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Synthesis and Characterization of Metronidazole loaded with Go/ZnO

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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" وَقُلْ رَبِّ زِدْنِي عِلْمًا "

صدق الله العلي العظيم

سورة طه الآية (114)

الإهداء ...

وصلت رحلتنا الجامعية إلى نهايتها بعد تعب ومشقة
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 .

فكر وفكر

بسم الله الرحمن الرحيم الرحيم، والحمد لله رب العالمين الذي وفقنا وأعاننا على إنهاء هذا البحث والخروج به بهذه الصورة المتكاملة، فبالأمس القريب بدأنا مسيرتنا التعليمية ونحن نتحسس الطريق برهبة وارتباك، فرأينا أن (الصيدلة) هدفًا ساميًا وحبًا وغاية تستحق السير لأجلها، وإن بحثنا يحمل في طياته طموح شباب يحلمون بصناعة مستقبل جميل يليق ببلدهم.

وانطلاقًا من مبدأ أنه لا يشكر الله من لا يشكر الناس، فإننا نتوجه بالشكر الجزيل للأستاذ المعلم "الدكتورة أسماء هاشم حمادي" و"الاستاذة صبا عبد المنعم حبيب" اللذان رافقانا في مسيرتنا لإنجاز هذا البحث وكانت لهما بصمات واضحة من خلال توجيهاتهما وانتقاداتهما البناءة والدعم الأكاديمي.

الشكر موصول أيضا الى الاساتذة اعضاء لجنة المناقشة الذين تفضلوا بقراءة هذا البحث.

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Abstract:

in the present work, Zinc Oxide nano powder is synthesized through thermal precipitation as a chemical method in alkaline media then loaded on Graphene-Graphite Nanocomplex oxide by emigration method at room temperature. Further, microscopic techniques such as SEM established the formation of a multi-shaped ZnO-GO complex with an average size of 20-40 nm. XRD characterization was employed to analyze showed purity and phase of complex yield. In addition to this, ZnO-GO complex nano-powder conjugated with metronidazole (drug) through chemical method.

Chapter One

Introduction

1. INTRODUCTION

The emergence of infectious diseases promises to be one of the leading mortality factors in the healthcare sector. Although several drugs are available on the market, newly found microorganisms carrying multidrug resistance (MDR) against which existing drugs cannot function effectively, giving rise to escalated antibiotic dosage therapies and the need to develop novel drugs, which require time, money, and manpower. Thus the exploitation of antimicrobials has led to the production of MDR bacteria, and their prevalence and growth are a major concern. Novel approaches to prevent antimicrobial drug resistance are in practice. Nanotechnology-based innovation provide physicians and patients the opportunity to overcome the crisis of drug resistance. Nanoparticles have promising potential in the healthcare sector .Thus, nanotechnology provides an excellent opportunity to treat drug-resistant microbial infections. Numerous antibiotics have been used to inhibit the growth and kill of microbes, but the development of resistance and the emergence of side effects have severely limited the use of these agents. Due to the development of the nanotechnology, nanoparticles are widely used as antimicrobials. Silver and chitosan nanoparticles have antifungal, antiviral and antibacterial properties, and many studies confirm the antifungal properties of silver nanoparticles. Nowadays, the use of nanoparticles in the diagnosis and treatment of infectious diseases has developed due to less side effects and also the help of these particles in effective drug delivery to the target tissue. Liposomes are also used as carriers of drug delivery, genes, and modeling of cell membranes in both animals and humans. The ability of these liposomes to encapsulate large amounts of drugs, minimize unwanted side effects, high effectiveness and low toxicity has attracted the interest of researchers[1-2] .

1.1 Nanoparticles:

Nanoparticles NPs are tiny materials having size ranges from 1 to 100 nm. They can be classified into different classes based on their properties, shapes or sizes. The different groups include fullerenes, metal NPs, ceramic NPs, and polymeric NPs. NPs possess unique physical and chemical properties due to their high surface area and nanoscale size. Their optical properties are reported to be dependent on the size, which imparts different colors due to absorption in the visible region. Their reactivity, toughness and other properties are also dependent on their unique size, shape and structure. Due to these characteristics, they are suitable candidates for various commercial and domestic applications, which include catalysis, imaging, medical applications, energybased research, and environmental applications[3]. Heavy metal NPs of lead, mercury and tin are reported to be so rigid and stable that their degradation is not easily achievable, which can lead to many environmental toxicities. Nanoparticles (NPs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least . Depending on the overall shape these materials can be 0D, 1D, 2D or 3D . The importance of these materials realized when researchers found that size can influence the physiochemical properties of a substance e.g., the optical properties. A 20-nm gold (Au), platinum (Pt), silver (Ag), and palladium (Pd) NPs[4].

