

## Synthesis of ZnO Nanoparticle and Utilized as a Drug Carrier to Treat Leukemia

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**Abstract:** This study includes two parts, and the first was the preparation of the Zn(II) complex by reacting N-[4-(5-((Z)-[(5-oxo-2-sulfanyl-4,5-dihydro-1H-imidazol-1-yl)imino]methyl)furan-2-yl)phenyl]acetamide with ZnCl<sub>2</sub>. The complex was characterized by using microscopic analysis such as UV-Vis spectrum, LC-MS, FTIR spectrophotometer, measurements of conductivity, magnetic susceptibility, and atomic absorption. The second part was the preparation of the ZnO nanoparticles by dissolving the Zn(II) complex in HNO<sub>3</sub> and HCl and its use as a drug transporter to treat leukemia. FSEM, TEM, and XRD were examined for the characterization of ZnO nanoparticles that will be used in the synthesis of most medicines and drugs in the future.

**Keywords:** zinc(II) complex; ZnO nanoparticles; carrier for anti-cancer drugs; leukemia; microscopic analysis

### ■ INTRODUCTION

During the past decades, new trends have emerged widely known as nanotechnology, where they include the ability to manufacture new or improved properties, which are controlled by nanotechnology, which may include these characteristics, electrical delivery thermal, visual response, flexibility, corrosion resistance as well in the vital and medical fields with special treatment properties against cancer cells [1]. Where the focus of scientists in various fields has become a deep influence on all specialists in all scientific fields, such as engineering, physics, and medical biology. Because of the new properties of nanomaterials that have been discovered, nanoparticles can be used in catalysts, functional coatings, medicine, and vital medicine [2].

Nanomaterials play an important role in medicine and pharmaceutical sciences, where nanomaterials affect levels of cytotoxicity in living systems. Therefore, nanomaterials have been used in biological applications because it was discovered that they have potential in the future in the future in bio-diagnostics (bio-characterization devices), treatments, and drug delivery [3]. It was used as a drug delivery compound because it controls drug release for a prolonged period. It also has

the ability to deliver proteins, peptides, and DNA transporters in gene therapy to its potential in recruiting disabled members [4-6].

Nano-oxide is used in various medical and industrial sectors, for example, in pharmaceuticals and cosmetics usages [7]. Also, it has different types of usages to treat various skin diseases besides its ability to absorb the light of ultraviolet rays. All previous studies gave us evidence that ZnO nanoparticles exhibit anticancer and antibacterial activities. Besides ZnO nanoparticles, leukemia cells were investigated to show that compounds can be drugs besides gene delivery, biosensing, and cancer treatment [8-9]. ZnO is a hopeful and multiple functional inorganic material for a great area implementation. Moreover, it has bio-safe properties which own photo-oxidizing besides photocatalysis effects on chemicals and biological compounds [10-11]. ZnO is a non-toxic substance that has biological and therapeutic importance, so it has been used in the synthesis of most medicines and drugs.

Leukemia is a kind of blood cancer that can be classified depending on the type of mutated precursor cell for example lymphoid or myeloid, and how quickly the disease progresses either acute or chronic.

Accordingly, leukemia can include acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) [12]. Human Leukemia 60 (HL60) is a hematopoietic model system *in-vitro* that has been utilized for a long time to study normal myeloid differentiation and leukemia biology [13]. The aim of this research is to synthesis of nano-carriers that serve as carriers for the transport of leukemia drugs.

## ■ EXPERIMENTAL SECTION

### Materials

The materials used in this study were zinc chloride anhydrous ( $\text{ZnCl}_2$ , 99% purity, Sigma-Aldrich, USA), high-quality absolute ethanol and trimethylamine (99% purity, Fluka), hydrochloric acid (HCl, 37%, Sigma-Aldrich, USA), and the ligand used was synthesized with the same procedure used in the reference [14].

