Ministry of Higher Education and Scientific Research
Babylon University
Collage of pharmacy

Synthesis of ZnO Nanoparticles and loaded on Doxorubicin

Research project

Provided to Babylon University as part of the requirements for obtaining a bachelor's degree for the Collage of pharmacy

By

Noora Gheni Hameed
Mariam Qasim Ali
Russul Abbas Heydar

Supervisor
Assist. Prof. Dr. Fatima Al-Zahraa jabbar

1444 **2023**

بسم الله الرحمن الرحيم

يَا أَيُّهَا الَّذِينَ آمَنُوا كُونُوا قَوَّامِينَ لِلَّهِ شُهَدَاءَ بِالْقِسْطِ وَلا يَجْرِمَنَّكُمْ شَنَآنُ قَوْمٍ عَلَى أَلَّا تَعْدِلُوا اعْدِلُوا هُوَ أَقْرَبُ لِلتَّقْوَى وَاتَّقُوا اللَّهَ إِنَّ اللَّهَ خَبِيرٌ بِمَا تَعْمَلُونَ

صدق الله العظيم
الآية 8 من سورة المائدة

الإهـداء

الى خالق اللوح والقلم وبارئ الذر والنسم وخالق كل شيئ من العدم الى القاف والنون وكان خير الرُسل وما كادوا ليزلقوه بأبصارهم إلا هو ذكر للعالمين .. نبي الرحمة

الى تراجمة وحي الله ومهبط سره

الى السادات الاطهار وعروته الوثقى ..اهل بيت النبوه الى مراد قلبي .. الأقرب لي من نفسي

المغيب عن الأبصار والكامن بعين البصيرة

كلماتي المسطرة ترجمان عنائي للوصول إليك بزادي العلمي.. صاحب العصر والزمان

لعلي أفي تلك الأبوه حقها ، وإن كان لايوفى بكيل ولا وزن ، فأعظم مجدي انك لي أب .. ابي الحبيب

الى وطني الأول ومدرستي الاولى .. الى تلك الحبيبة ذات القلب النقي والنظرة الدافئة الى من تفتش سعادتها في سعادتي .. امي الحبيبه

الى من اشاركهم لحظاتي .. الى من يفرحون لنجاحي وكانه نجاحهم اخوتي بكل حب اهديكم هذه الجهد المتواضع

شكر وتقدير

أحمد الله حمداً كثيراً، وأصلِّي وأسلّم على سيدنا وشفيعنا (محمد) وعلى آله الطيبين الطاهرين وأصحابه المنتجبين على إنجاز هذا الجهد المتواضع.

ولا يسعني في هذا المقام إلا أن أتقدم بجزيل شكري وعظيم امتناني إلى الدكتوره (فاطمة الزهراء جبار) في جامعة بابل كلية الصيدلة الإشراف على بحثي أولاً، ولما أبداه من رعاية ونصح وتوجيه وتصويب ثانياً، حيث كان لسديد آرائه وقيمة ملاحظاته اليد الطولى والكأس المعلى في إظهار هذا البحث بالصورة التي هي عليه الآن شكلاً وموضوعاً فجزاه الله عنا خير جزاء وأمد في عمره.

كما أتقدم بجزيل شكري وامتناني إلى أساتذة جامعة بابل كلية الصيدلة لرعايتهم واهتمامهم وحرصهم العالي على إنجاز متطلبات البكالوريوس بنجاح ودقة.

كما أتقدم بشكري وتقديري إلى كل من ساعدني في إنجاز هذا البحث وأمدني بالمصادر وعززني بالمراجع.

List of Content

Chapter one

1. Introduction:

Nanotechnology is the science that deals with a matter at dimensions in Nano-scale. This science studies synthesis, characterization, and applications of materials on a Nano-scale [1]. Nano-materials was defined as those materials, which have size but 1-100 nm dimension. Nano-materials are of typically tremendous interest [2]. The chemical and physical properties of Nano-materials show unique properties compared to the bulk metals of the same components such as their melting temperature, color, charge capacity, and magnetic properties [3].

Nano materials were classified as zero dimensional, one dimensional, two dimensional, three-dimensional nanostructures [4].

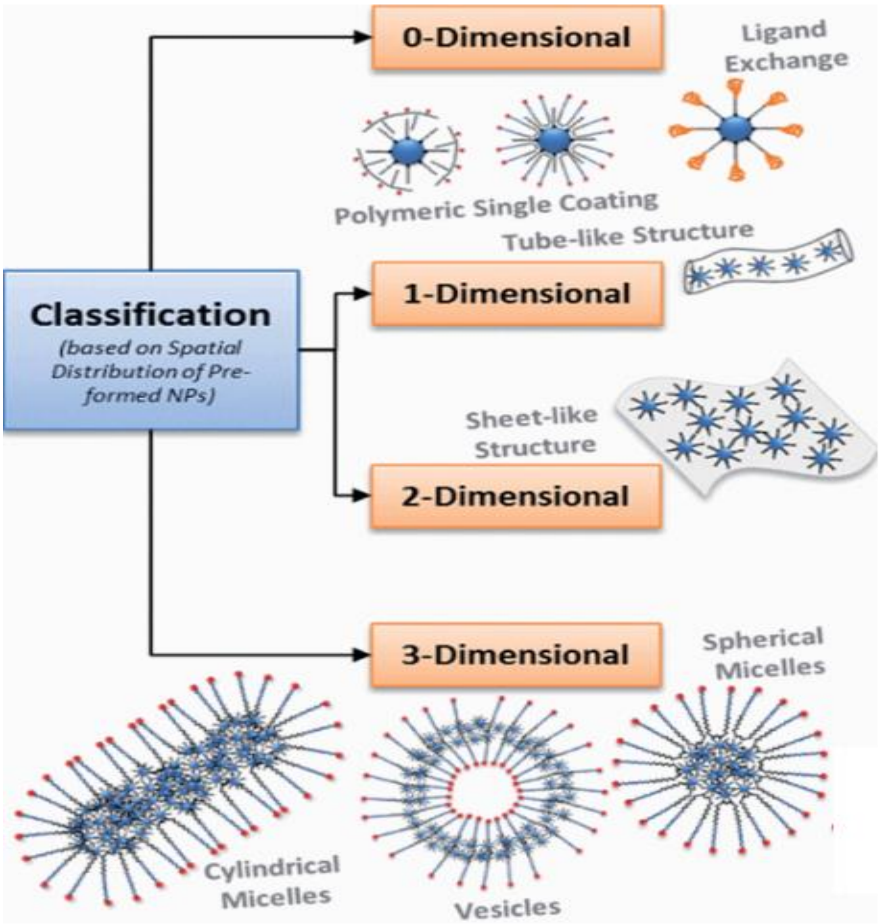


Figure (1-1): classification of Nano- materials

1-1. Nanoparticles Types

Nanoparticles types are commonly divided in two main groups: organic and inorganic. The first group includes micelles, dendrimers, liposomes, hybrid and compact polymeric nanoparticles. The second group includes fullerenes, quantum dots, silica and metal nanoparticles [5].