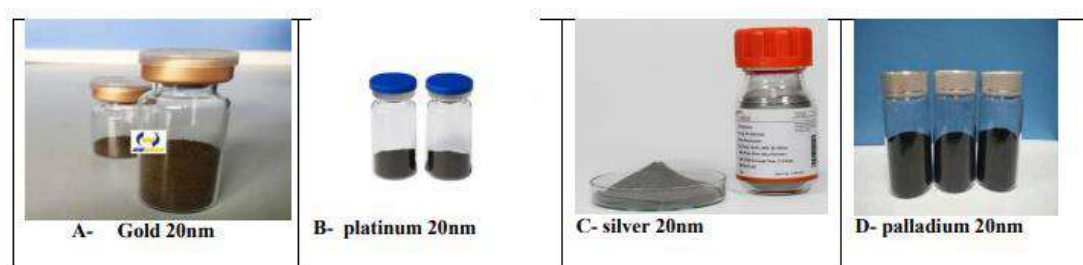


Fig (1): Examples of some materials NPs

ZnO NPs is of highest interest because they are inexpensive to produce, safe and can be prepared easily . Moreover, they possess several novel properties, such as large band gap (3.37eV) and high exciton binding energy (60 meV), high refractive index, binding energy, large thermal conductivity, high catalytic activity, antibacterial, UV filtering properties, anti-inflammatory, wound healing [5-6]. ZnO has also high biocompatibility and fast electric transfer kinetics, such phenomena encourage the use of this material as a biomimic membrane to immobilize and modify the biomolecules [7]

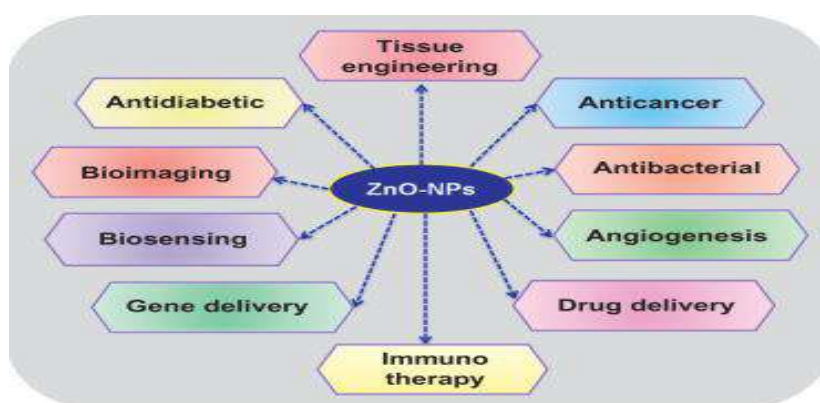


Fig (2):Applications of ZnO-NPs.

NPs are not simple molecules itself and therefore composed of three layers i.e. (a) The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants and polymers. (b) The shell layer, which is chemically different material from the core in all aspects, and (c) The core, which is essentially the central portion of the NP and usually refers the NP itself [8]. Owing to such exceptional characteristics, these materials got immense interest of researchers in multidisciplinary fields. The NPs can be employed for drug delivery, chemical and biological sensing, gas sensing, CO₂ capturing, and other related applications [9].

1.2 Composite between nanoparticles:

Composite nanoparticles are advanced materials having recently gained increasing attention due to their scientific and technological importance. They find a wide variety of applications such as catalysts with huge activity and specificity, metal semiconductor junctions, optical sensors, and modifiers of polymeric films for packaging. From a scientific point of view the composition and the atomic order of the aggregates, in addition to size, are pivotal factors in determining their properties and functionalities, while the nanoscale regime confers to them structural and electronic degrees of freedom which are inaccessible to bulk materials. The first indispensable stage to develop novel nanotechnologies is the preparation of tailored composite nanostructures. Virtually all the possible physicochemical phenomena have been employed to reach this objective allowing to set up a huge number of protocols each one with its specific advantages and disadvantages. The aspects of novel preparation methods are addressed in two manuscripts. S. Bagheri et al. describe a novel synthesis of anatase titanium dioxide nanoparticles using egg white solution via solgel method and characterized them through a high number of techniques. The main advantage of using egg white proteins as a gelling agent is that it can provide long-term stability for nanoparticles by preventing particle agglomeration. They demonstrated that egg white solution is a reliable and cheap green gelling agent that can be used as a matrix in the sol-gel method to synthesise tiny size TNPs. V. Ovchinnikov and A. Shevchenko describe a costeffective fabrication of random noble-metal nanostructures with a feature size of the order of 10 nm on a large-area dielectric substrate. The technique allows to create metal nanoislands on a nanopatterned dielectric template with an enhanced adhesion between the metal and the dielectric. The use of the adhesion layer (that makes the structures stable) is

important in view of variety of optical and other potential applications of the structures. The results of their work can be of interest in regard to the development of new approaches to self-organization based nanofabrication of extremely small metal and metal-dielectric nanostructures on large-area substrates. Due to their peculiarities, the production of nanomaterials is a promising strategy to fuel novel technological applications[10]. Two manuscripts, highlighting enhanced properties for applicative purposes, are good examples of the potentialities of these materials. M. C. Carrera et al. studied the permeation of CO₂ in films of high density polyethylene (HDPE) and organoclay modified with polyvinylalcohol (MMTHDTMA/PVA) obtained from melt blending. They showed that the incorporation of the modified organoclay generated an exfoliated nanocomposite structure with a significant effect on the barrier properties of HDPE. Notably the mechanical properties remain equal to those of pure polyethylene, but with an increase in barrier properties to CO₂ allowing to obtain nanocomposites of HDPE competitively used in packaging. Q. X. Li et al. prepared a novel Pd/C catalyst with enhanced performance for direct ethanol fuel cell. They characterized the catalysts by XRD and TEM showing that they are spherical and homogeneously dispersed on carbon. Finally, the theoretical/computational approach has been faced by P. Shabanzadeh et al. In their investigation, they applied the accuracy of artificial neural network training algorithm to study the effects of different parameters on the synthesis of Ag nanoparticles performed in the interlamellar space of montmorillonite (MMT) by chemical reduction technique. Using the best performing artificial neural network, they predicted the optimum conditions of AgNO₃ concentration, MMT d-spacing, and reaction temperature. By compiling these papers, we hope to enrich our readers and researchers with respect to the most recent progresses in the field of nanoscale multicomponent particles[11].

