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Drug Loading on CuO nanoparticles synthesized by chemical method

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بسم الله الرحمن الرحيم
رب اشرح لي صدري
ويسر لي أمري

الاهداء

وصلت رحلتنا الجامعية إلى نهايتها بعد تعب ومشقة..
وها نحن ذا نختم بحث تخرُّجنا بكل همَّة ونشاط،
ونحن ممتنين لكل من كان له فضل في مسيرتنا،
وساعدنا ولو باليسير،
الأبوين ، والأهل ، والأصدقاء ، والأساتذة المُبجَّلين..
أُهديكم بحث تخرُّجي

شكر و تقدير

اشكر الله عز وجل و أحمده على توفقيه لنا
لإنجاز هذا البحث
كما أتقدم بخالص الشكر الى الاساتذة المشرفين
"الدكتورة اسماء هاشم حمادي" و"الدكتورة
صبا عبد المنعم حبيب" و "الأستاذ غسان جاسم محمد"
على ارشادهم وتوجيههم لنا
الشكر موصول ايضا الى الاساتذة اعضاء لجنة المناقشة
الذين تفضلوا بقراءة هذا البحث الاخير اشكر كل من ساهم من
قريب أو بعيد في انجاز هذا البحث

Contents:

Abstract:

in the present work capper oxide nano powder sis synthesized through thermal reduction as a chemical method in ascorbic acid media. Further, microscopic techniques such as SEM established the formation of multi-shaped copper complex with an average size of 25-30 nm. XRD characterization was employed to analyzed phases and partials sizes were in average 19.7 nm for CuO complex yield.

In addition to this, CuO complex nano-powder conjugated with Oxytetracycline (antibiotic)through chemical method. Later, antibacterial potential of Cu element..

Chapter One

Introduction

1.1 nanomaterials

Nanomaterials mainly refer to some particles with certain physical or chemical properties or biological effects, whose external size or internal size or surface structure are within the nanoscale range (1 nm-100 nm) [1] . Nanomaterials can be divided into organic and inorganic nanomaterials, of which organic nanomaterials include nanofibers, nanotubes, liposomes, and polymer nanoparticles and inorganic nanomaterials include elementary substances, alloys, silica, and quantum dots [2] .

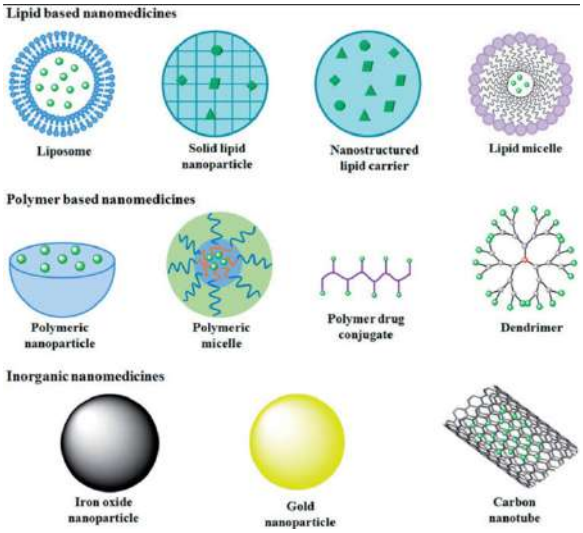

Fig1: Different types of nanomaterials

Nanomaterials can effectively transport and load drugs since they have the largest specific surface area among all the known materials at present. At the same time, nanomaterials have good biocompatibility and biodegradability, and they can accumulate in human organs with less

side effects [3] . In addition, nanomaterials have the properties of slow release, which can reduce drug concentration and toxic side effects [4] . Compared to traditional drugs which are with defects such as being ubiquitous in poor stability, apt to deform and inactivate, short biological half-life, and low bio-availability, and unable to easily go through the physiological barrier, biological nanomaterials play a role that cannot be ignored in the field of biological medicine, such as in diagnosis, treatment, repair, or replacement of the damaged organization. For example, the nanoparticles with small size are easy to be swallowed up by cells; nanodrugs of large specific surface area and more functional groups or active centers can realize a large load of specific drugs; nanomaterials with the characteristics of porous, hollow, multilayer structures are easy for control and release of drugs, so as to change the half-life period of drugs in the body and prolong the action time of drugs. With the deepening of research on nanomaterials, nanomaterials have been developed from being only the delivery carrier of drugs to a new type of materials which are with certain biological effects and can participate in the treatment of diseases [5] . With the continuous innovation of nanomaterials, the physicochemical properties and structural characteristics of nanodrugs are enriched and the multifunctional nanomaterials have great application potential in the field of biomedicine.

