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Study effect of nano semiconductor on anticancer activity

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بسم الله الرحمن الرحيم
وعلمك ما لم تكن تعلم
وكان فضل الله عليك عظيما

إهداء

وصلت رحلتنا الجامعية إلى نهايتها بعد تعب ومشقّة

وها نحن ذا نختم بحث تخرّجنا بكل همّة ونشاط ،

نحن ممتنين لكل من كان له فضل في مسيرتنا

وساعدنا ولو باليسير . .

الأبوين ، الأهل ، الأصدقاء والأساتذة المبجلين ..

أهديكم بحث تخرجي .

شكر و تقدير

نحمد الله عز و جل الذي وفقنا في إتمام هذا البحث العلمي ، و الذي ألهنا الصحة و العافية و العزيمة فالحمد لله حمدا كثيرا نتقدم بجزيل الشكر و التقدير إلى :"الدكتورة اسماء هاشم حمادي" و الدكتورة "صبا عبد المنعم حبيب" و "استاذ غسان جاسم محمد " على كل ما قدموا لنا من توجيهات و معلومات قيمة ساهمت في إثراء موضوع دراستنا في جوانبها المختلفة ، كما نتقدم بجزيل الشكر إلى أعضاء لجنة المناقشة الموقرة الذين تفضلوا بقراءة هذا البحث.

Contents:

Abstract:

Despite the emergence of cancer decades ago, it is still one of the most dangerous diseases that cause panic to the patient for several reasons, the sudden attack on the body, its discovery by chance, the difficulty of surviving it, the most important of which are the side effects caused by anti-cancer drugs such as general weakness, loss of appetite, hair and head loss, And thinness, etc., and even psychological damage. But with the advent of the nanotechnology revolution for delivered of semiconductors on drugs, gave hope to survive this disease. For example, zinc oxide is industrially prepared is high purity, low toxicity, and environmentally friendly. In our research, zinc oxide was synthesized by precipitation method by heating it at 60-70 °C as a thermal condition. The optical properties of the product are Nano- homogenous white powder. Instrumental characteristics such as XRD, SEM, and FT-IR proved all optical properties like morphology. Additional, microscopic techniques such as SEM established the formation of multi shaped zinc oxide with an average size of 14-93 nm. XRD characterization was employed to analyzed phases and partials size were in range (28-59.5nm) for ZnO yield.

CHAPTER ONE

INTRODUCTION

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LITERATURE REVIEW

1.1 Introduction:

Nanotechnology is a fast-growing science of nanosized particle growth and consumption, measuring size in the nanometre range. Additionally, nanotechnology is the practice of systemically characterising, manipulating and arranging matter at the nanometre (1 to 100 nm) scale, which has brought about a revolution in science, technology, engineering, drug discovery and therapy (1).

American physicist Richard Feynman is considered the father of nanotechnology. He introduced the ideas and concepts behind nanotech in a 1959 talk titled "There's Plenty of Room at the Bottom." Feynman did not use the term "nanotechnology," but described a process in which scientists would be able to manipulate and control individual atoms and molecules.

Modern nanotechnology truly began in 1981, when the scanning tunneling microscope allowed scientists and engineers to see and manipulate individual atoms (2).

1.2 Applications of the Nanoscience

NPs can be used in variety of application

I - Nanomaterials: Solar Energy Conversion

II - Nanoelectronics

III- Nanomedicine

IV – Nanoneurobiophysics

V - Nanosensors

VI- Electrochemical Sensors

VII- Molecular Modeling Applied to Nanobiosystems

VIII -Transports: New materials with different properties for car industry and aeronautics.

III. Nanotechnology is used in field of medicine for drug delivery, treatment, diagnostic & monitoring techniques, bio sensors, antimicrobial techniques, cell repair and control of the biological system are some of the applications.

In Diabetics: Developed NP containing insulin attached to matrix. Nanotechnology uses in Treatments Heart Diseases This is still under research. NP is a protein produced by translation and used to attach damaged regions of arteries as well asto break blood clots. NPs are tried to direct under magnetic field to deliver proteins to right place in arteries. in eye diseases like diabetic retinopathy, retinoblastoma, retinitis pigmentosa. Treatment of TB required continuous and frequent drug supply to the cells (to improve bioavailability, reduce dosing frequency and drug administration methods in TB treatment).(3)

NP can cross blood brain barrier (BBB) so it can use to deliver drugs to brain tumors ,Alzheimer's disease ,inborn metabolic errors like lysosomal storage disease, infectious diseases and aging.

nanotechnology provides advanced capable of promoting the adhesion and proliferation of stem cells and accelerating stem cell differentiation in a controlled manner in tissue engineering (4).

nanotechnology drug delivery system successfully used in drug delivery in the treatment of cancer, asthma, and hypertension as well diabetics.

The nanoparticulates drug delivery system offers plenty of advantages, improves efficiency and safety by controlling the rate, time and place of release of drug in the body, and improves patient compliance.NP attached drug delivery method leads to improved duration of drug circulation, bio-availability of the drug and control drug releasing at the particular site. Ultimately NP with drug incorporation enhancing the ability to use highly toxic, poorly soluble, unstable drugs. Nanotechnology capable of production biodegradable, biocompatible, targeting and stimulate responsive carriers such as liposomes, nanofabricated materials (fullerenes, carbon) nanotubes, silicon, silica) mental (gold, silver, iron, platinum, quantum dots) and polymers (micelles, dendrimers) (5).

fundamental advantages of nanotechnology for cancer treatment is tumor targeting enhanced permeability and retention (EPR) effect to increase the concentration of nanoparticles (NPs) in the tumor. increase bioavailability, reduce the toxicity of chemotherapy drugs, release hydrophobic or hydrophilic chemotherapy drugs into the bloodstream, and achieve cytotoxic effects against cancer cell (6).

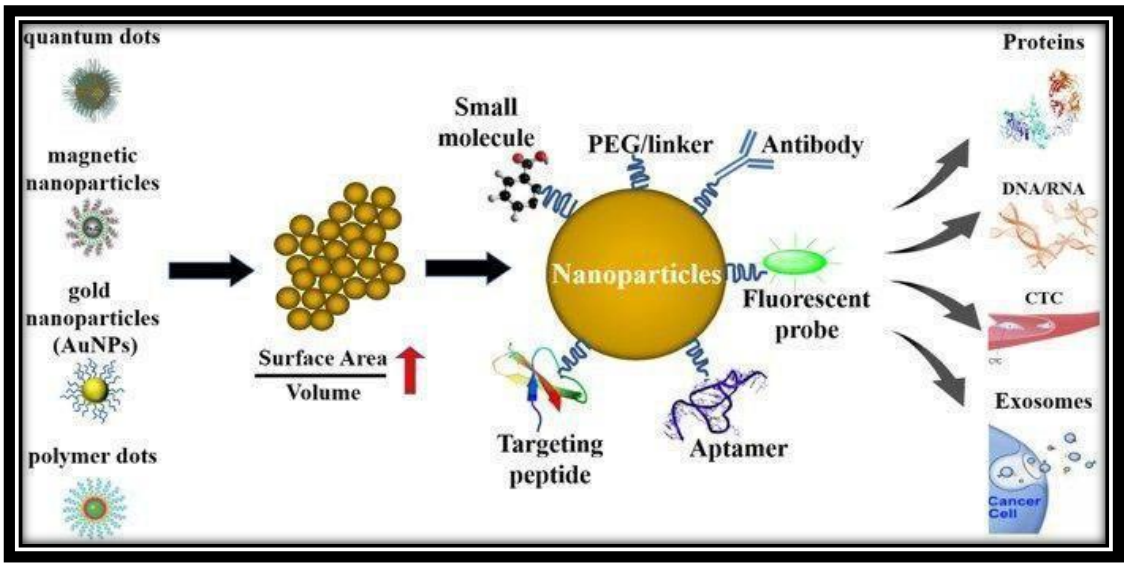


Fig.1. Nanotechnology improves cancer detection and diagnosis

1.2 Types of Cancer Treatment

The most common treatments are, chemotherapy, immunotherapy therapy, targeted therapy and there are other therapies like surgery, hormonal and laser thearapy. Here is an overview of the different treatments for cancer and how they work.