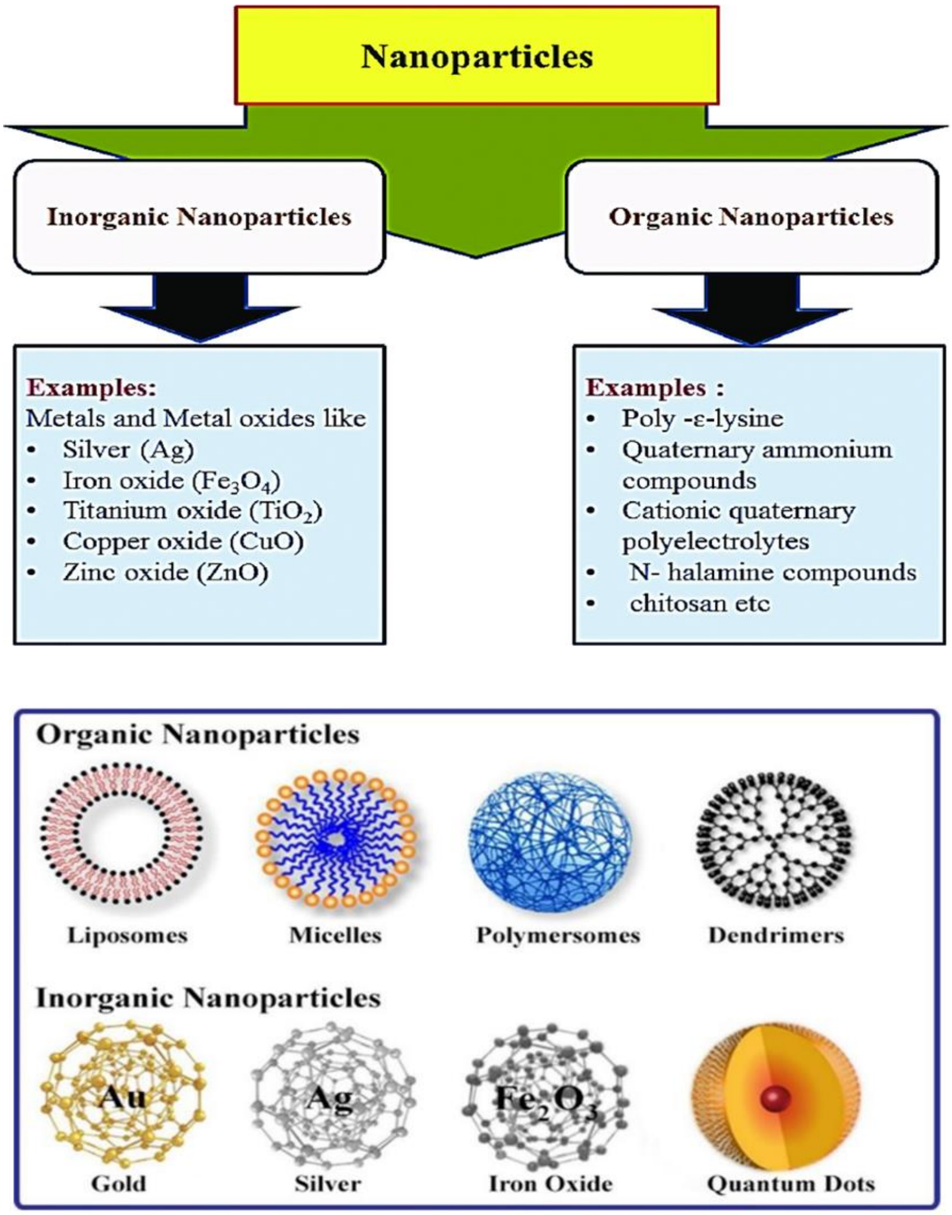


Figure (1-2): Nanoparticles types.

1-2. Zinc oxide

Organic compound zinc oxide, also known as zincite, is an insoluble white powder. Zinc oxide is a wide-band gap semiconductor made by the II-VI group. Due to the presence of zinc interstitials or oxygen vacancies, its native doping is n-type [6].

ZnO has mainly three crystal forms Cubic rock salt (B1), Hexagonal wurtzite (B4), and Cubic zinc blende (B3). Hexagonal wurtzite form is the most common and most stable structure at room temperature [8].

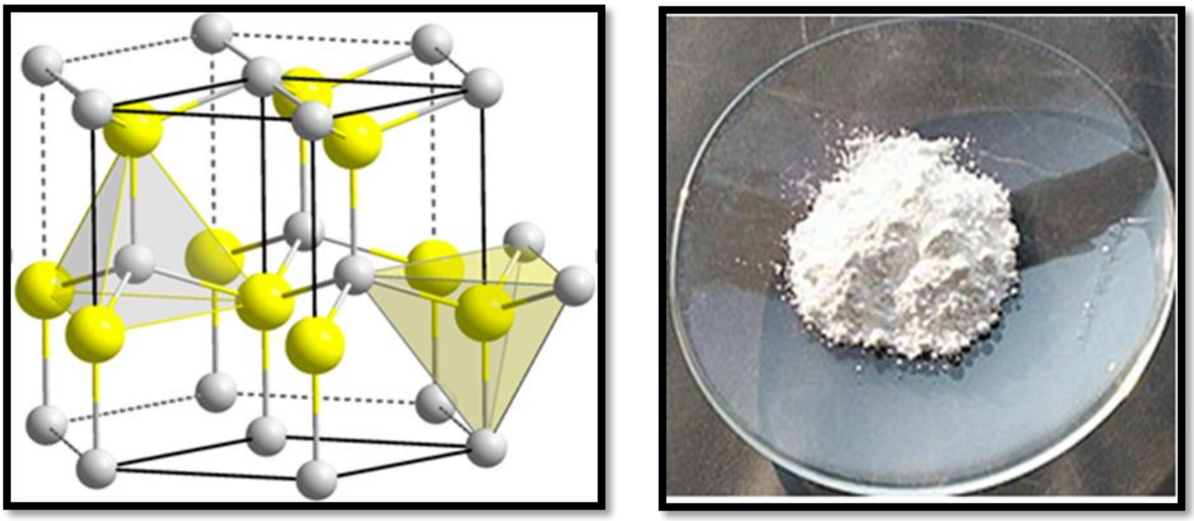


Figure (1-3): The hexagonal wurtzite structure model of ZnO

Some of its other characteristics include high electron mobility, transparency, and room-temperature luminescence. These properties make it ideal for various applications, such as flexible electronics and liquid crystal displays [9].

Table 1: Some of the important properties of ZnO [10].

Properties	Values
Crystal structure	**Rock salt, Zinc blende, and Wurtzite**
Stable phase at 27^{0}C	**Wurtzite**
Band gap (eV)	**3.37 at room temperature**
Electron Mobility ($cm^{2}Vs^{-1}$)	**2.5-300 (Bulk ZnO), 1000 (Single nanowire)**
Exaction Binding Energy	**60 meV**
Density	**5.606 g/cm^{3}**
Refractive Index	**2.0041**
Electron Effective Mass (me)	**0.26**
Relative Dielectric Constant	**8.5**
Melting point	**1975 ^{o}C**
Boiling point	**2360 ^{o}C**
Electron Diffusion Coefficient	**5.2 cm^{2} s^{-1} (Bulk ZnO), 1.7 × 10-7 cm^{2} s^{-1} (Particulate Film)**
Static Dielectric Constant	**8.656**

1-3. Characterization

The structure of ZnO NPs could be revealed by X-ray diffraction (XRD), field emission scanning electron microscope (FESEM), Fourier Transform Infrared Spectroscopy FTIR, Electronic Data Systems (EDS) and Atomic Force Microscope (AFM) [11].