### Instrumentation

The instrumentations used in this study were FTIR spectroscopy (Shimadzu FTIR 8400S), LC-MS (SCIEX 3200 QTRAP), X-ray diffraction spectroscopy (Phillips PANalytical X'Pert), field emission scanning electron microscopy (FESEM Tescan Mira3), transmission electron microscopy (TEM Philips em208s 100 kV), and atomic absorption flame (Analytik Jena NovAA 350).

### Procedure

#### **Synthesis of the complex $[\text{Zn}(\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3\text{S})\text{Cl}_2]\cdot\text{H}_2\text{O}$ and ZnO nanoparticles**

A ligand (0.068 g, 0.000201 mol) was dissolved in ethanol (25 mL) while  $\text{ZnCl}_2$  (0.0273 g, 0.000201 mol) was dissolved in ethanol. The solution of  $\text{ZnCl}_2$  and one drop of trimethylamine were added to the solution of ligand. The mixture was refluxed for 1 h and then cooled the produce of reaction at standard circumstances of 25 °C. The gained precipitate was collected before it filtered and then dried, Yield: 80.64%, m.p decomposition above 350 °C, metal percentage % Calc. (Found). For  $\text{C}_{16}\text{H}_{16}\text{Cl}_2\text{N}_4\text{O}_4\text{SZn}$ :Zn, 13.16 (12.95). After that, the 0.05 g from the complex  $[\text{Zn}(\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3\text{S})\text{Cl}_2]\cdot\text{H}_2\text{O}$  has been dissolved in HCl and  $\text{HNO}_3$  mixture [15]. The solution was heated at 150 °C for 15 min to completely dissolve the

chemicals. The mixture was transferred to a 25 mL volumetric vial and diluted with deionized water. The solution was filtered prior to measurement.

#### **Treatment of leukemia cell line**

The HL-60 cells were grown in 96 flat well micro-titer plates, in a final volume of 200 mL for complete culture medium per each well. The microplate was covered by sterile parafilm with shacked slowly. The plates were incubated at 37 °C in a 5%  $\text{CO}_2$  atmosphere for 24 h. After incubation, the medium was removed and various concentrations of azacytidine drug (12.5, 25, 50, 100, and 200 mg/mL) in loaded ZnO nanoparticles were added to the wells. Plates were incubated at 37 °C in a 5%  $\text{CO}_2$  atmosphere for 24 h as the exposure time. After that, 10 mL of the MTT solution was added to each well. Plates were further incubated at 37 °C in a 5%  $\text{CO}_2$  atmosphere for 4 h. The media were carefully removed and then 100 mL of solubilization solution was added per each well for 5 min.

## ■ RESULTS AND DISCUSSION

### Characterization of the Complex $[\text{Zn}(\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3\text{S})\text{Cl}_2]\cdot\text{H}_2\text{O}$

The FTIR spectrum complex exhibited an occurrence of shifting in the vibration of stretching for C=O from  $1667\text{ cm}^{-1}$  in ligand to  $1617\text{ cm}^{-1}$  in complex and azomethine group from  $1596\text{ cm}^{-1}$  in ligand to  $1600\text{ cm}^{-1}$  in complex, which was a good proof on the coordination of ligand and the metal ion from the nitrogen atom of an azomethine group and oxygen atom of the carbonyl. On the other hand, a new band appeared with a weak intensity at  $533\text{ cm}^{-1}$  refers to M-N stretching vibration, and M-O appeared at  $462\text{ cm}^{-1}$  [16-17]. While the band at  $325\text{ cm}^{-1}$  refers to the M-Cl [18].

UV-Visible spectrum for Zn(II) complex was characterized and displayed two peaks around 287 and 339 nm, which resulted from  $\pi$  to  $\pi^*$  and appeared as the third peak at 358 nm, referring to the n to  $\pi^*$  transition. Also displayed a new peak at 421 nm, referring to charge transfer, but some shifting besides turning in the form of the bands were contrasted with bands for the free ligand that appeared at 284 and 363 nm, resulting from  $\pi$  to  $\pi^*$

and  $n$  to  $\pi^*$  transition, respectively. These results became proof of the coordination link between the active site atom for the ligand and the transition metal ion. The spectrum of Zn(II) complex was illustrated by not finding the visible absorption band because of the absence of  $d$  to  $d$ , which can be referred to as the full saturation of  $d$  shell ( $d^{10}$ ). For the same reason, the prepared Zn(II) complex has diamagnetic properties and conductivity data for dissolved samples in DMSO solvent at room temperature displayed that it was not an ionic compound [19-20].