Chemotherapy is basically used to control the disseminated subclinical disease and also, for ele-mentary lesion treatment; moreover, this method could be followed by other modalities. For preventing drug resistance, different drug classes and modes of action are used in chemotherapy. The purpose of cytotoxic chemotherapy is to eradicate tumor cells while sparing normal tissue, the sensitivity of tumors varies by histology and class of

drugs with high cure rates in tumors that are highly sensitive to the drug administrate (7).

I. Alkylating agents

This medicine works directly on DNA to keep the cell from reproducing itself. These drugs will kill cells in all phases of the cell cycle. examples of alkylating agents are Chlorambucil, Cyclophosphamide, Cisplatin, and Carboplatin.

II. Nitrosoureas

A group of drugs that act similar to that of alkylating agents. These drugs slow down or stop enzymes that help repair DNA. They do travel into the brain, though many chemotherapy medicines do not. Examples are Carmustine and Lomustine.

III. Anti-metabolites

Drugs that interfere with a cell's RNA and DNA. Anti-metabolites work when cells are dividing. Examples are Fluorauracil, Methotrexate and Fludarabine.

V. Plant alkaloids and natural products

Medicines that are made from natural products. This group of drugs can block a cell's ability to divide and become two cells,

and to repair damage to cells. Examples are Vincristine, Paclitaxel, and Topotecan.

IV. Anti-tumor antibiotics

Anti-neoplastic drugs that are made from micro-organisms. These antibiotics do not act like the antibiotics used to treat infections. They may work in all phases of the cell cycle. They either break

up DNA strands or slow down or stop DNA synthesis that cells need to grow. Examples are Bleomycin, Doxorubicin and Mitoxantrone (8).

Immunotherapy is a type of cancer treatment that relies on the body's ability to fight infection (immune system). It uses substances made by the body or in a lab to help the immune system work harder or in a more targeted way to fight cancer. This helps your body get rid of cancer cells.

Immunotherapy works by: Stopping or slowing the growth of cancer cells Preventing cancer from spreading to other parts of the body Boosting the immune system's ability to get rid of cancer cells These drugs are designed to seek and attack certain parts of a cancer cell. Some have toxins or radioactive substances attached to them. Immunotherapy is given by IV (9)

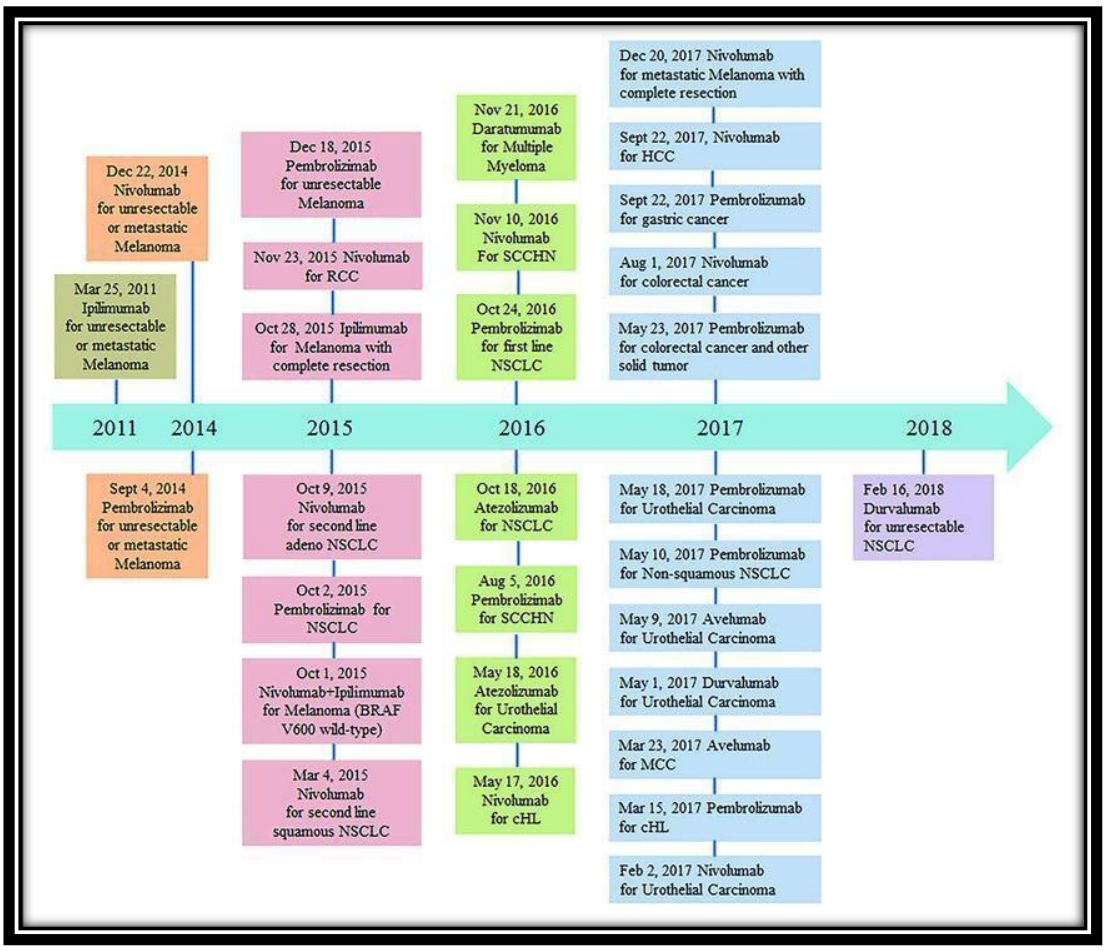


Fig.2 FDA approval timeline of immune checkpoint inhibitors for the treatment of malignancies .