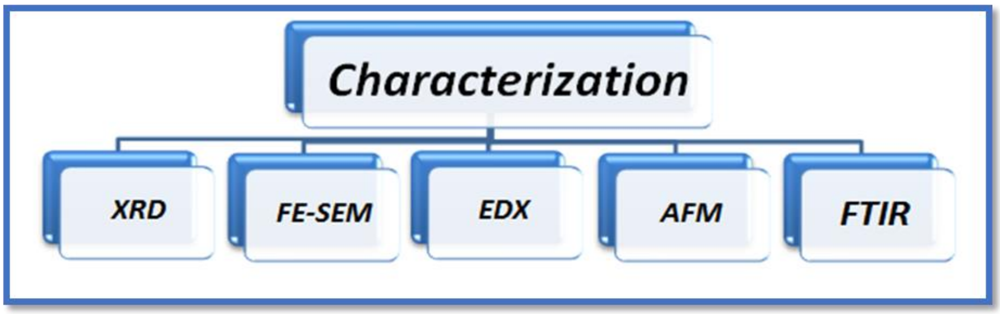


Figure (1-4): Analytical techniques for ZnO NPs characterization

1-3-1. XRD:

X-ray diffraction (XRD) was used to determine the crystalline structure and grain sizes of the synthesized ZnO NPs, The technique provides valuable information of structure, different phases and preferred crystal orientation [12]

1-3-2. FESEM:

Field Emission Scanning Electron Microscopy technique can provide very high resolution and 3-dimensional images of a sample surface. FE-SEM used for studying the morphology of pure and metaled ZnO NPs .This technique has three-dimensional representation, high resolution, and clear images [13].

1-3-3. FTIR:

Is an analytical technique used to identify organic, polymeric, and, in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties, FTIR data show the peaks between 4000 and 400 cm^{-1} of the functional groups of –OH, C-O, –C-H-, and Zn-O bonds [14].

1-3-4. Energy-Dispersive Spectroscopy (EDS):

As one of the widely used analytical methods for the analysis of elemental composition of solid matter, EDS is a common technique for the analysis of elemental composition of a specimen.

This technique give Information of relative or absolute concentration of all elements can be determined.

The purity of nanoparticles was also determined energy-dispersive X-ray spectroscopy (EDS) has recently gained significant importance regarding its application to the chemical analysis of nanoparticles, especially in conjunction with the use of a scanning electron microscope (SEM) and the use of the transmission operation mode of SEM (STEM-in-SEM) [15].

1-3-5. AFM:

Atomic force microscopy (AFM) analysis is a commonly used technique for the determination of the size of NPs. AFM gives us insight about the roughness of ZnO NPs The grain size of NPs was observed from tip-corrected AFM measurements and the shape of ZnO NPs were determined [16].

1-4. Applications of ZnO NPs

ZnO NPs can be used as an additive in various products, such as cosmetics, plastics, ceramics, glass, rubbers, food supplements, paints, batteries, ferrites, and first-aid tapes. Although it can be produced naturally, most zinc oxide is synthetically produced [17].

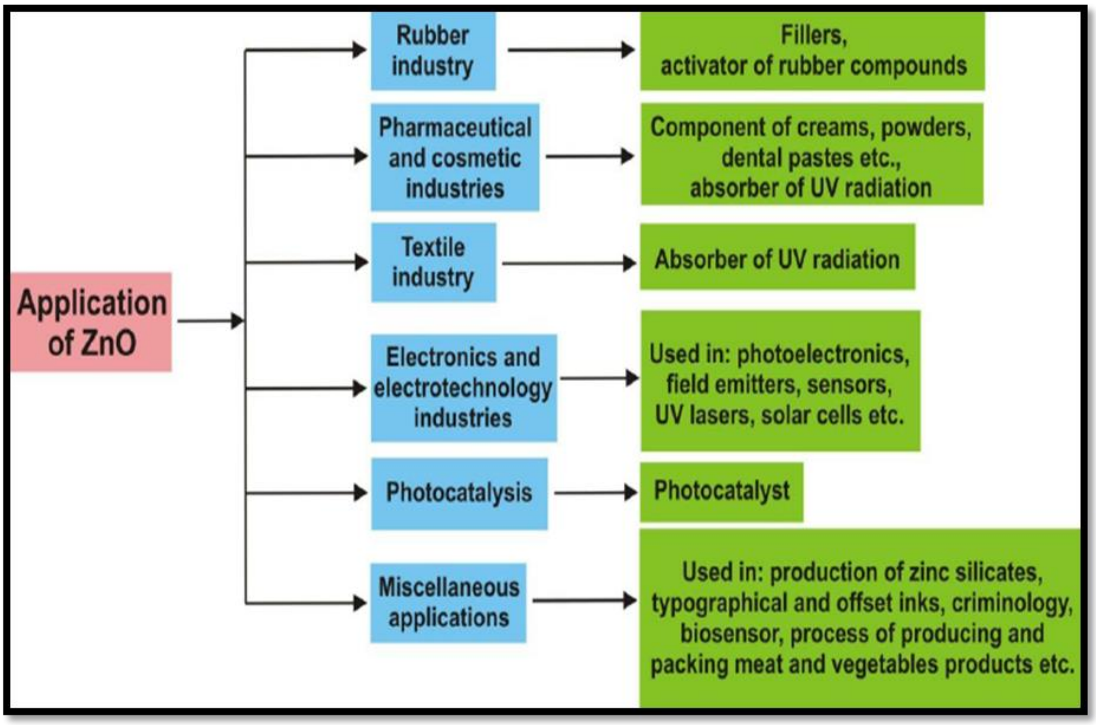


Figure (1-5): Applications of ZnO NPs

1-5. Medical Applications of ZnO NPs [18]

1. Anticancer activity
2. Gene delivery
3. Antibacterial Activity
4. ZnO NPs for Diabetes Treatment
5. Anti-Inflammatory Activity
6. Biomedical imaging
7. Drug delivery
8. Bio-sensor