The liquid-chromatography mass spectrum of the complex, Fig. 1 showed a peak,  $m/z = 496.1$  g/mol assigned to the molecular of the complex that confirms the suggested structure  $[\text{Zn}(\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3\text{S})\text{Cl}_2] \cdot \text{H}_2\text{O}$ , where conformable approximately with the theoretical calculation that equal 496.7 g/mol.

## Investigation of the ZnO Nanoparticles

### X-ray diffraction

X-ray diffraction (XRD) is a technique that aims to describe crystalline materials and provide information about the structure and characteristic appearance such as average grain size, crystallinity, defects of crystals as well as identifying the different chemical phases that may be present in the sample. The diffractogram was compared with the standards in the database of the International Center for Diffraction Information.

By analyzing the XRD of the prepared nanoparticles of zinc metal, the sharply appearing peaks were

determined (Fig. 2), as it was noted that 6 distinct and different. Diffraction peaks appeared at the  $2\theta$  angles, with the match card reference card in the international database (JCDPS Card No:1451). The MDI Jade is sacrificed at the (tops) and collected, which is viewed in Table 1. We focused on each hkl as described to identify the structure of ZnO nanoparticles. The XRD results of prepared ZnO nanoparticles showed a hexagonal crystal structure of type (Wurtzite) belonging to the space group P36 mc with the next trellis constants:  $a = b = 3.2498 \text{ \AA}$ ,  $c = 5.2066 \text{ \AA}$ , also  $\alpha = \beta = 90^\circ$  and  $\gamma = 120^\circ$ .

### The nanoparticle scanning electron microscope (FESEM) and transmission electron microscope (TEM)

FESEM's purpose is to analyze the constitutional morphological qualities of the surface of the prepared