Targeted therapy

Molecularly targeted therapy is one of the major modalities of medical treatment for cancer. As a form of molecular medicine, targeted therapy blocks the growth of growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth. rather than by

simply interfering with all rapidly dividing cells as in the case of traditional chemotherapy

There are 2 main types of targeted therapy drugs (9):

A) Monoclonal Antibodies

Antibody drugs are man-made versions of immune system proteins that have been designed to attack certain targets on cancer cells. Monoclonal antibodies exert their anticancer effect through a variety of mechanisms. 1. By recruiting host immune functions to attack the target cell. 2. By binding to ligands or receptors thereby interrupting essential cancer cell processes. 3.By carrying a lethal payload such as radioisotope or toxin to the target cell. They are used in targeted therapy for the delivery of active therapeutics, prodrug activation enzymes, and chemotherapy toxins

B) Small Molecule Inhibitors

Small drugs constitute a pill that is taken orally. As they are smaller chemical components than monoclonal antibodies, the body absorbs them better. typically interrupt cellular processes by interfering with the intracellular signaling of tyrosine kinases. Tyrosine kinase signaling initiates a molecular cascade that can lead to cell growth, proliferation, migration, and angiogenesis in normal and malignant tissues. EGFR, HER2/neu and VEGF receptors are tyrosine kinases. (10)

1.3 Nanocarriers used in drug delivery system

Nanotechnology has been actively integrated as drug carriers over the last few years to treat various. Nanocarriers have been used to circumvent the problems associated with conventional antitumor drug delivery systems, including their nonspecificity, severe side effects, burst release and damaging the normal cells. Nanocarriers improve the bioavailability and therapeutic efficiency of antitumor drugs, while providing preferential accumulation at the target site. nanocarriers such as polymeric nanoparticles, micelles, dendrimers, solid lipid nanoparticles, quantum dots, and magnetic nanoparticles (7).

Nanocarriers can be used to either passively or actively target cancer cells. Indeed, nanocarriers can extravasate into tumor tissues via leaky tissues through the enhanced permeability and retention (EPR) effect (passive targeting). Due to the dysfunctional lymphatic drainage in tumors, nanocarriers accumulate within these tissues and allow drug release within the vicinity of cancer cells (4).

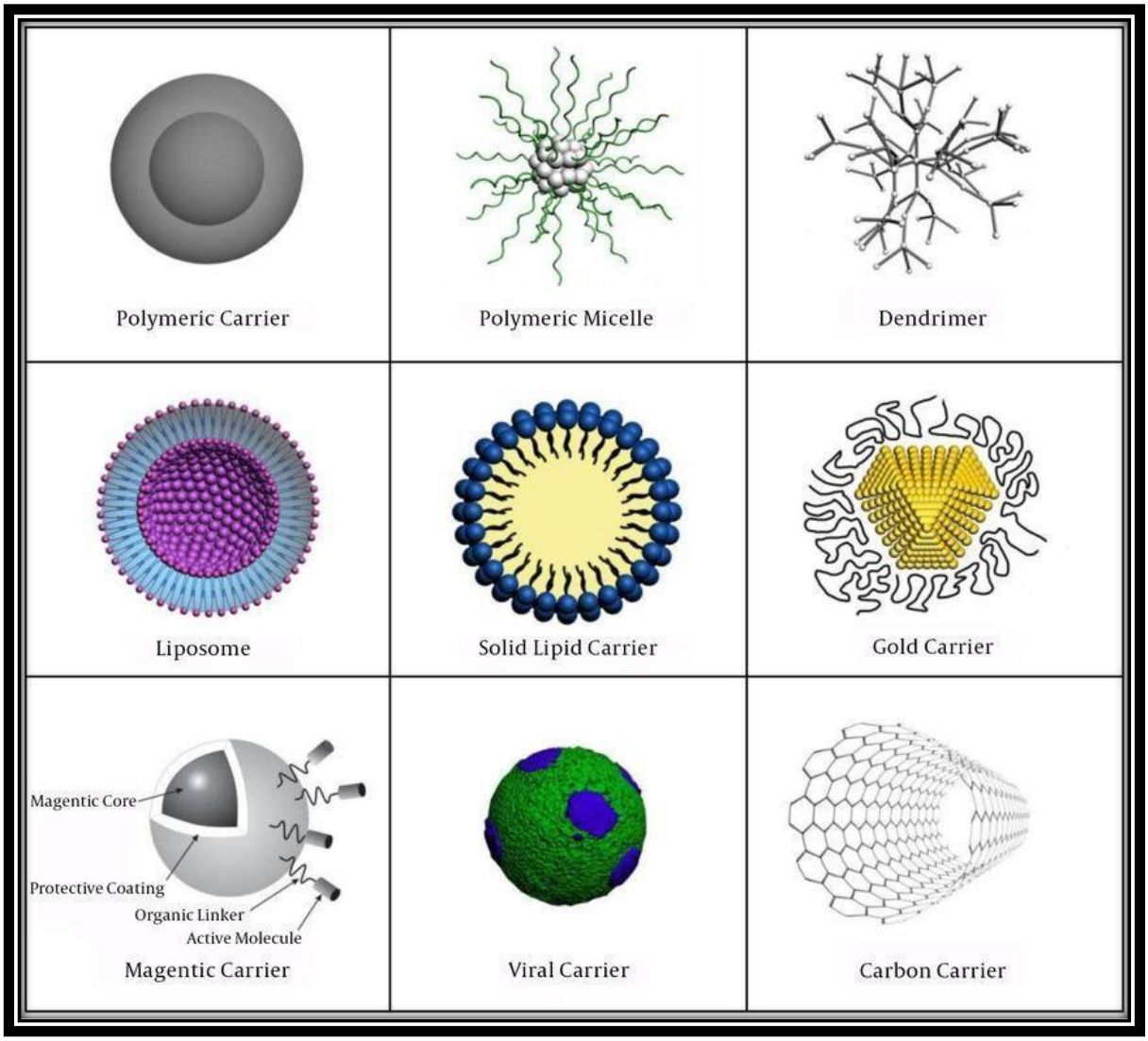


Fig:3 Different Types of Nanocarriers Have Used for Drug Delivery

1.4 zinc oxide Nanoparticles

Zinc oxide nanoparticle is the second most abundant metal oxide after iron and it is inexpensive, safe, and as well as it can be prepared easily. Physical and chemical behaviors of zinc oxide nanoparticles can be easily turned by changing the morphology by using different synthesis routes or different

precursors or different materials to produc the nanomaterial. Zinc oxide nanoparticle is one of the inorganic compounds of group II–IV semiconductor for analytical sensing applications. appears to be white powder and insoluble in water. has an energy band of 3.37 eV and a bonding energy of 60 meV, which provides its excellent chemical, electrical, and thermal stabilities، also has optical, electrical, and photocatalytic properties. its low toxicity and high UV-absorption making it a good candidate to be used in the biomedical field. also has a hard and rigid structure. they act as a good surface material. Zinc oxide nanoparticle is naturally known as a strong resistance of microbes. Due to these reasons zinc oxide nanoparticle is extensively used for biological labelling, biological sensing, drug delivery, gene delivery, and nanomedicine. Food and drug administration has approved zinc oxide as a safe material. also, can solubilize in an acidic environment therefore, Zinc oxide exists in the following phases: hexagonal quartzite, cubic zinc blende, and cubic rock salt. The wurtzite structure is most common and stable at ambient conditions due to its ionicity that resides

exactly at the borderline between the covalent and the ionic materials (10).