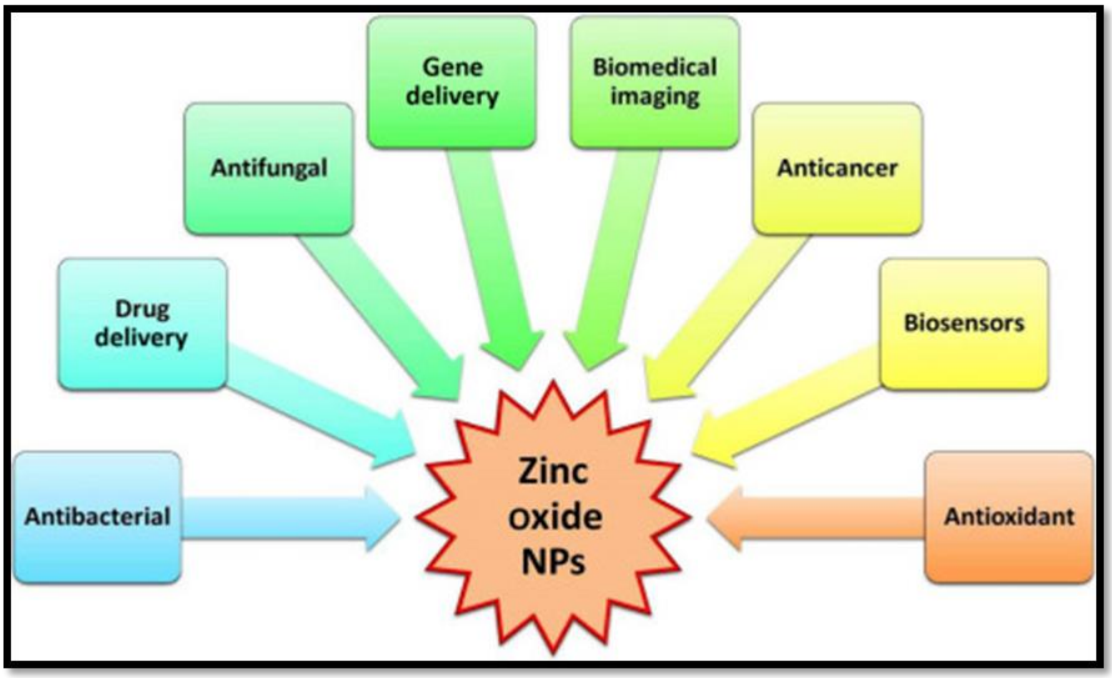


Figure (1-6): Medical Applications of ZnO NPs.

1-6. Doxorubicin:

Doxorubicin is a chemotherapy drug and is a treatment for many different types of cancer. Doxorubicin is also known as Adriamycin [18].

Doxorubicin may be used to treat soft tissue and bone sarcomas and cancers of the breast, ovary, bladder, and thyroid. It is also used to treat acute lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, and small cell lung cancer. Doxorubicin is given by injection into a vein [19].

Figure (1-7): Chemical formula of DOX

1-7. Side effects of Doxorubicin [20]

- Side effects:
  - Nausea or vomiting
  - Pain at injection site
  - Temporary Low blood counts of white & red blood cells and platelets
  - Mouth sores
  - Alopecia (hair loss on scalp or body)
  - Eyes watering (first week of treatment)
  - Urine color red, red-brown, orange, or pink in first 1-2 days after dose administered

Chapter 2

2-1. Chemical Materials

The employed chemicals in this work are listed in Table 2-1. All the used chemicals were employed without further purification.

Table 2.1: Chemicals.

No	Chemicals	Company supplied	Purity %
1	Zinc nitrate ($Zn(NO_3)_2$)	S.D. Fine-chem. Ltd., India	99
2	Sodium chloride (NaCl)	Barcelona-spain	99
3	Sodium hydroxide (NaOH)	Alfa, Aesar	99
4	Ethanol	Alfa, Aesar	99.93
5	Doxorubicin	C27H29NO11	99

2-2. Instruments Analysis

Table (2-2): Instruments used in this work.

No	Apparatus	Company	Position of instrument
1	X-Ray Diffraction Spectroscopy	D5000 XRD6000, Shimadzu,Japan	Bagdad university, chemistry Department
2	Sonication bath	FALC-Italy	College of science, University of Babylon
3	Electrical Magnetic stirrer	Heidolph- Mr Hei-Standard- Germany.	College of science, University of Babylon
4	Centrifuge	EBA 20 Hettich	College of Pharmacy, University of Babylon
5	Fourier Transform Infrared spectrophotometer.	8400S, Shimadzu-Japan.	College of science, University of Babylon
6	Atomic force microscopy (AFM).	Sartorius Arium 611	College of science, University of Bagdad
7	furnaces	XIN YOO electronic components co. Ltd.	College of science , University of Babylon
8	pH meter.	Hanna- Rommana.	College of Pharmacy, University of Babylon
9	Filed Emission Scanning electron microscopy (FE-SEM)	INSPECT S50 FEL (USA)	Sharif University of Technology, Iran, Tehran,
10	Oven	Memmert-Germany.	College of science, University of Babylon
11	Water bath	Stuart- England.	College of science, University of Babylon

2-3. Synthesis of ZnO Nanosheets:

Modified simple reflex precipitation method is briefly summarized in figure (2-1). 5.6 g of Zn (NO3)2 was dissolved in 250 ml of deionized water. Aqueous solution of NaOH (1 M) was drop wise added to the solution under stirring with speed of 1250 rpm at room temperature until pH of the solution reached 13.

Afterward the white suspension was refluxed at about 110 C0 for 3 h. The precipitate was separated and washed two times with deionized water and ethanol to remove the unreacted reagents and dried in an oven at 70C0 for 24 h. Then white powder was crushed by using ceramic mortar .The final product was obtained by calcination of the precipitate at 2000C for 1 h. The reactions for obtaining ZnO NPs are written as the following:

$$Zn(NO_3)_2 + 2NaOH \rightarrow Zn(OH)_2 + 2NaNO_3 --------(2-1)$$

$$Zn(OH)_2 \xrightarrow{reflexing} ZnO + H_2O ------------(2-2)$$

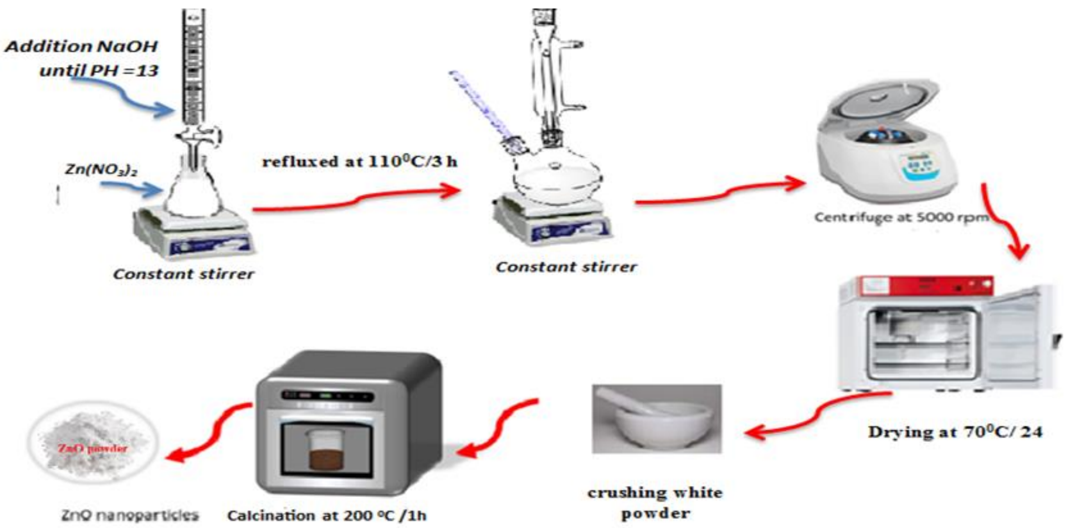


Figure (2-1): Schematic Diagram of the steps used for synthesizing ZnO nanosheets.

2-4. Loading of Doxorubicin on the surface of ZnO nanosheets

Doxorubicin (0.5 mg) was dissolved in 500 mL deionized distilled water and stirred at 600 rpm for 10 min to obtain a transparent solution.