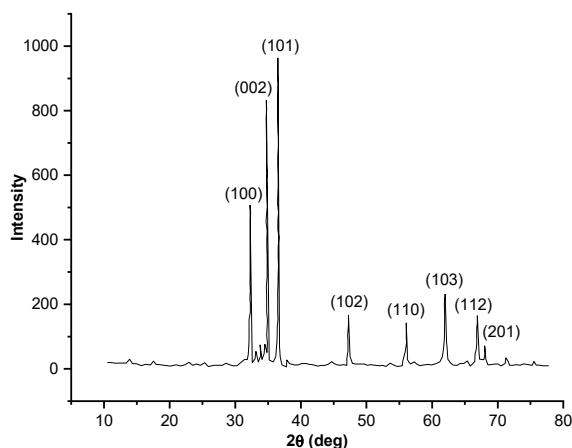


Fig 2. The spectrum of XRD for the ZnO nanoparticles

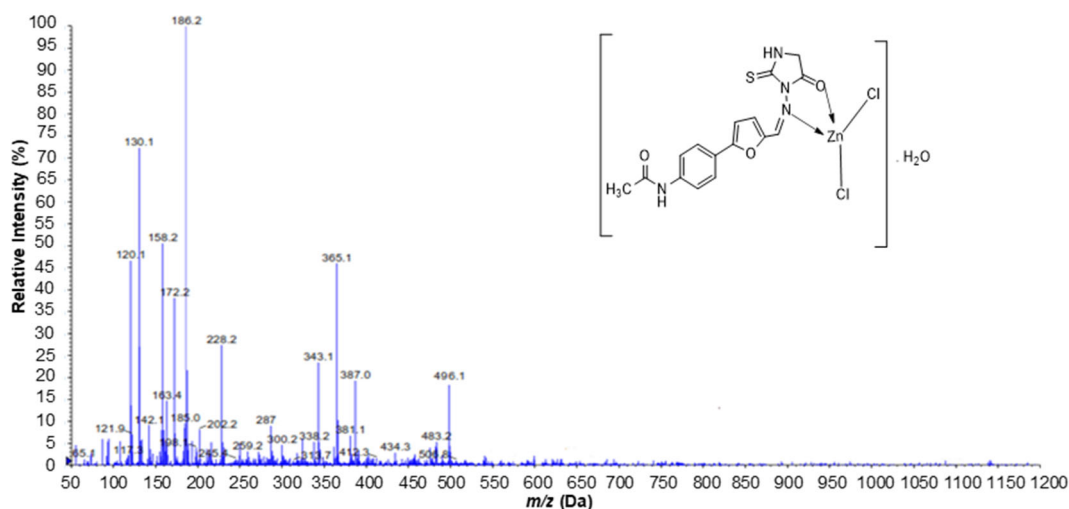
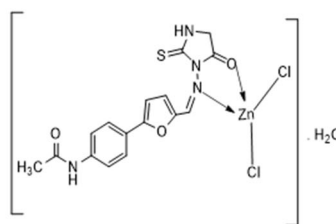
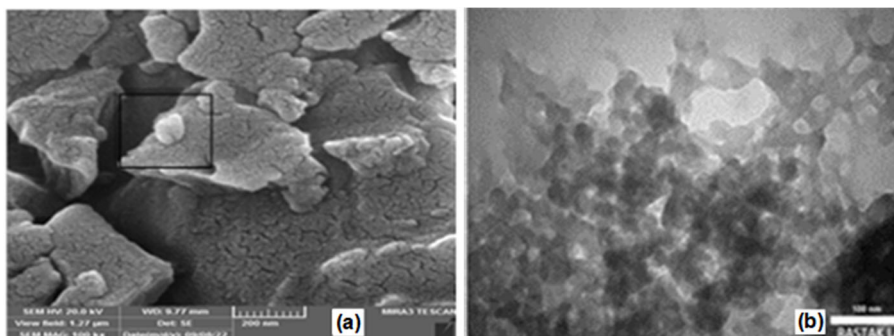


Fig 1. LC-MS of  $[\text{Zn}(\text{C}_{16}\text{H}_{14}\text{N}_4\text{O}_3\text{S})\text{Cl}_2] \cdot \text{H}_2\text{O}$



**Table 1.** The angle  $2\theta$  and crystalline levels for ZnO nanoparticles

Angles ( $2\theta$ )	31.99	34.84	36.20	47.14	55.97	61.95	66.94	67.96
Crystal levels (hkl)	100	002	101	102	110	103	112	201

**Fig 3.** (a) The FESEM images and (b) TEM image of ZnO nanoparticles

nanoparticles after being installed on glass slides and imaging. There is 100 nm in size of ZnO nanoparticles. The microimage did not show them as a clear in high magnification image, however, we saw there were composed nanoparticles thus we referred to it by a small text box on the FESEM original picture for the ZnO nanoparticles which it referred to it in Fig. 3(a).

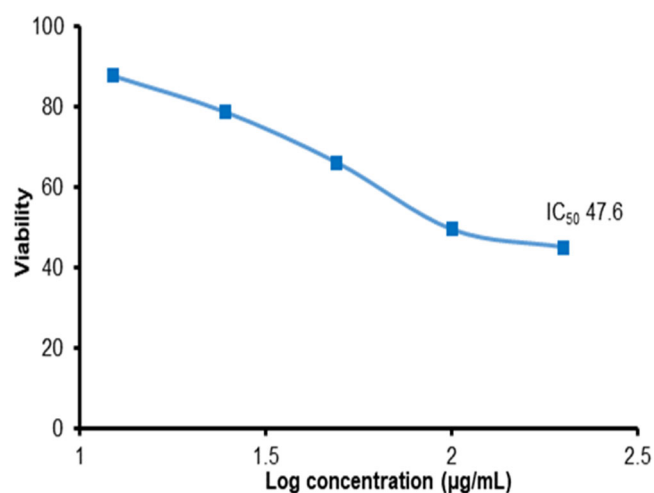
The TEM technology gives a clear, high-resolution, magnified and three-dimensional image of the surfaces of nanoparticles that was examined by a TEM, where an image of the prepared ZnO nanoparticles with a size of 100 nm was shown in Fig. 3(b).