1.3 Zinc oxide nanoparticles (ZnO NPs):

as one of the most important metal oxide nanoparticles, are popularly employed in various fields due to their peculiar physical and chemical properties. ZnO NPs are firstly applied in the rubber industry as they can provide wearproof of the rubber composite, improve performance of high polymer in their toughness and intensity and antiaging, and other functions [12-13]. Because of the strong UV absorption properties of ZnO, they are increasingly used in personal care products, such as cosmetics and sunscreen [14]. In addition, ZnO NPs have superior antibacterial, antimicrobial, and excellent UV- blocking properties. Therefore, in the textile industry, the finished fabrics by adding ZnO NPs exhibited the attractive functions of ultraviolet and visible light resistance, anti- bacteria, and deodorant [15]. Apart from the applications mentioned above, zinc oxide can also be used in other branches of industry, including concrete production, photocatalysis, electronics, electrotechnology industries, and so on [16]. It is generally known that zinc as an essential trace element extensively exists in all body tissues, including the brain, muscle, bone, skin, and so on. As the main component of various enzyme systems, zinc takes part in body's metabolism and plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis . Nano-ZnO, with small particle size, makes zinc more easily to be absorbed by the body. Thus, nano-ZnO is commonly used as a food additive. Moreover, ZnO is graded as a "GRAS" (generally recognized as safe) substance by the US Food and Drug Administration (FDA) [17]. With these properties, ZnO NPs have received more attention in bio medical applications. Compared with other metal oxide NPs, ZnO NPs with the comparatively inexpensive and relatively less toxic property exhibit excellent biomedical applications, such as anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation;

wound healing; and bio- imaging .Herein, in this review, we will summarize the methods of synthesis and recent exciting progress on the use of ZnO NPs in the biomedical fields. [18–19]

1.3.1 Basic Crystal Structures of ZnO:

Zinc oxide crystallizes in three forms i.e. hexagonal wurtzite, cubic zincblende, and cubic rock salt. The wurtzite structure is most stable at ambient conditions and thus most common. The zincblende form can be 3 stabilized by growing ZnO on substrates with cubic lattice structure. In both cases, the zinc and oxide centers are tetrahedral. The rock salt (NaCl-type) structure is rarely observed and it is only observed at relatively high pressures of about 10 Gpa. Despite of these three basic forms zinc oxide can be induced to form a very large variety of crystalline shapes using specialized growth[20].

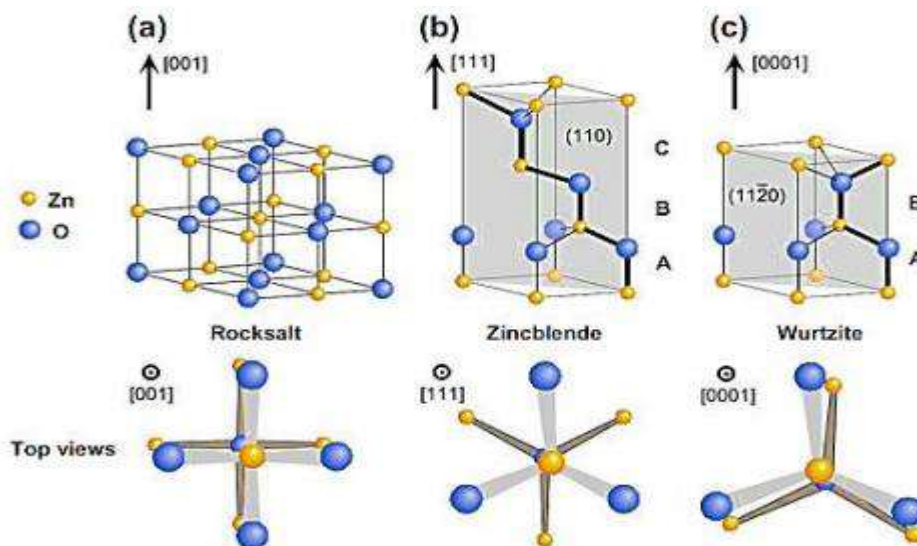


Fig (3) : Stick and ball representation of ZnO crystal structures (a)cubic rocksalt (B1),(b) cubic zincblende (B3) and (c) hexagonal wurtzite(B4).

1.4 Graphene oxide (GO):

Graphene oxide (GO) is an oxidized derivative of graphene, a fascinating carbon material that has spurred significant interest in the last 10 years. GO contains a large number of oxygen bonds, such as hydroxyl and epoxy functional groups on the hexagonal network of carbon atoms and carboxyl groups at the edges. These abundant oxygen functional groups not only assist the dispersion of GO in water as a stable colloidal suspension but also provide active sites for functionalization and hybridization with other materials, especially metal and metal oxide through both electrostatic and coordinate approaches. Various metal/metal oxide and GO composites have been synthesized and applied in different fields, such as biosensors, capacitors, and photocatalysts. Recently, the antibacterial properties of GO have been reported also. GO shows obvious antibacterial activity at concentrations of around $40 \mu\text{g mL}^{-1}$. In the meantime, GO possesses good biocompatibility. Therefore, we aim to elucidate that the composite of ZnO NPs and GO may reach more superior antibacterial ability compared to ZnO NPs or GO alone[21].