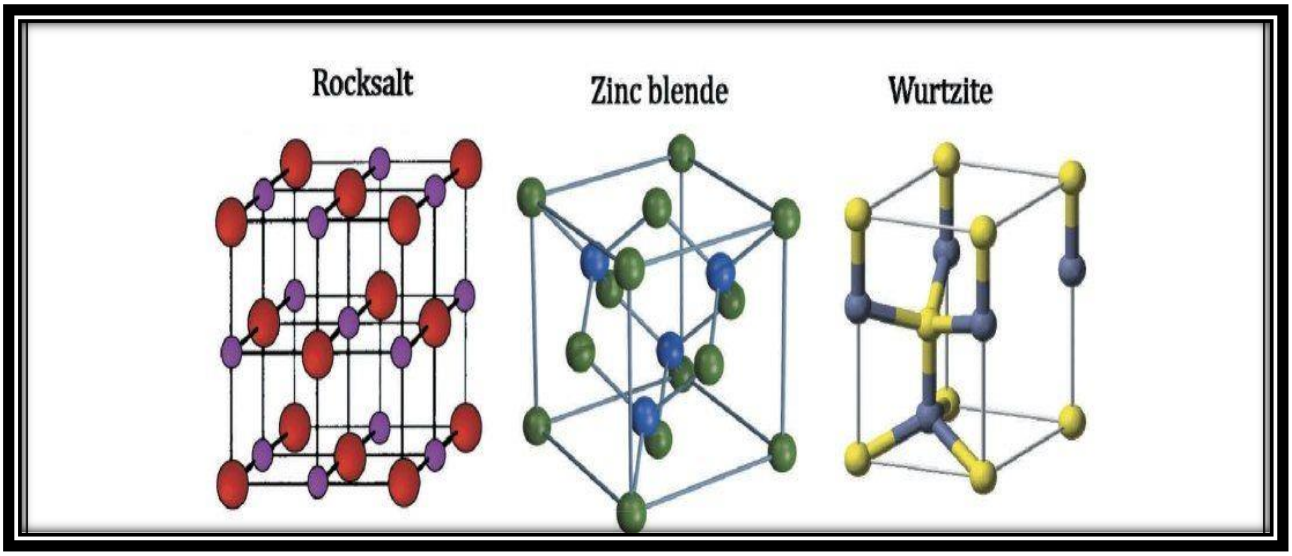


Fig4: various crystals structures of ZnO

1.5 Dox drug :Pharmaceutical and medical applications

Doxorubicin is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Doxorubicin is classified as an "anthracycline antibiotic. routinely used in the treatment of several cancers including breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, sarcoma, and pediatric cancers.

Pharmacokinetic data

Bioavailability 5% (by mouth)

Protein binding 75%

Metabolism Liver

Elimination half-life Mean: 1–3 hours

Excretion Urine (5–12%), faeces (40–50%)

There are two proposed mechanisms by which doxorubicin acts in the cancer cell (i) intercalation into DNA and disruption of topoisomerase-II-mediated DNA repair and (ii) generation of free radicals and their damage to cellular membranes, DNA and proteins. In brief, doxorubicin is oxidized to semiquinone, an unstable metabolite, which is converted back to doxorubicin in a process that releases reactive oxygen species.

Reactive oxygen species can lead to lipid peroxidation and membrane damage, DNA damage, oxidative stress, and triggers apoptotic pathways of cell death. Alternatively, doxorubicin can enter the nucleus and poison topoisomerase-II, also resulting in DNA damage and cell death.

Themechanism of cardiotoxicity of doxorubicin i) iron-related free radicals and formation of doxorubicinol metabolite and (ii) mitochondrial disruption.

The proposed principal mechanisms of doxorubicin cardiotoxicity are increased oxidative stress, as evident from increased levels of reactive oxygen species and lipid peroxidation Decreased levels of antioxidants and sulfhydryl groups , inhibition of nucleic acid and protein synthesis , release of vasoactive amines , altered adrenergic function and decreased expression of cardiac-specific (11)

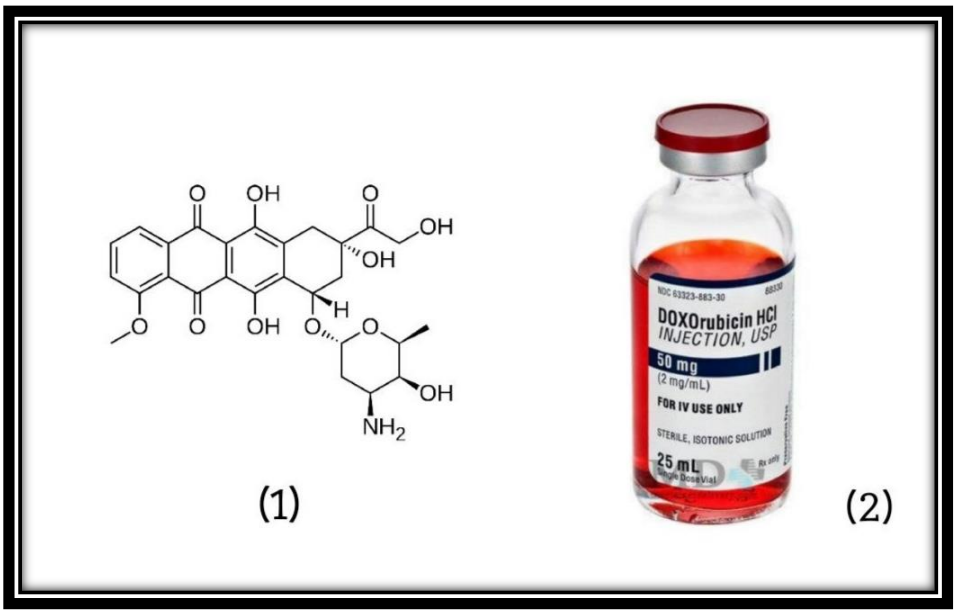


Fig5: (1) Chemical structure of doxorubicin, (2) Doxorubicin Injection

1.6 zinc oxide _Based Drug Delivery Systems for Cancer Therapy

Drug formulation in biocompatible nanoforms is emphasized in pharmaceutical nanotechnology, which provides advantages in drug delivery. NPs improve drug efficiency and safety by improving bioavailability, providing targeted drug delivery, improving drug stability, and extending the drug's impact on the

target tissue. ZnO is used in current drug delivery systems due to its ease of manufacture, low cost, customiz- able structure, non-toxicity, high drug-loading capacity, programmable drug release ability, and targeted delivery. Porous ZnO structures such as porous nanotubes, porous nanobelt, porous nanorods, and porous cages have been successfully used in targeted drug delivery systems. In order for the drug to be delivered efficiently, surface functionalization of NPs can be done, which is achieved using various agents, i.e., ligands, linker chains, drugs, and markers. Through specific molecular interactions such as receptor _ligand-based interactions NPs accumulate in cells, which through endo/lysosomal escape on receiving an appropriate stimulus, release the drug, destroying its cognate target. Various types of internal and external stimulus are also involved in targeted delivery of anticancerous drugs (12).

1.7 Drug Delivery For Doxorubicin

The initial development of the liposomal drug delivery system showed great promiseThe liposomes showed signs of leakage, easy recognition and removal from the circulatory system via the reticuloendothelial system (RES).The pathways consist of uptake by the cells of the bone marrow, spleen and liver from the drug-infused liposomes in the circulation, followed by drug metabolism and removal, drug leakage fromthe liposomes as well as the rapid elimination of the drug in free form, and finally the accumulation of drug-loaded liposomes in the tissue. Furthermore, nanoparticles have been modified to act as carriers for doxorubicin, there is a better safety profile with

liposomal Dox formulations. Compared to patients treated with conventional Dox.