### The Route Treatment of Leukemia Cell Line Using ZnO Nanoparticles as a Drug Transporter: Rules for Guiding Treatment

To direct the drug to cancer cells using nanotechnology accurately, the study concluded that this is achieved through 3 main rules, the first of which is that the dose of chemotherapy is placed in nano-carriers, which are nanomaterials that are used as a drug transport unit to direct it to cancer cells only [9,21]. The second is that there are so-called “chemical ligands” on the surface of these vectors, and their function is to identify the third element in the process, which is the “receptors” that are present in a large amount on the surface of cancerous cells, but it is not present on healthy ones. When the ligands and receptors unite together, the drug is emptied into the cancer cells with extreme precision, without reaching the

**Table 2.** Mean value of the HL-60 concentration

Drug concentration	HL-60	
	Mean	SD
200.00	44.98	6.45
100.00	49.42	4.48
50.00	66.09	1.97
25.00	78.67	1.58
12.50	87.62	2.95

**Fig 4.** The curve measurement of effectiveness against HL-60

healthy cells [22]. In this research, the ZnO nanoparticles have been used as a drug transport unit against leukemia as shown in Table 2. Where it gave clear effectiveness and effect as the concentration of drug-loaded on ZnO nanoparticles increased, as shown

in Fig. 4. The curve was drawn between the log of concentration on the x-axis and the survival rate of cancer cells on the y-axis, where the line of leukemia represented the HL-60 cell line. The test was conducted on cancer cells, and the result was a decrease in the percentage of cancer cells with an increase in the concentrations of the drug, where the results obtained, which is the highest table, show that the ratio has been reduced to 50%.

## ■ CONCLUSION

The ZnO nanoparticles were synthesized from Zn(II) tetrahedral complex with HCl and HNO<sub>3</sub> and characterized. The ZnO nanoparticles were found in 100 nm size and they can be used as a drug carrier to treat leukemia after combining it with drugs, where the ratio has been reduced to 50%.