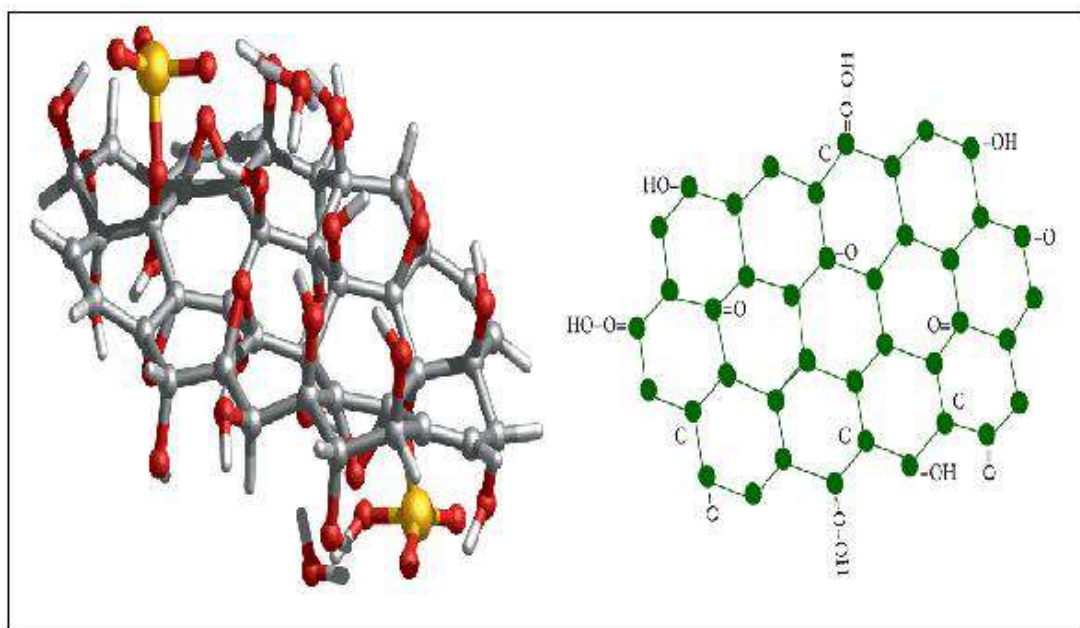


Fig (4) : Structure of Graphene Oxide

1.5 ZnO/GO composite:

Herein, employing a facile one-pot reaction, we synthesized ZnO/GO composites. A typical bacterium, *Escherichia coli*, was used to evaluate the antibacterial activity and HeLa cells for the cytotoxicity study. The results showed that the composites have a much stronger ability to kill bacteria at low concentrations, which do not affect the viability of the HeLa cells at all. Synthesis and Characterization of the ZnO/GO Composites. GO sheets are graphene basal planes covered mostly with epoxy and hydroxyl groups, while carbonyl and carboxyl groups are located at the edges. These oxygen-containing groups, acting as anchor sites, enable the in situ formation of nanostructures on the surfaces and edges of the GO sheets[22]. In the reaction, zinc ions from reactant zinc acetate bound to oxygen atoms of negatively charged oxygen-containing functional groups on GO by the electrostatic force and coordination reaction, acting as anchor sites for the growth of ZnO NPs. With the addition of OH⁻, a large number of crystal nuclei formed at the anchor sites in a short time. Then, ZnO NPs grew larger along the planes and edges of the GO sheets to form the ZnO/GO composites. Finally, ZnO NPs uniformly anchored on the GO sheets. The density and size of ZnO NPs on GO can be regulated by adjusting the quantity of the reactants (GO and zinc ions) and by altering the reaction time[23].

1.6 Metronidazole:

Metronidazole is a commonly used antibiotic, belonging to the nitroimidazole class of antibiotics. It is frequently used to treat gastrointestinal infections as well as trichomoniasis and giardiasis, and amebiasis which are parasitic infections. Metronidazole has been used as an antibiotic for several decades with added

antiparasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections. It is available in capsule form, tablet form, and topical form, and suppository preparations for the treatment of various infections[24-25] .

Type	Small Molecule
Chemical Formula	$C_6H_9N_3O_3$
Molecular weight	mol/g 171
Brand Names	Flagyl , Metroloction , Vandazole , Flagystatin

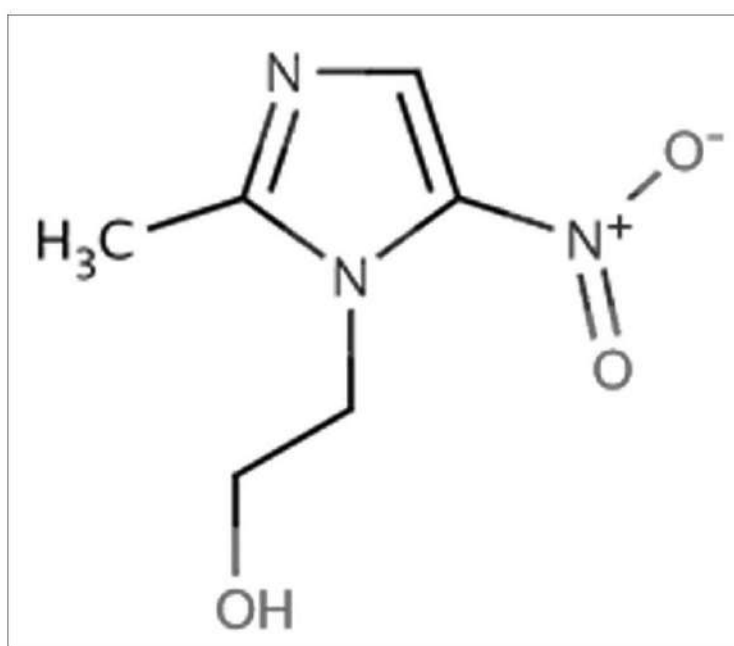


Fig (5) : Structure of Metronidazole drug

Dosage Forms & Strengths:

Capsule 375mg

Tablet 250mg , 500mg

tablet, extended-release 750mg

infusion solution 500mg/100mL



Metronidazole is indicated for the treatment of confirmed trichomoniasis caused by *Trichomonas vaginalis* (except for in the first trimester of pregnancy) and the patient's sexual partners, certain types of amebiasis, and various anaerobic infections. The above anaerobic infections may occur on the skin and skin structures, the abdomen, the heart, reproductive organs, central nervous system, and the respiratory system. Some may also be present in the bloodstream in cases of septicemia. Common infections treated by metronidazole are *Bacteroides* species infections, *Clostridium* infections, and *Fusobacterium* infections, as well as *Peptococcus* and *Peptostreptococcus* infections. Topical formulations of metronidazole are indicated for the treatment of inflammatory lesions of rosacea[17,20] .

DOSING OF METRONIDAZOLE		
Condition	Adult Dosing (18-64 years)	Child Dosing
Bacterial Infections	7.5mg/kg of body weight given every 6 hours for seven to ten days	As determined by the physician, based on the child's age and body weight
Amebiasis Infection	500 - 700mg thrice daily for five to ten days	
Trichomoniasis infection	250mg three times a day for seven days	

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1.6.1 Mechanism of action:

Metronidazole diffuses into the organism, inhibits protein synthesis by interacting with DNA, and causes a loss of helical DNA structure and strand breakage . Therefore, it causes cell death in susceptible organism[22] .

1.6.2 side effects:

Dizziness, headache, stomach upset, nausea, vomiting, loss of appetite, diarrhea, constipation, or metallic taste in your mouth may occur . This medication may cause your urine to turn darker in color. This effect is harmless and will disappear when the medication is stopped and Toxicity The oral LD50 of metronidazole in rats is 5000 mg/kg [25] .

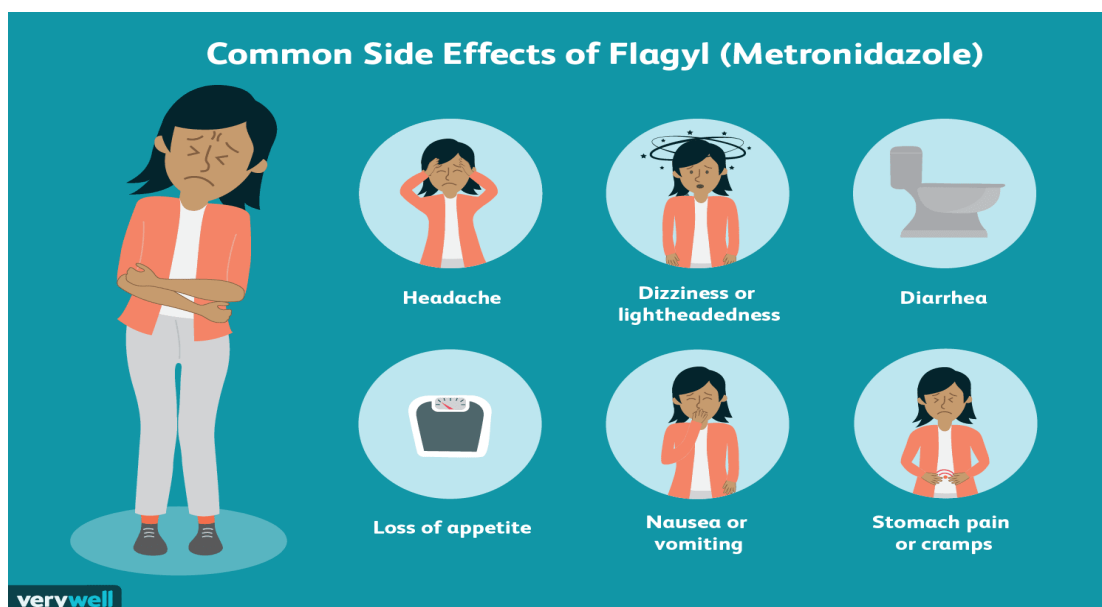


Fig (6) :common side effects of metronidazole

1.7 Drug Delivery:

Drug delivery refers to approaches, formulations, manufacturing techniques, storage systems, and technologies involved in transporting a pharmaceutical compound to its target site to achieve a desired therapeutic effect. Principles related to drug preparation, route of administration, site-specific targeting, metabolism, and toxicity are used to optimize efficacy and safety, and to improve patient convenience and compliance. Drug delivery is aimed at altering a drug's pharmacokinetics and specificity by formulating it with different excipients, drug carriers, and medical devices[26]. There is additional emphasis on increasing the bioavailability and duration of action of a drug to improve therapeutic outcomes. Some research has also been focused on improving safety for the person administering the medication. For example, several types of microneedle patches have been developed for administering vaccines and other medications to reduce the risk of needlestick injury[27]. Drug delivery is a concept heavily integrated with dosage form and route of administration, the latter sometimes being considered part of the definition. While route of administration is often used interchangeably with drug delivery, the two are separate concepts. Route of administration refers to the path a drug takes to enter the body whereas drug delivery also encompasses the engineering of delivery systems and can include different dosage forms and devices used to deliver a drug through the same route [29]. Common routes of administration include oral, parenteral (injected), sublingual, topical, transdermal, nasal, ocular, rectal, and vaginal, however, drug delivery is not limited to these routes and there may be several ways to deliver medications through other routes.[30] Since the approval of the first controlled-release formulation in the 1950s, research into new delivery systems has been progressing, as opposed to new drug development which has been declining. Several factors may be contributing to

this shift in focus. One of the driving factors is the high cost of developing new drugs. A 2013 review found the cost of developing a delivery system was only 10% of the cost of developing a new pharmaceutical . A more recent study found the median cost of bringing a new drug to market was \$985 million in 2020, but did not look at the cost of developing drug delivery systems. Other factors that have potentially influenced the increase in drug delivery system development may include the increasing prevalence of both chronic and infectious diseases as well as a general increased understanding of the pharmacology, pharmacokinetics, and pharmacodynamics of many drugs[30].