And allow subsequent reaction with dispersed ZnO-NPs (0.1 mg/500 mL) in distilled water of 1:1 volume ratio and sonicated for 15 min. Both solutions were mixed under continuous stirring at 600 rpm for 90 min at room temperature. The solution was then incubated for 60 min at room temperature after centrifugation; the supernatant was discarded, washed with ethanol, and dried in a vacuum oven overnight. Figure 2-2 explained Schematic Diagram of the steps used for loading of Doxorubicin on the surface of ZnO Nano sheets.

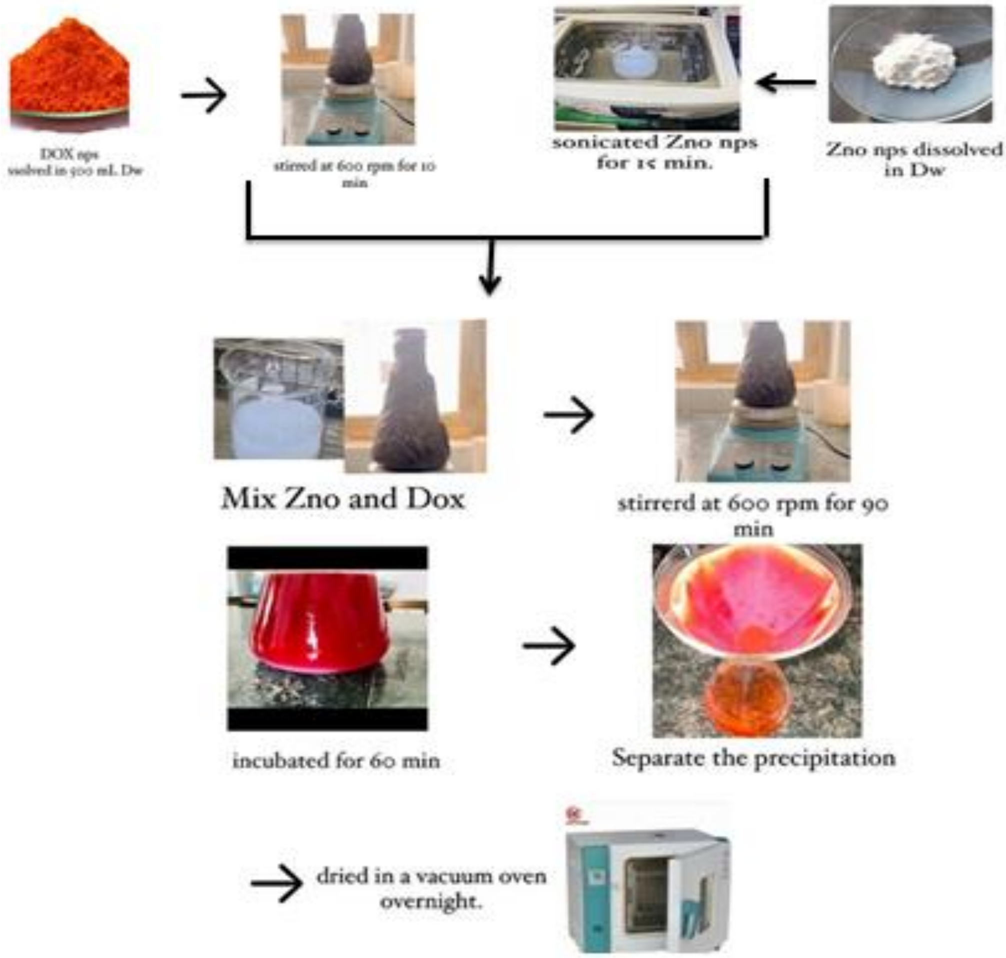


Figure (2-2): Schematic Diagram of the steps used for loading of Doxorubicin on the surface of ZnO Nano sheets.

Chapter 3

Results and Discussion

3. Characterization

3-1. X-ray diffraction analysis

XRD analysis is used to provide insight into the crystalline structure and crystallite size of particles. The XRD peaks at 2θ = 31.8355°, 34.5207°, 36.2871°, 47.5808°, 56.6487°, 62.8917°, 66.4115°, 67.9331°, 69.1842° and 77.0106° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), (201) and (202) crystal planes and hexagonal crystal geometry(20) . The XRD pattern for the synthesized ZnO NPs is shown in Fig (3-1).

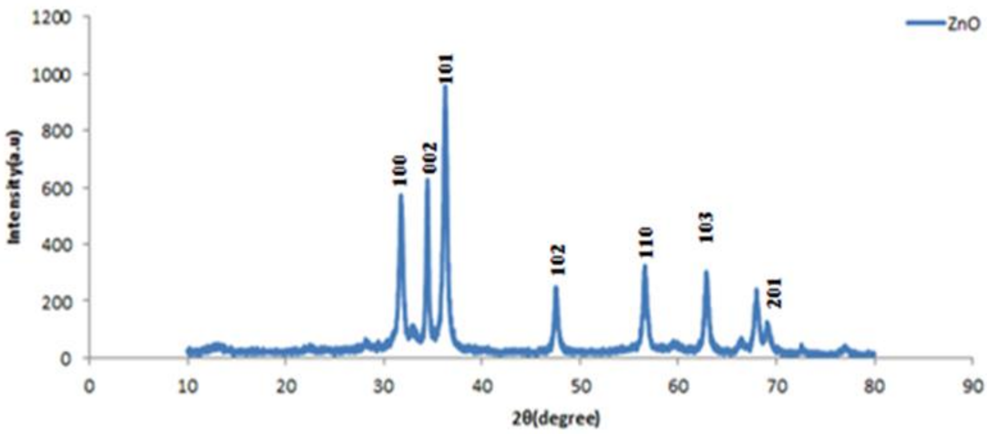


Figure (3-1): XRD pattern of ZnO nanosheets

The average particle size of the NPs was determined by using the Scherrer equation:-

$D=k\lambda/\beta Cos\theta$

where D is the crystallite size of the particle, K represents the Scherrer constant, which is equal to 0.9, λ is the wavelength of light used for diffraction (λ = 1.54 Å), β is the FWHM (full width at half maximum) of the diffraction peak and θ is the angle of reflection(21). The average crystallite sizes (D) in nm were calculated by using Scherrer's formula which estimated to be about 31.077 nm

3-2. Field Emission Scanning Electron Microscopy (FE-SEM)

Field Emission Scanning Electron Microscopy technique used for studying the morphology of pure and metaled ZnO NPs and can provide very high resolution and 3-dimensional images of a sample surface. FE-SEM used for studying the morphology of pure ZnO NPs .This technique has three-dimensional representation, high resolution, and clear images(22). The results of this analysis showed ZnO samples have an individual sheets structure with the particles size ranging between 18.56-41.94 nm. as shown figure (3-2).