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## ■ REFERENCES

- [1] Iqbal, A., Iqbal, K., Qin, W., and Mateen, M., 2019, Recent revolutions in nanoscience and nanotechnology with its application's, *Adv. Nanosci. Nanotechnol.*, 3 (3), 1–6.
- [2] Huang, C., Chen, X., Xue, Z., and Wang, T., 2020, Effect of structure: A new insight into nanoparticle assemblies from inanimate to animate, *Sci. Adv.*, 6 (20), eaba1321.
- [3] Nazeruddin, G.M., Prasad, S.R., Shaikh, Y.I., and Prasad, N.R., 2015, A brief review: Science at nanoscale, *Int. J. Nanomater. Nanostruct.*, 1 (1), 16–32.
- [4] Richards, D.A., Maruani, A., and Chudasama, V., 2017, Antibody fragments as nanoparticle targeting ligands: A step in the light direction, *Chem. Sci.*, 8 (1), 63–77.
- [5] Pudlarz, A., and Szemraj, J., 2018, Nanoparticles as carriers of proteins, peptides and other therapeutic molecules, *Open Life Sci.*, 13, 285–298.
- [6] Lee, M., and Kim, S., 2005, Polyethylene glycol-conjugated copolymers for plasmid DNA delivery, *Pharm. Res.*, 22 (1), 1–10.
- [7] Hahn, Y.B., 2011, Zinc oxide nanostructures and their applications, *Korean J. Chem. Eng.*, 28 (9), 1797–1813.
- [8] Mirzaei, H., and Darroudi, M., 2017, Zinc oxide nanoparticles: Biological synthesis and biomedical applications, *Ceram. Int.*, 43 (1, Part B), 907–914.
- [9] Zhang, Y., Nayak, T.R., Hong, H., and Cai, W., 2013, Biomedical applications of zinc oxide nanomaterials, *Curr. Mol. Med.*, 13 (10), 1633–1645.
- [10] Mohd Yusof, H., Mohamad, R., Zaidan, U.H., and Abdul Rahman, N.A., 2019, Microbial synthesis of zinc oxide nanoparticles and their potential application as an antimicrobial agent and a feed supplement in animal industry: A review, *J. Anim. Sci. Biotechnol.*, 10 (1), 57.
- [11] Sirelkhatim, A., Mahmud, S., Seeni, A., Mohamad Kaus, N.H., Ann, L.C., Mohd Bakhori, S.K., Hasan, H., and Mohamad, D., 2015, Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism, *Nano-Micro Lett.*, 7 (3), 219–242.
- [12] Dozzo, A., Galvin, A., Shin, J.W., Scalia, S., O'Driscoll, C.M., and Ryan, K.B., 2023, Modelling acute myeloid leukemia (AML): What's new? A transition from the classical to the modern, *Drug Delivery Transl. Res.*, 13 (8), 2110–2141.
- [13] Basu, J., Madhulika, S., Murmu, K.C., Mohanty, S., Samal, P., Das, A., Mahapatra, S., Saha, S., Sinha, I., and Prasad, P., 2023, Molecular and epigenetic alterations in normal and malignant myelopoiesis in human leukemia 60 (HL60) promyelocytic cell line model, *Front. Cell Dev. Biol.*, 11, 1060537.
- [14] Ward, H.A., Musa, T.M., and Nasif, Z.N., 2022, Synthesis and Characterization of some transition metals complexes with new ligand azo imidazole derivative, *Al-Mustansiriyah J. Sci.*, 33 (2), 31–38.

- [15] Budavari, S., 1996, *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals*, 12<sup>th</sup> Ed., Merck, Whitehouse Station, New Jersey.
- [16] Numan, A.T., Sanak, K.A., Atiyah, E.M., and Sadiq, S.A., 2015, Synthesis and characterization of new bidentate chalcone ligand type (NO) and its Mn<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup> and Cu<sup>II</sup> complexes with study of their antibacterial activity, *Diyala J. Pure Sci.*, 11 (3), 25–42.
- [17] Al-Shemary, R.K.R., 2017, Microwave preparation, spectral studies and antimicrobial activities evaluation of Mn<sup>(II)</sup>, Ni<sup>(II)</sup>, Hg<sup>(II)</sup>, Co<sup>(II)</sup> and Cu<sup>(II)</sup> complexes with Schiff base ligand, *Ibn Al-Haitham J. Pure Appl. Sci.*, 30 (3), 58–67.
- [18] Bader, A.T., Al-Abdaly, B.I., and Jassim I.K., 2019, Synthesis and characterization new metal complexes of heterocyclic units and study antibacterial and antifungal, *J. Pharm. Sci. Res.*, 11 (5), 2062–2073
- [19] Alias, M.F., and Seewan, A.N., 2013, Synthesis, spectral study, theoretical treatment and biological activity of some transition metal complexes with 2-amino acetic acid-6-chloro benzothiazole, *Diyala J. Pure Sci.*, 9 (4), 93–103.
- [20] Lateef, H.S., Jarullah, A.A., and Faraj, F.L., 2019, Cytotoxicity effecting of new ligand (LCl) and it's complexes on a breast cancer, *Int. J. Pharm. Res.*, 11 (4), 1–10.
- [21] Krug, H.F., and Wick, P., 2011, Nano toxicology: An interdisciplinary challenge, *Angew. Chem., Int. Ed.*, 50 (6), 1260–1278.
- [22] Kaur, G., Kaur, P., and Kaur, R., 2019, Quinoline: Its synthesis and pharmacological activities, *Pramana Res. J.*, 9 (6), 1718–1748.