In the recent past, metal and metal oxide nanomaterials have received a great deal of attention and have mobilized various synthetic routes [6-7]. The wide range of efficiency and versatility of these nanomaterials have further promoted their designing and has also initiated the tailoring of suitable size and morphology of metal and metal oxide NPs. Synthesis of nanoscale transition metal oxides [8], such as tin oxide, iron oxide ,titanium oxide, copper oxide, etc., are of significant

interest owing to their enhanced catalytic activity, high thermal conductivity, excellent antibacterial/antimicrobial activity, easy availability of economical & up-scalable synthetic routes and non-toxicity.

The advantages of nanomaterials are their large surface area and small particle size , making them excellent for synthesizing pharmaceutical formulations [9]. Metal oxide nanoparticles have recently come up as a promising research area due to their vast range of applications [10] . Copper oxide nanoparticles (CuO NPs) have been widely studied nanoformulation owing to their intriguing physical, biochemical, and pharmacological features [11] . Cu-based products have been permitted for human use by the United States Environmental Protection Agency (USEPA) since February 2008. These nanoparticles are widely explored because they are essential trace element and have significant roles in metabolism and physiological processes[12].

Fig2: copper

Fig3:powder CuO nanoparticles

1.2 Nanocarriers

It has to be noted that more than two-thirds of prescribed antibiotics are ineffective against intracellular pathogens [13]. Instead of looking for new conventional antibiotics, some groups are seeking to use nanotechnology in order to improve the therapeutic index of not only antimicrobial drugs that are already on the market, but also those that will be developed in the future. In fact,delivery of antibiotics to localities within the host where infections are difficult to treat, such as into cells, is a promising solution to treat intracellular infections. In this regard, the use of nanodevices able to enter intracellularly through similar endocytic/phagocytic pathways to bacteria may represent an interesting approach to allow the targeted delivery of antibiotics intracellularly. In the past few decades, the design of nanodevices employed to carry and protect drugs has been mainly considered in oncology, but also for the treatment of infectious diseases [14] . With the help of nanocarriers, not only can the drug be efficiently delivered to the infection site (i.e. intracellular), but also the amount and frequency of dosage can be controlled, thereby preventing toxicities related to therapy.

Criteria/guidelines for the design of efficient drug nanocarriers

More than 40 years of research in the drug delivery field have enabled refinement of the specifications for the design of optimal nanocarriers for pharmaceutical drugs . First of all, a safe and pharmacologically efficient vehicle has to be biocompatible and biodegradable, but it also needs to:

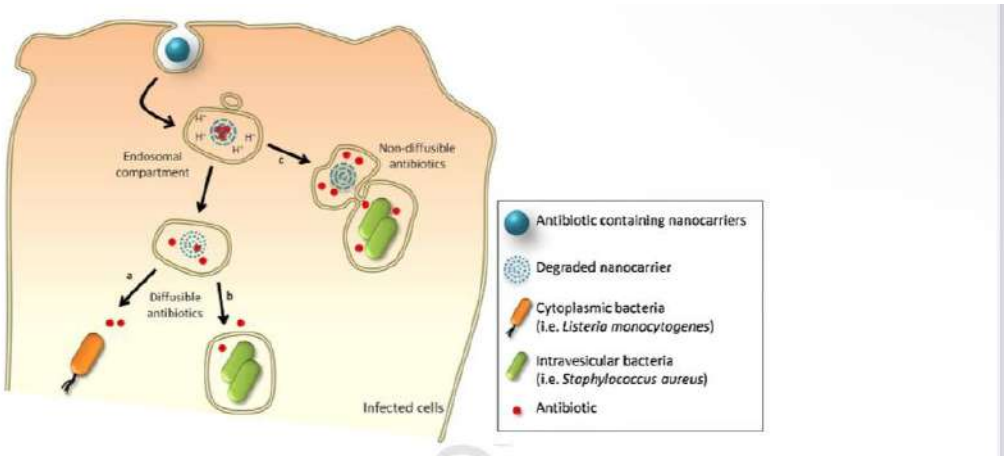

Fig4:Intracellular delivery of antibiotics to treat intracellular infections

- Have a high drug payload in order to achieve drug therapeutic concentrations at the diseased tissues or cells, eventually allowing sustained drug delivery;
- Prevent the 'burst release' of the encapsulated drug before reaching the target site, hence decreasing side effects on healthy tissues;
- Be inert against the encapsulated drug and protect it from possible degradation/metabolization; and

- Be able to deliver the drug at the right place in the body.