Fig (7): Different routes of drug delivery.

Chapter two

Method and Literature

review

Table 2.1. A simple review and comparison of results of some articles on Zinc Oxide different applications prepared by nanotechnology.

NO.	Synthesis method	Characterization	Application	Refs.
1	Sol-Gel	1-FESEM 2-XRD 3- EDX	anti-corrosion, anti-bacteria	[31]
2	Green synthesis of zinc oxide	1- UV–Visible. 2- FT-IR 3- XRD 4- SEM	Anti-bacterial	[32]
3	Precipitation	1-FT-IR 2-XRD 3-TEM	photocatalytic degradation	[33]
4	The direct thermal precipitation technique	1- XRD. 2- EDX	indicate antibacterial activity towards the Gram-positive Staphylococcus.	[34]
5	polymer nanocomposites	SEM FT-IR	antibacterial capability	[35]
6	Emulsion	ESM XRD	preparing ultrafine ceramic powder having controlled morphology and composition	[36]
7	Green synthesis of zinc oxide	UV – visible FTIR TEM	technological applications, because of its exceptional optical and electrical properties, such as thin-film transistors, gas sensors, transparent conductors, biomedical and piezoelectric applications	[37]

2.2. Synthesis of ZnO nanoparticles

The direct thermal precipitation technique has been used in preparing zinc oxide NPs.

Using deionized water, the preparation of KOH and zinc nitrate (0.4M and 0.2M, respectively) took place at room temperature, marginally adding the aqueous solution to the zinc nitrate while constantly stirring, followed by controlling the temperature at

60 °C for 120 min, forming a white precipitation. The resulting mixture is centrifuged at 500 rpm for 20 minutes, followed by a triple wash in deionized water and absolute alcohol. Kept at 300, 500°C for 2 hours, the formation of zinc oxide is facilitated using a custom prepared tubular muffle furnace with no calcination.[39]

2.2 Synthesis ZnO/GO Composites

Prepare the ZnO/GO composite, approximately 10 mg GO was dispersed into 150 mL of distilled water and sonicated for 10 min, Zinc Oxide powder was added to the GO suspension with continuous stirring. After that, it was sonicated for 60 min. , the suspension containing GO and ZnO particles. After that, the composite was processed under stirring for 12 h. The composites were prepared using ratios of ZnO and GO. This mass ratios was (25:1) [38].

2.3 loading metronidazole on ZnO/GO NPs

The loaded ZnO nanoparticles with metronidazole . To prepare the ZnO/ GO/ metronidazole , approximately 7.5 mg of metronidazole were dissolved in 10 mL of a mixture of metronidazole and 20 mg ZnO and sonicated for 4 h. The solutions were stirred for 24 h at room temperature [39].

Chapter three

Results and discussion

3.1 Morphological Analysis SEM

The SEM was used to study the morphologies of the as-prepared GO, ZnO, and ZnO/GO nanomaterials, Figure 8(A) displays the SEM images at a higher magnification, and demonstrates the formation of particles with a size of rang (35-70)nm by Image J software. 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. The graphene sheets are not perfectly flat but intrinsically microscopic roughening and out-of-plane deformations (wrinkles). Besides, some dispersed GO sheets could connect randomly to each other that brought about a porous structure with numerous cavities or holes, which provides additional possibilities to form ZnO-GO nanohybrid between precursor and GO. Figure 8(B) clearly shows that ZnO nanoparticles appear as granule-like nanostructures and the powder aggregation at different levels. It can be seen from Figure 8(C) that the surface of GO is covered rigorously by ZnO crystals, demonstrating a good combination between GO sheet and ZnO nanoparticles. The ZnO-GO catalyst shows a cross-linked flaky structure and excellent dispersibility without agglomeration, and the structure presents an average thickness of about rang (20-40) nm. It can be observed obviously that the ZnO-GO nanocomposites collapsed into a smaller average sizes. It can be seen that the calcined samples have better dispersion.