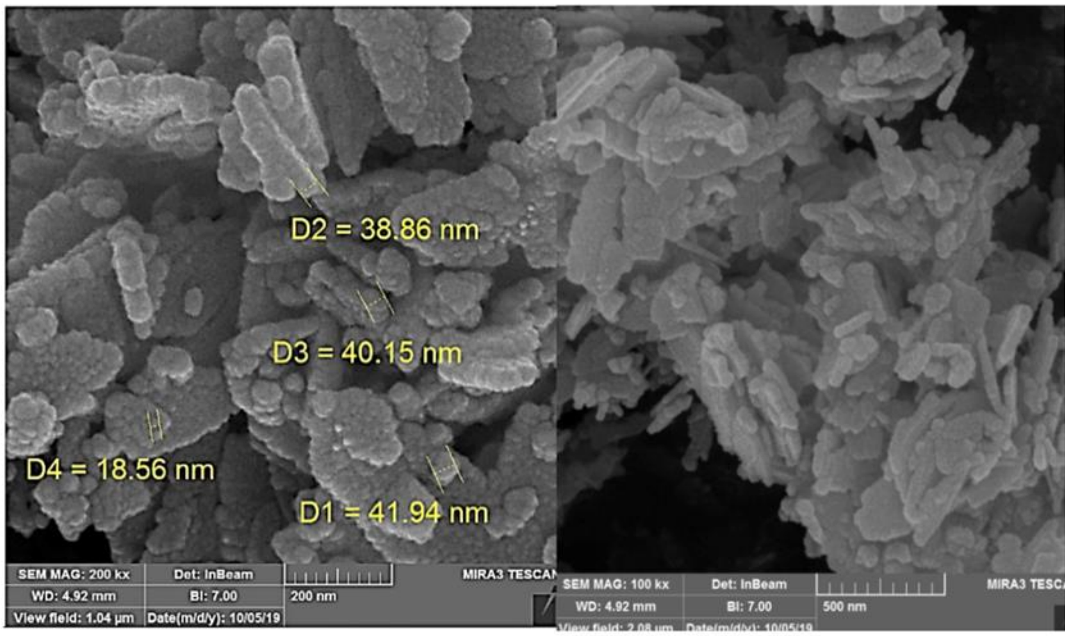
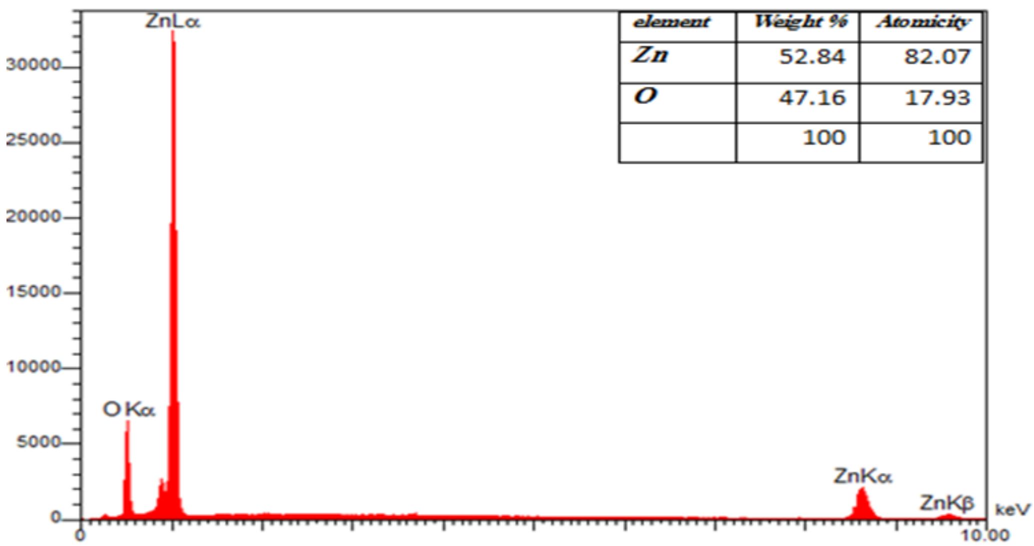


Figure (3-2): FE- SEM images of pure ZnO nanosheets

3-3. Energy-dispersive X-ray Spectroscopy (EDX)

Energy dispersive X-ray spectroscopy (EDX) is a common technique for the analysis of elemental composition of a specimen. This technique give Information of relative or absolute concentration of all elements can be determined The purity of nanoparticles was also determined. Figure 3-3 shows the EDS spectrum of ZnO NPs. FE-EDS was used to determine the elemental composition present in the samples. EDX characterization of ZnO-NPs shows that each element has a specific atomic percentage Zn (82.07%) and O (17.93%). This result has confirmed that the ZnO NPs have high purity(23).



element	Weight %	Atomicity
Zn	52.84	82.07
O	47.16	17.93
	100	100

Figure (3-3): FE- SEM-EDX spectrum of ZnO nanosheets.

3-4. Atomic Force Microscopy (AFM)

The atomic force microscopy was done to identify the topological appearance, the average grain size and other surface parameters of pure and metaled ZnO nanostructures (24). The figure 3-4 shows 2D and 3D AFM images of the sample produced in this work The surface roughness was measured using atomic force microscopy (AFM).The average diameters was obtained are 35.89 nm .