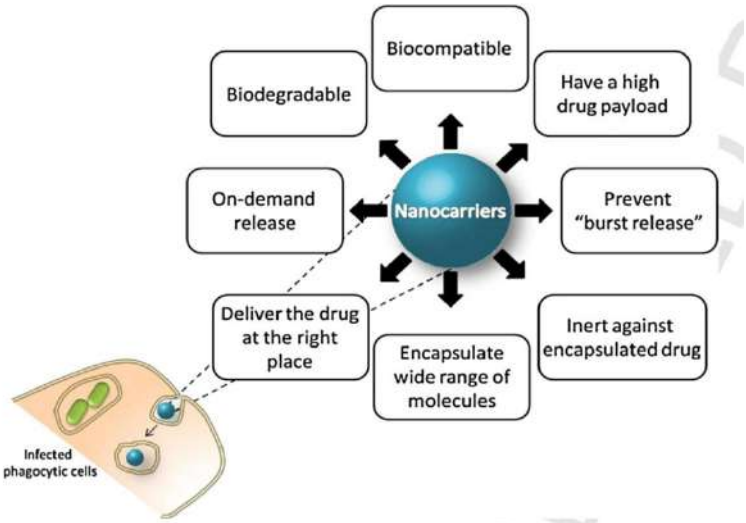

Fig5: Characteristics of an ideal nanocarrier for intracellular drug delivery

So far, different kinds of vehicles have been explored as drug delivery platforms, such as liposomes, micelles, dendrimers, nano- tubes and nanoparticles (i.e. polymeric nanoparticles, solid lipid nanoparticles, polymersomes, etc.)[15]

Liposomes

The best known and most widely investigated platform for drug transportation is the liposome. Although discovered in the mid 1960s , they were introduced as drug delivery vehicles in 180 the 1970s, and in 1995 the first liposomal pharmaceutical product, Doxil , received US Food and Drug Administration (FDA) approval [16].

Liposomes are spherical vesicles with a diameter ranging from 20nm to several micrometres, consisting of one or more lipid bilayers surrounding aqueous spaces [17] . One of their main advantages is that they are made from natural, non-toxic, non-immunogenic and biodegradable

lipid molecules (mainly phospholipids) and they can encapsulate or bind diverse drug molecules into or onto their membranes. These lipid-based drug nanocarriers are flexible since they can carry hydrophilic or hydrophobic drugs, or both together.

Polymeric nanoparticles

Polymeric nanoparticles were developed later than liposomes in order to improve stability and drug payload.Polymeric nanoparticles are solid particles made of a polymer matrix (nanospheres) or a polymer shell (nanocapsules) with a size between 10 nm and 1000, although most of those reported in the literature display a diameter ranging from 50 nm to 350 nm [18].A wide range of bioactive agents can be either physically entrapped or encapsulated in nanoparticles or can be chemically conjugated to a polymer.Polymers used for nanoparticle construction are either natural or synthetic. Natural polymers include albumin, collagen, gelatin, chitosan, haemoglobin and alginate, but their use in the medical field has decreased due to higher costs and/or doubtful purity. The second group consists of synthetic biodegradable and biocompatible polymers such as poly(amides), poly(amino acids), poly(alkyl- -cyanoacrylates), poly(esters) and poly(ortho esters) . Among them, poly(lactide) (PLA), poly(glycolide) and especially the copolymers of poly(lactide-co-glycolide) (PLGA) have generated remarkable interest in drug delivery [19]. The main advantage of these degradable polymers is their ability to break down into biologically tolerable molecules that are metabolised and removed from the body via normal metabolic pathways.

Solid lipid nanoparticles (SLNs)

SLNs may be considered as ‘hybrid’ nanodevices between polymer nanoparticles and liposomes. In contrast to liposomes, SLNs are rather amorphous and do not display a bilayer structure; they are made up of a solid lipid core stabilized by surfactants. Some advantages of SLNs are their long-term stability, their scaling-up feasibility, the possibility to incorporate lipophilic but also hydrophilic drugs, as well as their biocompatibility . In fact, according to certain studies, even in the presence of surfactants, SLNs display lower cytotoxicity than PLA/PLGA nanoparticles [20].

Other nanocarriers

Polymersomes

Terpenoid-based nanoparticles

Dendrimers

are highly ordered and regularly branched globular macromolecules with a well defined core–interior–periphery architecture that distinguishes them from traditional polydisperse linear polymers . The properties of these molecules are dominated by the presence of functional end groups on the molecular surface, and the nature of these peripheral groups governs the solubility and related biological properties such as biocompatibility[21].