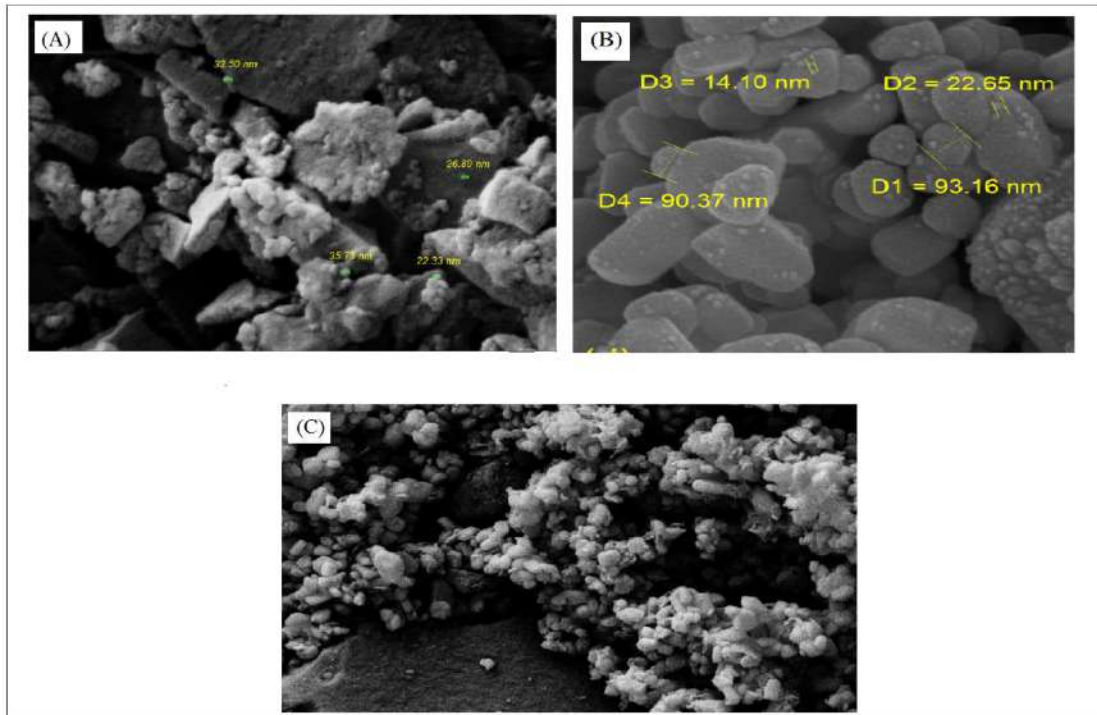


Fig 8: SEM images of (A) GO, (B) ZnO, (C) ZnO-GO nanocomposite

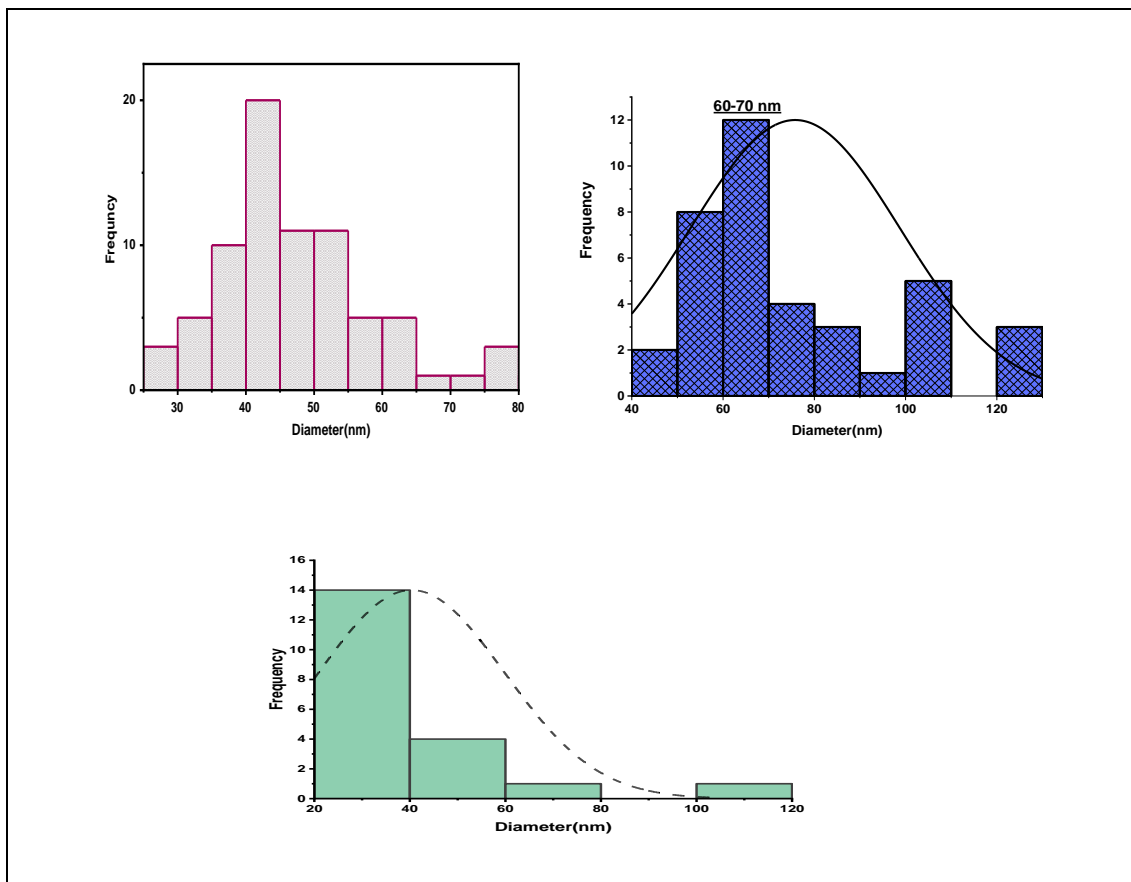


Fig 8: Histogram show the distribution of (a) GO, (b) ZnO, (c) ZnO-GO nanocomposite

Fig. 9 exhibits the SEM imaging of the metronidazole- ZnO-GO. The surface shape of metronidazole has altered after being loaded with ZnO-GO. As we can see in Fig. 9B, the NPs manifested as granules with a light color which are evenly spread over the surface of metronidazole. In Fig. 9B, we can see the SEM micrograph of the ZnO-GO after reaction with metronidazole. The morphology of these ZnO-GO was altered completely, and the greatest available holes and pores on the surface of ZnO-GO were occupied by metronidazole particles. When comparing Fig. 9A with Fig. 9B, we can see the significant change in the morphology of metronidazole-ZnO-GO.