1. 8 METHODS OF synthesis zinc oxide Nanoparticles

	Synthesis method	**Characterization**	**Application**	Refs.
ZnO	1.Physical methods 2.Chemical methods (Thermal precipitation method) 3.Biological Method 4.Microfluidic	X-ray Diffraction (XRD) FT-IR analysis FESEM analysis	1.Pharmaceuticals Soap Ointment Dental inlays Food powders 2.Cosmetics—hair and skin care products Powders Creams UV radiation-blocking sunscreen lotions Burn ointments 3.Medical devices Surgical/industrial adhesives Mastics Sealants	(13) (14) (15) (16) (17) (18) (19)

Each method has different advantages and disadvantages

Precipitation method

	Advantages	Disadvantage	Refrence
Precipitation method	Monetarily cheap The cost of the apparatus and equipment inexpensive high production yield. High quality of production More controllablesize particle obtained can be Control easily. Rapid	Expensive reagents (Surfactants) Need to wash And filter Nucleation and growth occur simultaneously due to the rapid reaction, making it difficult to study the detail growth process	(20) (21) (22) (23) (24) (25)

CHAPTER TWO

MATERIAL AND METHODS

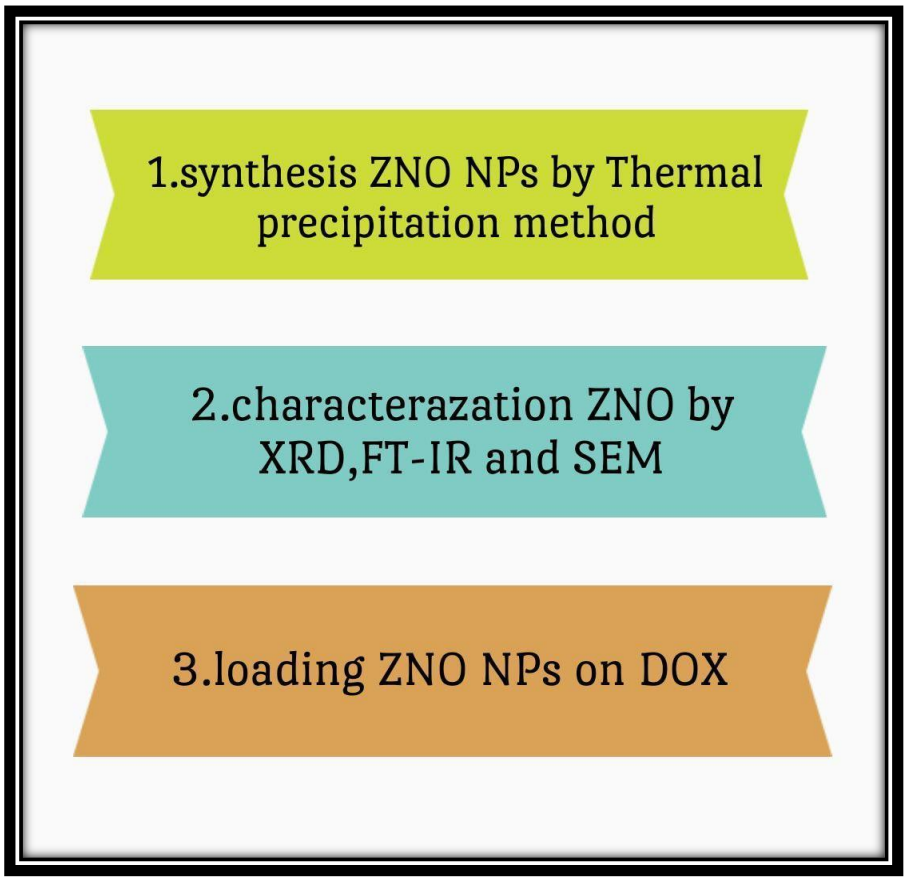
Material and Method

2.1 Chemical used

Name of chemical	Assay	Supplier	Purpose of use
KOH	96%	India	Synthesis ZnO media
Zinc nitrate	98%	India	NP synthesized
Ethanol	99.9%	Spain	NP synthesized
DOX			Model application (drug)

2.2Chart of drug delivery experimental

In order to give an overview of the experiments carried out in this study, a flowchart of the experiment showed our level of work.



2.3 Synthesis of zinc oxide nanoparticles

(A)-Synthesis of ZnO nanoparticles

ZnO nanoparticles were prepared by direct thermal- precipitation method. Briefly, an aqueous solution of 0.2 M of zinc nitrate and

0.4 M of KOH were prepared with deionized water respectively. At room temperature, the KOH solution was gradually purred into zinc nitrate solution with constant stirring with controlling the temperature to 60 0C for 120 min to form a white precipitation. After centrifuging the obtained mixture at 5000 rpm for 20 min and it was washed three times with deionized water and at last with absolute alcohol. Furthermore, the acquired precipitates were claimed at 500oC for 2 h onacustom made tubular muffle furnace and without calcination to facilitate formation of ZnO.

2.4 loading DOX on ZnO NPs

The loaded ZnO nanoparticles Doxorubicin (DOX) . To prepare the ZnO/DOX, approximately 1.0 mg of OTC were dissolved in 10 mL of a mixture of DOX and 25 mg ZnO. which was used as the drug carrier. The reaction was processed under stirring for 12 h with dark conditions. (26)

Results & Discussion

Results & Discuss

1- Characterization of the Synthesis ZnO

3.1.1The SEM with EDX analysis

Figure (6) display the Scanning Electron Microscope (SEM) photograph for zinc oxide, where zinc nitrate has been used in its preparation as the starting material at a temperature of 500^{o}C. The morphological investigation of the prepared ZnO powder took place by means of scanning electron microscopy (SEM)(27).

Figures 6 (a, b, and c) illustrate the formation of nanoparticles resulting from the decomposition, as they appear in form of faceted crystals (Fig. 3d). One of this material's characteristics is its relatively higher porosity.

Figure 1 displays the SEM images at a higher magnification, and demonstrates the formation of particles with a size of 14nm. It also provided a clearer idea about the particle separation, as the particles are seen to be separated smoothly, without being highly affected by agglomeration.

