. Y., "Diverse photoinduced dynamics in an organic charge-transfer complex having strong electron-Phonon interactions", *Accounts of chemical research*, Vol. 47(12), 3494-3503 (2014).
23. Stephanos J. J., "Addison A.W.Chapter 10", *Electronic Spectroscopy, Electrons, Atoms, and Molecules in Inorganic Chemistry*, 585-645 (2017).
24. Casini, A., Kelter, G., Gabbiani, C., Cinellu, M. A., Minghetti, G., Fregona, D., and Messori, L., "Chemistry, antiproliferative properties, tumor selectivity, and molecular mechanisms of novel gold (III) compounds for cancer treatment: a systematic study", *JBIC Journal of Biological Inorganic Chemistry*, Vol. 14(7), 1139-1149 (2009).
25. Socrates, G., "Infrared and raman characteristic group frequencies: Tables and charts", *John Wiley & Sons*, Vol. 283, 366 (2004).
26. Al-Zinkee, J. M. M., and Jarad, A. J., "Synthesis, spectral Studies and microbial evaluation of azo dye ligand complexes with some transition metals", *Journal of Pharmaceutical Sciences and Research*, Vol. 11(1), 98-103 (2019).
27. Mohammed, L. A., Mehdi, R. T., and Ali, A. A. M., "Synthesis and biological screening of the gold complex as anticancer and some transition metal complexes with new heterocyclic ligand derived from 4-Amino antipyrine", *Nano Biomed. Eng*, Vol. 10(3), 199-212 (2018).
28. Edalati, K., Shakiba, A., Vahdati-Khaki, J., and Zebarjad, S., M., "Low-temperature hydrothermal synthesis of ZnO nanorods: Effects of zinc salt concentration, various solvents and alkaline mineralizers", *Materials Research Bulletin*, Vol. 74, 374-379 (2016).
29. Ibrahim, O. B., Mohamed, M. A., and Refat, M. S., "Nano sized schiff base complexes with Mn (II), Co (II), Cu (II), Ni (II) and Zn (II) metals: Synthesis, spectroscopic and medicinal studies", *Can. Chem. Trans*, Vol. 2, 108-121 (2014).
30. Parsaee, Z., Bahaderani, E. J., and Afandak, A., "Sonochemical synthesis, in vitro evaluation and DFT study of novel phenothiazine base Schiff bases and their nano copper complexes as the precursors for new shaped CuO-NPs", *Ultrasonics Sonochemistry*, Vol. 40, 629-643 (2018).