1.3 copper oxide nanoparticles CuO NPs

As particles are reduced from a micrometre to a nanometre size, the resultant properties can change dramatically. For example, electrical conductivity, hardness, active surface area, chemical reactivity and biological activity are all known to be altered. The bactericidal effectiveness of metal nanoparticles has been suggested to be due to both their size and high surface-to-volume ratio. Such characteristics should allow them to interact closely with bacterial membranes, rather than the effect being solely due to the release of metal ions. In theory, metal nanoparticles could be combined with polymers or coated onto surfaces, which may then have a variety of potential antimicrobial applications. The antimicrobial properties of both silver and copper nanoparticles have been previously reported, and both of these have been coated onto or incorporated into various materials.

Copper oxide is a compound from two elements copper and oxygen, which are block d and block p elements in periodic table respectively. In a crystal copper ion is coordinated by four oxygen ions. Copper (Cu) and copper oxide (Cu_2O) nanoparticles have attracted considerable attention because copper is one of the most important in modern technologies and is readily available [22]. There is increasing interest on copper nanoparticles due to their optical, catalytic, mechanical and electrical properties. Copper oxide is widely used in the field of catalysis, superconductors, and ceramics as a kind of important inorganic materials. It can be used as a catalyst and catalyst support, as well as electrode active materials such as degradation of nitrous oxide with ammonia and oxidation of carbon monoxide, hydrocarbon and phenol in supercritical water. Copper oxide nanoparticle is a powder soluble in dilute acid, NH_4Cl, $(NH_4)_2CO_3$, potassium cyanide solution, insoluble in water, and it dissolves slowly in alcohols, ammonia solution. It can be reduced to metallic copper when meets hydrogen or carbon monoxide under high temperature. CuO

nanoparticle can also be used as burning rate catalyst in rocket propellant. Nano copper oxide shows superior catalytic activity and selectivity than that of the common copper oxide powder. The particle size of nanometre copper oxide is between 1-100 nm[23]. Compared with the ordinary copper oxide, nano CuO has peculiar physical and chemical properties such as: surface effect, superiority of the quantum size effect, volume effect and macroscopic quantum tunnelling effect in magnetic, optical absorption, chemical activity and thermal resistance, catalysis, and the melting point. Nano copper oxide attracts more and more people's attention, and become one of the most extensively used inorganic materials .

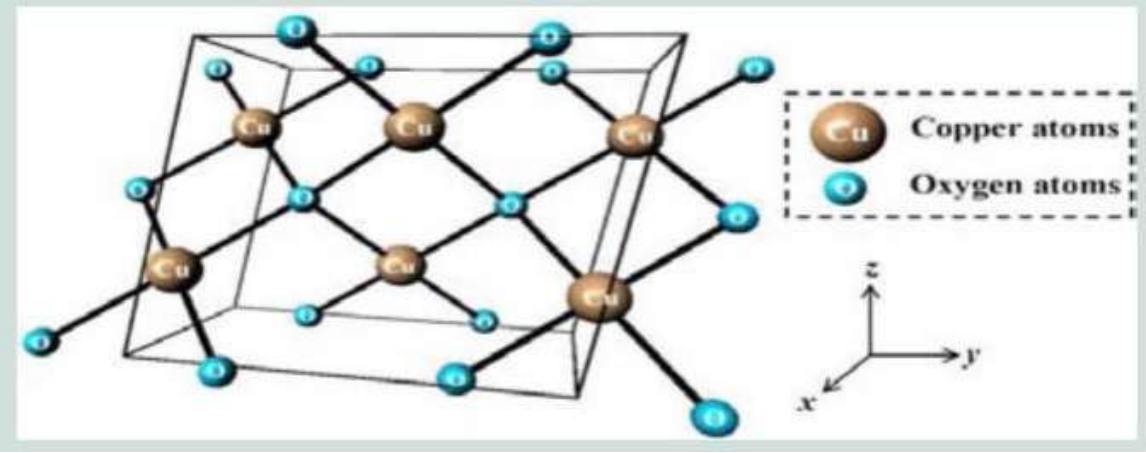

Fig6:Schematic representation of a monoclinic CuO unit cell

1.4 Antibacterial

Antibacterial activity is the most important characteristic of medical textiles, to provide adequate protection against microorganisms, biological fluids, and aerosols, as well as disease transmission.

The antibacterial activity is calculated dividing the number of bacteria present after 24 hours of cultivation onto a testing treated article/product (C) into the number of bacteria present after 24 hours of cultivation onto the corresponding untreated (without antimicrobial agent) article/product.

Antibacterial technologies are effective against a broad spectrum of harmful bacteria and they will typically incorporate several active ingredients, allowing for successful application in a wide variety of product types. Antimicrobial technologies actually minimize the presence of bacteria, mold, and fungi[24].