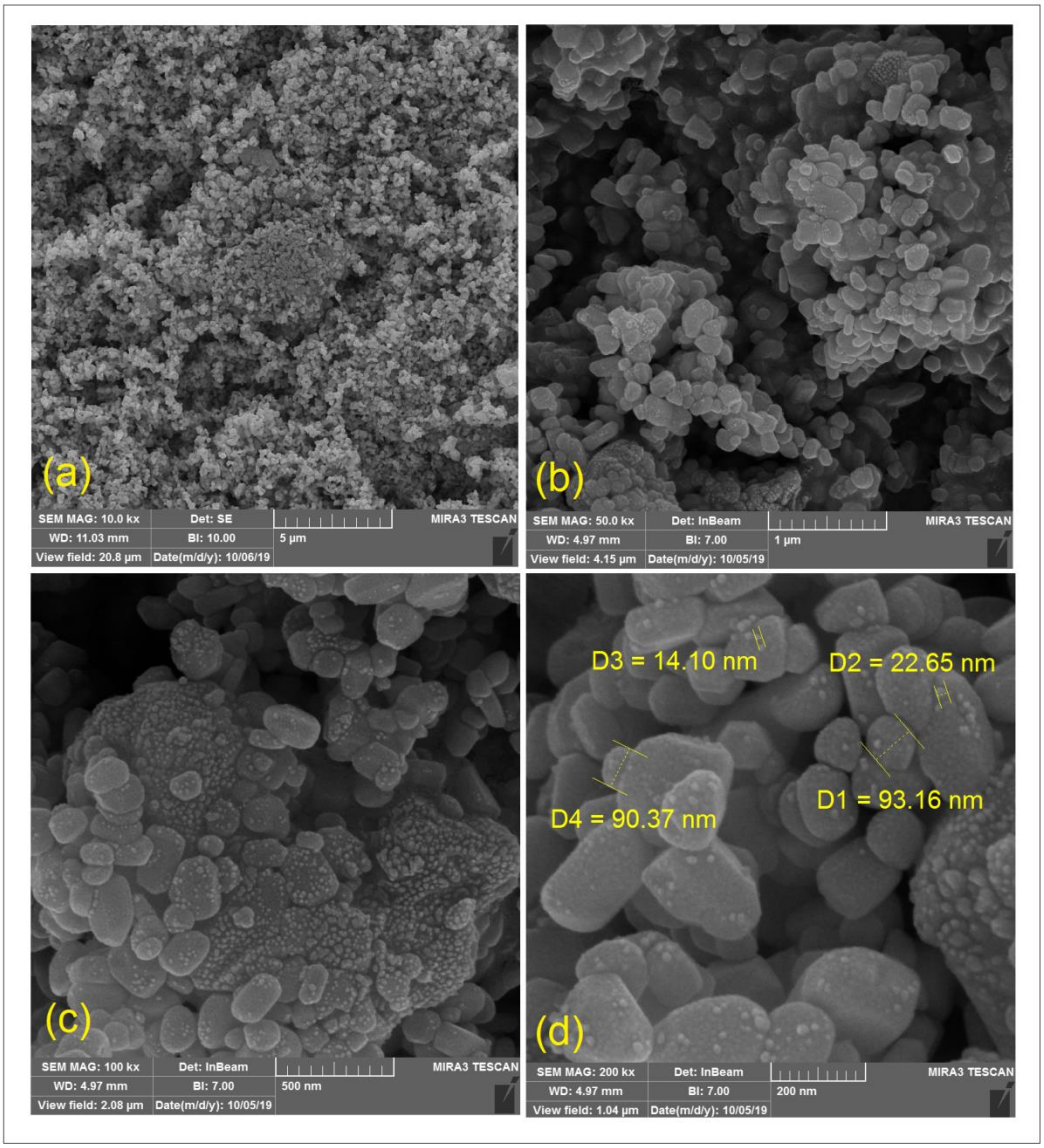


Fig .6: SEM of ZnO powder synthesized at 500ºC for different magnifications: (a) x 10 k, (b) x 50 k, (c) x 100 k, (d) x 200 k.

Figure 7 represents the EDX spectrum of ZnO nanoparticles. EDX spectrum displays four peaks that could be identified as zinc and oxygen. Therefore, the conclusion can be drawn that pure ZnO nanoparticles could be prepared through the use of the precipitation method.

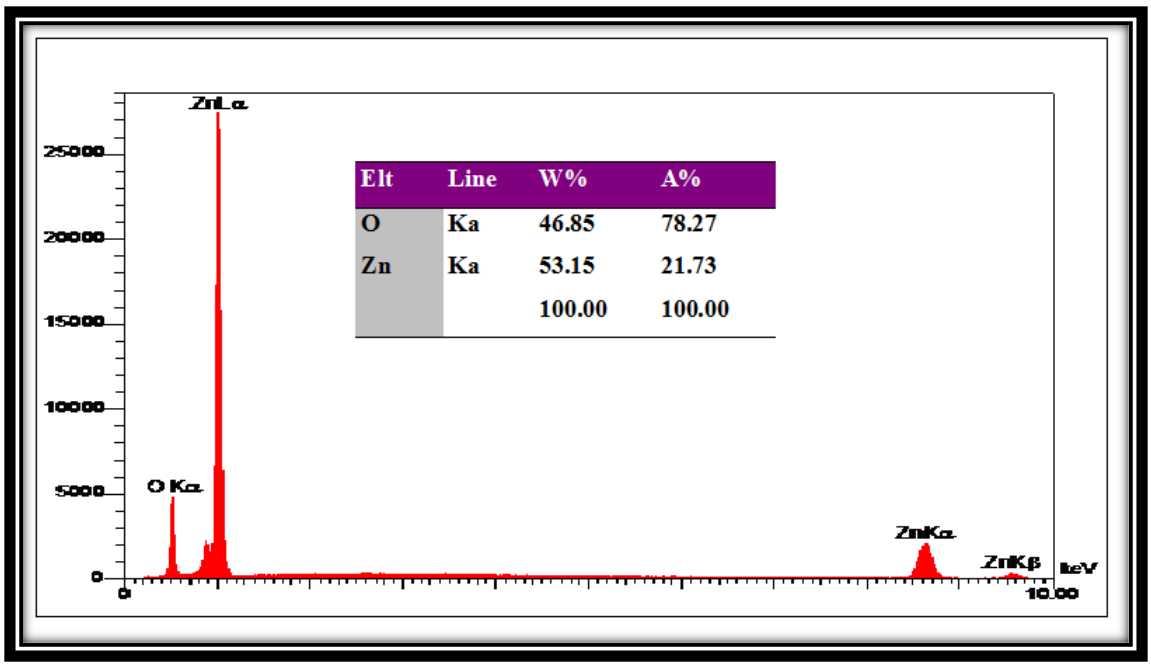


Fig .7: EDX of ZnO powder synthesized 500ºC.

3.1.2The XRD analysis

Figure 8 presents the results of X-ray diffraction for the ZnO after synthesis, showing wide peaks at (31.9, 34.5, 36.3, 56.7, and 62.9) characteristic to the ZnO structure. The remarkable breadth of line for such diffracting peaks indicates that the material falls within the nanometer span (28).

Figure 8: demonstrates the possibility of obtaining ZnO NPs through decomposing the zinc compound thermally. With the use of Scherrer's equation (29), the diameter of the crystallite domain (D) was taken through the XRD peaks:

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

where λ represents the wavelength of the incident X-ray beam (1.54 A° for the Cu Kα), θ stands for the Bragg's diffraction angle, and β represents how wide the X-ray pattern line is at half peak-height in radians (30). The average sizes of the particles of ZnO measured were about 34.06 nm, 28.1 nm, 59.5 nm at temperature 500 ^{o}C, respectively.