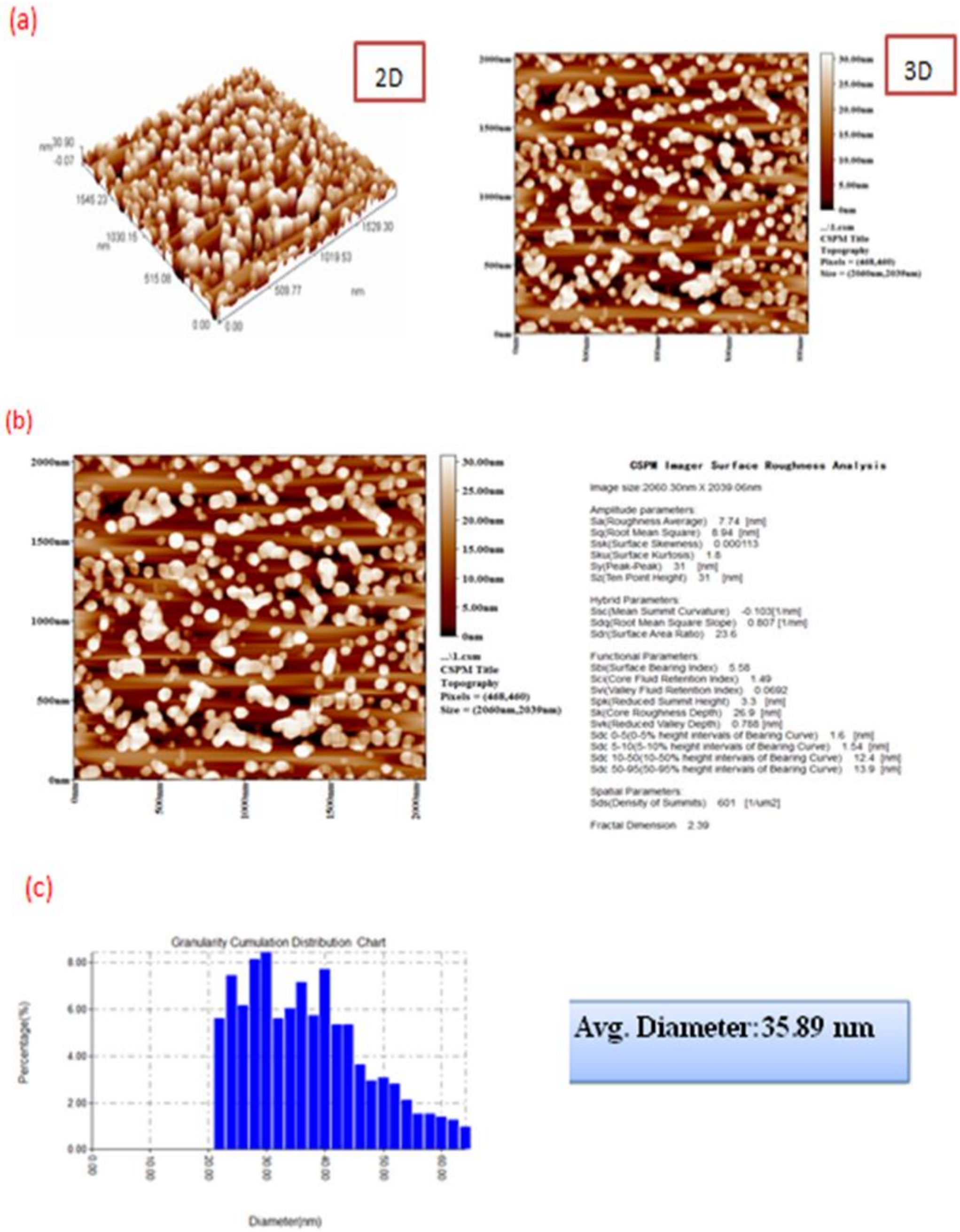


Figure (3-4): (a) 2D and 3D AFM images of the pure ZnO NPs (b) AFM surface roughness parameters of ZnO NPs, (c) AFM cross-section analysis of ZnO NPs.

Fourier Transform Infrared spectra (FTIR):

The FT-IR spectra of ZnO nanoparticles was measured in the range of 4000–400 cm^{-1} presented in Figure 3-5. The broadband around 3500 cm^{-1} assigned to the O-H stretching mode of the hydroxyl group, which represents the presence of a water molecule on the surface of ZnO nanoparticles**.** The two peaks at about ~ 1693 cm^{-1} and ~ 1568 cm^{-1} assigned to symmetric and asymmetric CO_2 stretching absorbed from the air atmosphere and can be neglected. metal oxides generally exhibit absorption bands in the fingerprint region below 1000 cm^{-1}.. The sharp peak observed in the range of 474 to 510 cm^{-1} was attributed to the Zn–O stretching bonds. This result indicates the successful production of ZnO nanoparticles (25).

The FT-IR spectra of DOX shows 3331 N–H stretch 3525 O–H stretch 2935, 2897 C–H stretch 1729 C=O stretch 1617, 1582, 1414 C=C ring stretch 1115, 1073 C–O–C stretch 805, 688 C=H bend, C=C ring bend (26).

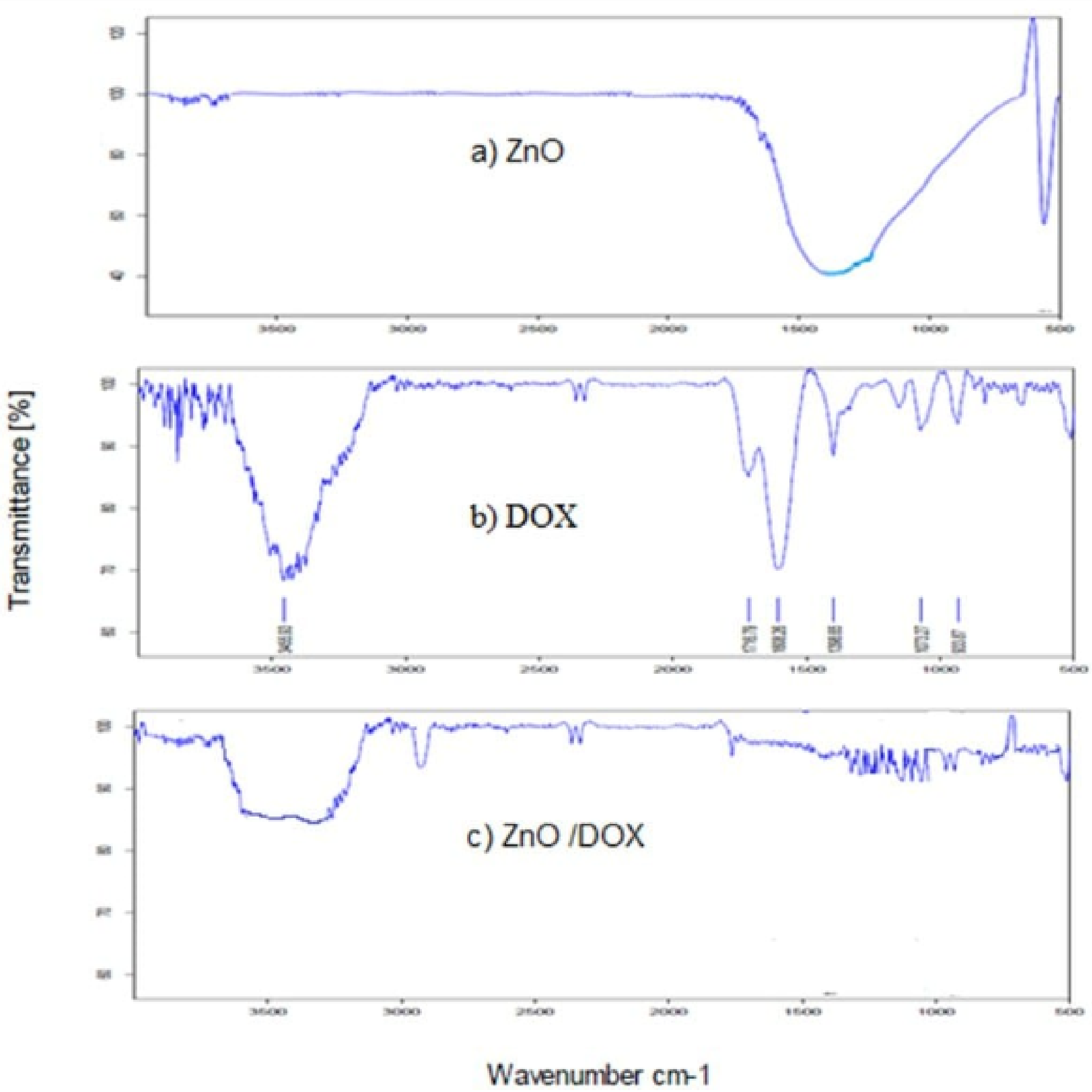


Figure (3-5): FT-IR spectra for ZnO nanosheets.

Fabrication of DOX-ZnO NCs

DOX-wrapped ZnO NCs were fabricated by a facile incubation protocol in this study. Although it is controversial whether or not free Zn2+ ions are a major contributor of cytotoxicity.

ROS generation from ZnO has been regarded as the major cell-death mechanism. To overcome the drawbacks of bare ZnO nanoparticles and improve their functionalities, the surface of ZnO nanoparticles has been engineered (27).

In this study, DOX (as a chemotherapeutic agent) was coated onto the outer surface of ZnO particles (Figure 3-6A). The different colors of DOX solution, ZnO NCs dispersion, and DOX-ZnO NCs dispersion, support the successful coating of ZnO NCs with DOX (Figure 3-6B). By combining DOX and ZnO into the single nanocarrier, the simultaneous arrival of DOX and ZnO in the tumor tissue and their efficient cellular entry may be possible.