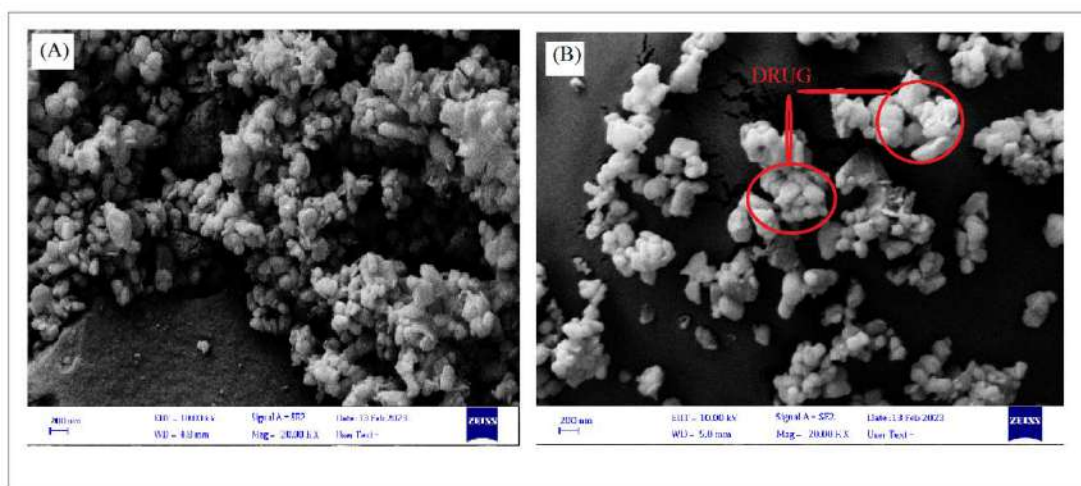
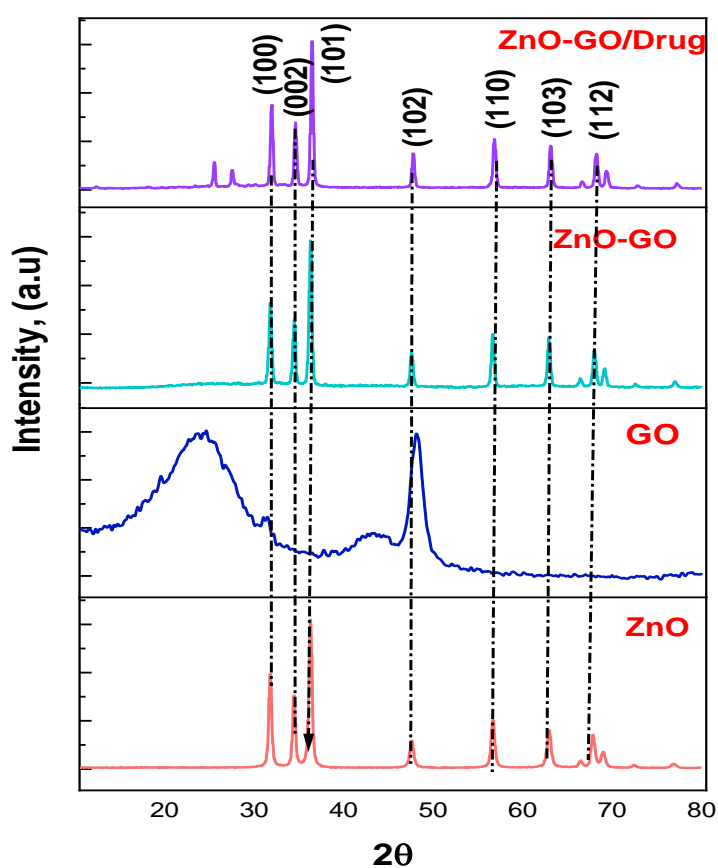


Fig 9: SEM images of(A) ZnO-GO nanocomposite, and (B) loaded ZnO-GO/metronidazole

3.2 X-ray diffraction pattern (XRD) :

Structural Analysis. In order to gain the crystal structure information of ZnO and ZnO-GO as well as the influences from different preparation conditions, the XRD measurements of the photocatalysts were conducted, as shown in Figure 10. The characteristic peaks at $2\theta = 31.7^\circ$, 34.4° , 36.2° , 47.5° , 56.7° , 63.0° , 66.4° , 68.1° , and 69.3° were observed from the XRD patterns of ZnO and ZnO-GO nanocomposites, corresponding to the planes (100), (002), (101), (102), (110), (103),

(200), (112), and (201), respectively (Figure 10), indicating the existence of ZnO . No existence of other phases or impurity was found, indicating the high purity of catalysts[40-41]. It is notable that the characteristic diffraction peak of GO at $2\theta = 24.6^\circ$ was not found in the XRD pattern of ZnO-GO, which was probably because ZnO crystals were covered by limited amounts of GO that changed its structure[42]. According to related literatures[43], the presence of grain boundaries in ZnO and ZnO-GO nanocomposition can be proved owing to the existence of amorphous superficial and intergranular layers between ZnO and ZnO/GO.



**Fig 10: X-ray diffraction (XRD) spectra of ZnO ,GO, ZnO-GO, and loaded ZnO-GO/
metronidazole**

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