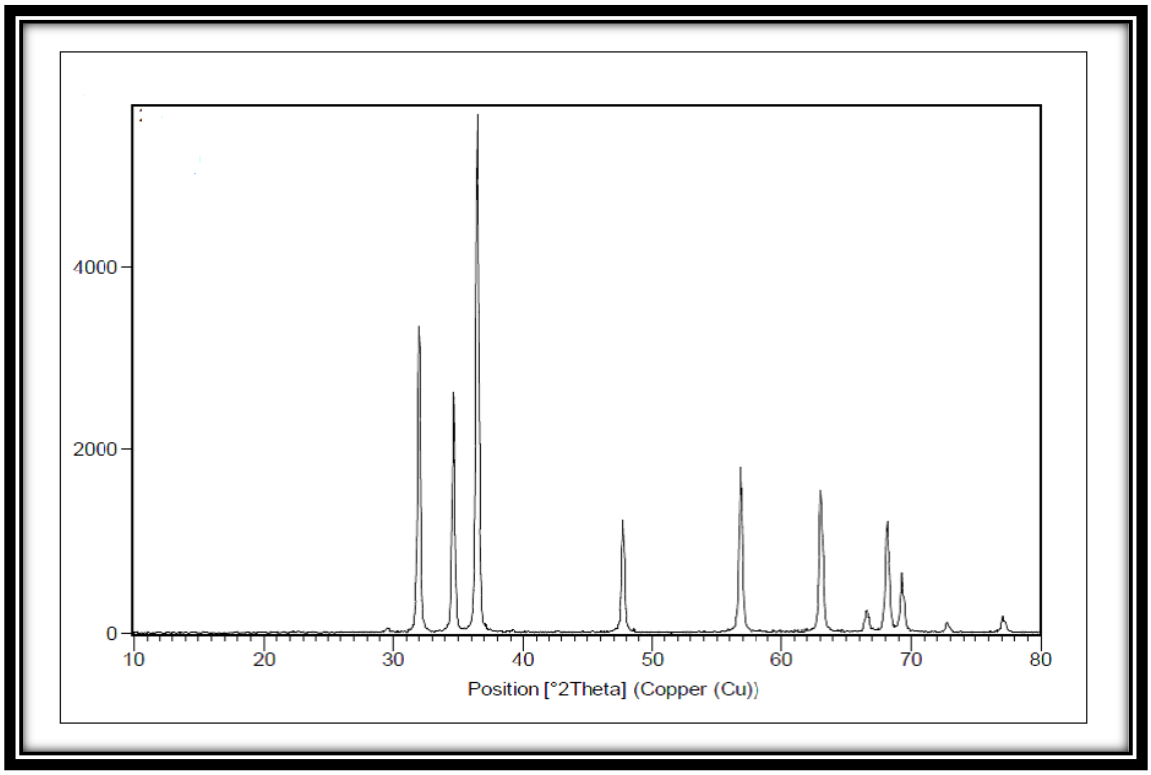


Fig .8: XRD of ZnO powder synthesized at 500^{o}C.

3.1.3The FTIR spectra analysis

FTIR spectra of ZnO nanoparticles (Figure 4) showed an intense peak at 400–500 cm^{-1} (Zn–O) and a broad peak at 3331–3441 cm^{-1} (O–H). FTIR spectra of DOX exhibited multiple peaks at 1285 cm^{-1}(C–O), 1414 cm^{-1} (C–C), 1617 cm^{-1} (N–H), 1730 cm^{-1} (C–O), 2933 cm^{-1} (C–H) and 3331–3441 cm^{-1} (O–H). The peaks

of the FTIR spectra of loading DOX–ZnO were slightly shifted to 1159 cm^{-1} (C–O), 1379 cm^{-1} (C–C), 1628 cm_1 (N–H), 1735 cm^{-1} (C–O) and 2936 cm^{-1} (C–H)(30-31).

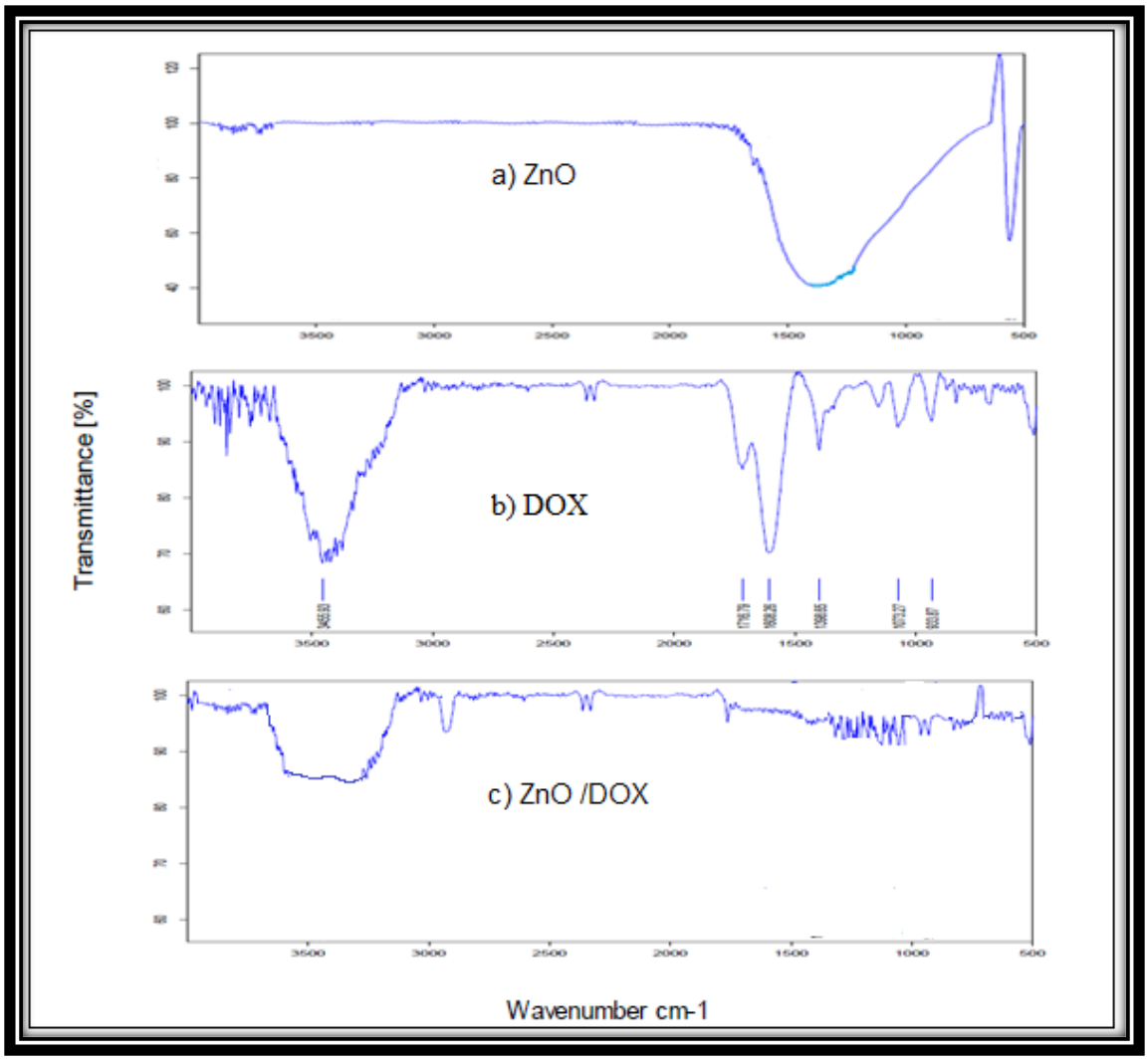


Fig: 9. FTIR for a) synthesized ZnO, b) DOX drug, and c) ZnO / DOX drug

References

References:

1. Mehra, Neelesh Kumar, and Keerti Jain. Dendrimers in Nanomedicine: Concept, Theory and Regulatory Perspectives. CRC Press, 2021.