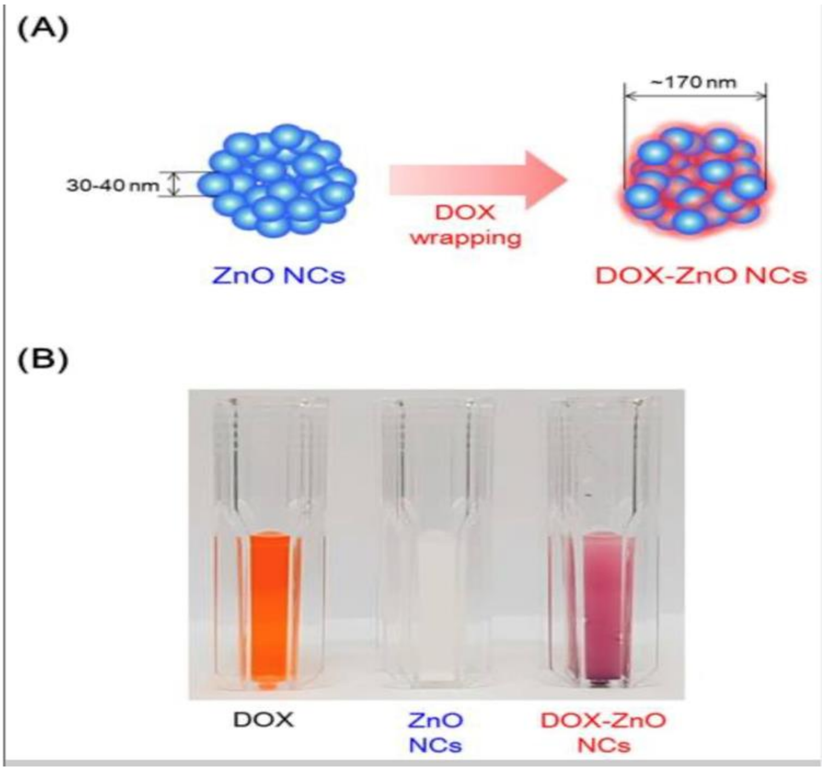


Figure (3-6): The different colors of DOX solution, ZnO NCs dispersion, and DOX-ZnO NCs dispersion(28).

ZnO Nanoparticles for Drug Delivery

Drug-loaded nanostructured materials such as ZnO nanoparticles, carbon nanotubes, mesoporous silica nanoparticles. ZnO is used in current drug delivery systems due to its ease of manufacture, low cost, customizable structure, non-toxicity, high drug-loading capacity, programmable drug release ability, and targeted delivery Porous ZnO structures [28].Various ZnO nanostructures could be employed successfully in loading and targeted delivery of anticancerous drugs [29].

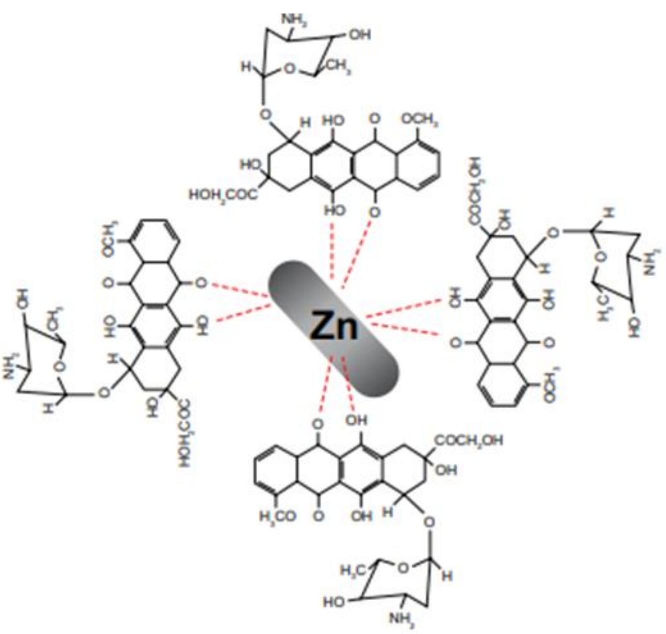


Figure (3-7): Dox loading onto the ZnO nanomaterial in the formation of a Dox-ZnO nanocomplex [30].

Mechanisms of anticancer activity of Dox-ZnO nanocomplexes

The molecular mechanism of anticancer activity induced by the synergistic effect of Dox-ZnO nanocomplexes in the cancer cells. ZnO–DOX composites were taken up by cells, they would be engulfed by the endosomes and lysosomes, so the DOX saturation in the cellular fluid was not reached; that is, more and more ZnO–DOX would be taken up continuously. Finally, the ZnO–DOX composites decomposed in the lysosomes to release high concentrations of DOX molecules, thus exhibiting higher cytotoxicity. Figure 14 schematically illustrates the possible synergizing processes of Dox-ZnO nanocomplexes as an integrated multimodal anticancer therapeutic agent [31].

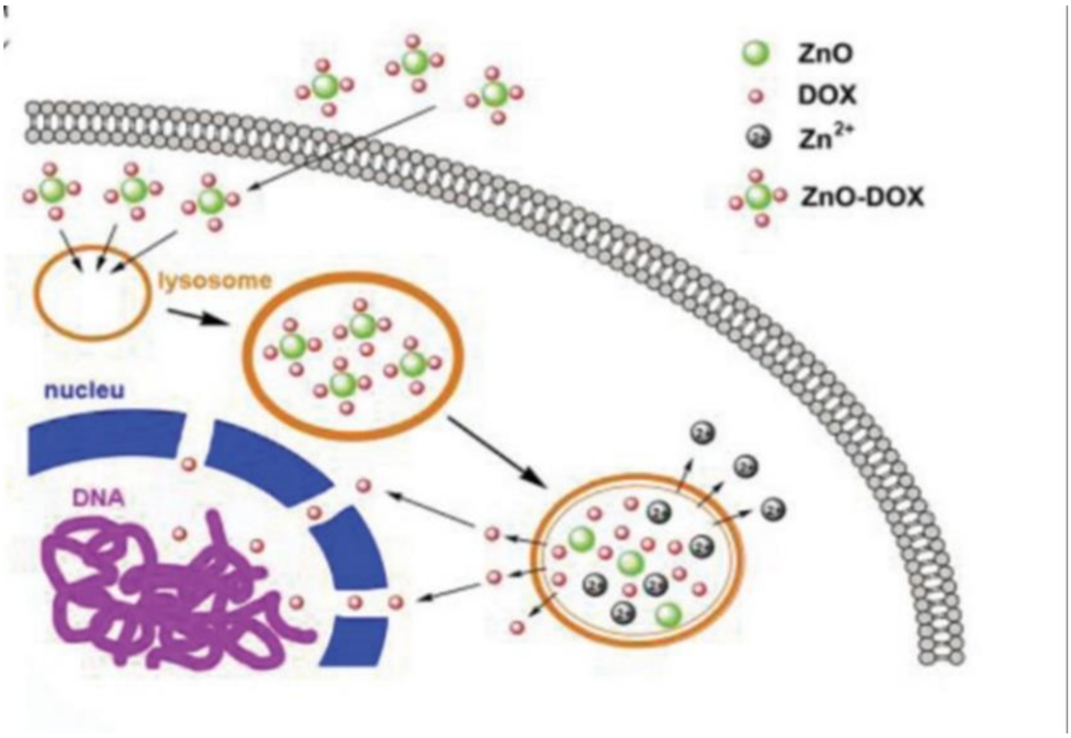


Figure (3-8): Schematic process of the DOX delivery from the ZnO@polymer–DOX composites in the cells [32].

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