2.Gupta, Swaroopa Rani N. "Advances in molecular nanotechnology from premodern to modern era." Int. J. Mater. Sci. Eng 2 (2014): 99-106.

3. Jayatilaka, Hasini. MECHANISTIC COUPLING OF CANCER CELL PROLIFERATION AND MIGRATION. Diss. Johns Hopkins University, 2016.

4. Yang, Yafeng, et al. "Applications of nanotechnology for regenerative medicine; healing tissues at the nanoscale." Principles of regenerative medicine. Academic Press, 2019. 485-504.

5. Debele, Tilahun Ayane, Cheng-Fa Yeh, and Wen-Pin Su. "Cancer immunotherapy and application of nanoparticles in cancers immunotherapy as the delivery of immunotherapeutic agents and as the immunomodulators." Cancers 12.12 (2020): 3773.

6. Thakur, Monika, Karuna Singh, and Renu Khedkar. "Amity Institute of Food Technology, Amity University, Noida, Uttar Pradesh, India." Functional and Preservative Properties of Phytochemicals (2020): 341.

7. Diao, Li. Unmanned Aerial Vehicle Assisted Health Care Resource Allocation in Disasters. Diss. Auckland University of Technology, 2019.

9. Dinesh, Malepati Venkata, Sobita Simon, and Sasya Nagar. "Biorational management of stem borer, Chilo partellus in maize." Journal of Pharmacognosy and Phytochemistry 7.4 (2018): 1142-1145.

8. BALANESCU, VALENTIN, et al. "54 Guide Cancer Therapeutic Drug." Cardio-Oncology Practice Manual: A Companion to Braunwald's Heart Disease E-Book (2022): 452.

10. Reig, Maria, et al. "BCLC strategy for prognosis prediction and treatment recommendation Barcelona Clinic Liver Cancer (BCLC) staging system. The 2022 update." Journal of hepatology (2021).

11. Anjum, Sumaira, et al. "Recent Advances in Zinc Oxide Nanoparticles (ZnO NPs) for Cancer Diagnosis, Target Drug Delivery, and Treatment." Cancers 13.18 (2021): 4570.

12. Rather, Gulzar Ahmed, et al. "The Role of Green Synthesised Zinc Oxide Nanoparticles in Agriculture." Sustainable Agriculture. Springer, Cham, 2022. 119-142.

13.Król, A.; Pomastowski, P.; Rafi ´nska, K.; Railean-Plugaru, V.; Buszewski, B. Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. Adv. Colloid Interface Sci. 2017, 249, 37–52.

14. Rasmussen, Kirsten, et al. "Developing OECD test guidelines for regulatory testing of nanomaterials to ensure mutual acceptance of test data." Regulatory Toxicology and Pharmacology 104 (2019): 74-83.

15. Khalafi, Tariq, Foad Buazar, and Kamal Ghanemi. "Phycosynthesis and enhanced photocatalytic activity of zinc oxide nanoparticles toward organosulfur pollutants." Scientific reports 9.1 (2019): 1-10.

16. Jin, Su-Eon, and Hyo-Eon Jin. "Synthesis, characterization, and three-dimensional structure generation of zinc oxide-based nanomedicine for biomedical applications." Pharmaceutics 11.11 (2019): 575.

17. Balaure, Paul Cătălin, et al. "In vitro and in vivo studies of novel fabricated bioactive dressings based on collagen and zinc oxide 3D scaffolds." International journal of pharmaceutics 557 (2019): 199-207.

18. Jin, Su-Eon, and Hyo-Eon Jin. "Synthesis, characterization, and three-dimensional structure generation of zinc oxide-based nanomedicine for biomedical applications." Pharmaceutics 11.11 (2019): 575.

19. Rai, Shweta, Neeraj Singh, and Sankha Bhattacharya. "Concepts on smart nano-based drug delivery system." Recent patents on nanotechnology 16.1 (2022): 67-89.

20. Roubdari, Maryam, Amir Marzban, and Syyad Hasan Talebi. "Engineering Students' ESP Problems at Islamic Azad Universities in Mazandaran Province using WhatsApp Application." Information and Communication Technology in Educational Sciences 12.47 (2022): 63-83.

21-Hammadi, Asmaa H., et al. "Synthesis, Characterization and Biological Activity of Zinc Oxide Nanoparticles (ZnO NPs)." Systematic Reviews in Pharmacy 11.5 (2020): 431-439.

22- P. Marilena ,M. Raul-Augustin ,M. Cristian, and B. Daniela "MESOPOROUS SILICA-CERIA COMPOSITES AS CARRIERS FOR DRUG DELIVERY SYSTEMS" Rev. Roum. Chim.,2016, 61(6-7), 557-563

23-P. Scherrer, "Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen." Mathematisch-Physikalische Klasse 2 ,1918, 98-100.

24-A. Aslinjensipriya, , et al. "Synthesis and characterization of ZnO nanoparticles by novel sol gel technique." AIP Conference Proceedings. Vol. 2244. No. 1. AIP Publishing LLC, 2020.

25- Suttirak Pairoj, Pattareeya Damrongsak, Badin Damrongsak ,et al. "ntitumor activities of carboplatin–doxorubicin–ZnO complexes in different human cancer cell lines (breast, cervix uteri, colon, liver and oral) under UV exposition". Artificial Cells, Nanomedicine, and Biotechnology. VOL. 49, NO. 1, 120–135, 2021.

26- Xiaoli Cai, Yanan Luo, , Weiying Zhang, et al. "pH-Sensitive ZnO Quantum Dots−Doxorubicin Nanoparticles for Lung Cancer Targeted Drug Delivery". ACS Appl. Mater. Interfaces, 8, 22442−22450, 2016.

27. Ojha, Brijesh, et al. "Nanotechnology: Introduction and Basic Concepts." Dendrimers in Nanomedicine: Concept, Theory and Regulatory Perspectives (2021): 1-17..

28Aremu, O. H., et al. "Synthesis and applications of nano-sized zinc oxide in wastewater treatment: a review." International Journal of Environmental Science and Technology 18.10 (2021): 3237-3256.

29. Aldeen, Thana Shuga, Hamza Elsayed Ahmed Mohamed, and Malik Maaza. "ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity." Journal of Physics and Chemistry of Solids 160 (2022): 110313.

30. Raha, Sauvik, and Md Ahmaruzzaman. "ZnO nanostructured materials and their potential applications: progress, challenges and perspectives." Nanoscale Advances 4.8 (2022): 1868-1925.

31. Kotresh, M. G., M. K. Patil, and S. R. Inamdar. "Reaction temperature based synthesis of ZnO nanoparticles using co-precipitation method: detailed structural and optical characterization." Optik 243 (2021): 167506.