The features of an ideal antibacterial drug are as follows:

- Selective target---target unique.
- Bactericidal---kills the bacteria.
- Narrow spectrum---does not kill normal flora.
- High therapeutic index---ratio of toxic level to therapeutic level.
- Few adverse reactions---toxicity, allergy.
- Various routes of administration.

The findings add strong support to a contact killing mechanism of copper oxides (CuO and Cu_2O) through which bacteria initially suffer severe damage to the cell envelope. Then further damage ensues by an independent pathway of each copper oxide nanoparticles. Formation of copper (I)-peptide complex from cuprous oxide (Cu_2O) and free radical's generation from cupric oxide (CuO) were identified as key sources of toxicity towards E.coli. Cu_2O rapidly inactivated Fumarase A, an iron sulphur cluster enzyme suggesting that cuprous state of copper binding to the proteins. This inactivation was not noticed in CuO. The percentage of biocidal / bacteriostatic activity is closely based on the oxidation state of copper oxides. In the case of E.coli, Cu_2O nanoparticles showed more efficient antibacterial activity and higher affinity to the bacterial cells. CuO nanoparticles produced significant ROS in terms of super oxides while Cu_2O did not. The diminishing defective emission peaks of Cu_2O after incubation with microbes strongly proposes the formation of protein complexes. This work is carried out to enable better understanding of the mechanistic pathways of copper oxide nanoparticles[25].

Oxytetracycline is a tetracycline antibiotic is a bacteriostatic antibiotic. Newly discovered, additional mechanisms of action include antioxidant, anti inflammatory and immynosupresive activity of oxytetracycline and other tetracyclines. These activities were the basis for developing therapy regimens with oxytetracycline in subantimicrobial doses. Due to its significant efficacy, limited adverse effects and low therapy costs, oxytetracycline at the dose of 500 mg per day is presently considered as therapy of choice in papulopustulous acne. Rosacea and perioral dermatitis are other indications. Topical oxytetracycline shows significant efficacy in primary and secondary skin infections with inflammatory reaction[26].

Once inside the cell, tetracyclines bind reversibly to the 30S ribosomal subunit at a position that blocks the binding of the aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex. Protein synthesis is ultimately inhibited, leading to a bacteriostatic effect.

Fig7: oxytetracycline

1.5 Loading oxytetracycline on CuO

The existence of antibiotics in the aquatic environment has been considered as one of the emerging environmental problem and attracted the attention of researchers due to their adverse effects on aquatic and terrestrial ecosystems even at extremely low concentrations. Moreover, the presence of antibiotics in aqueous systems may cause the evolution of antibiotic resistant superbugs . Some of the commonly used antibiotics have long half-lives and hence, persist in aqueous environment for a longer time. These antibiotics adversely affect the water quality and aquatic life. The presence of antibiotic residues in aqueous environment may lead to the development of antibiotic resistance genes and antibiotic resistant bacteria . The spread of antibiotic resistance may be due to the antibiotic residues present in wastewater [27]. Tetracyclines belong to the family of broad spectrum antibiotics.

Tetracyclines including OTC are not completely absorbed by humans and animals after oral and intravenous administration but a significant fraction of these drugs are excreted through urine

and faeces . Hence, these drugs enter the aqueous environment through soil, municipal wastewater and direct discharge of animal Wastewater . Various water treatment methods such as electro coagulation , fenton process , ozonation , photocatalytic degradation, membrane filtration and adsorption have been utilized for the removal or degradation of doxycycline from aqueous systems. Adsorption has proven to be an economic and effective technique and associated with advantages of low cost, availability of different adsorbents, easy handling, high efficiency and reuse ability[28].

Compared with the above methods, adsorption technology is a better choice for removing TCs because of its simplicity, ease of operation, high efficiency, relatively low cost, and no high toxicity by-products .The adsorbents that have been used to adsorb TC wastewater include zeolite , activated carbon, clays, and metal oxides. In the past decade, the development of novel metal adsorbents has gradually become a new research hotspot because of their remarkable adsorption performance for the removal of many organic or inorganic substances . Metal oxides are generally used as adsorbents to remove contaminants from water due to their high mechanical and thermal properties .Metal oxides commonly used in the current removal of antibiotics include iron oxides , aluminum oxides , manganese oxides , and composite metal oxides. It was found that Cu had great ability to chelate or complex with -COOH and -OH. It can be anticipated that a Cu-Mn binary oxide has the potential to oxidize and adsorb tetracycline simultaneously. Moreover, many studies have reported excellent performance of the removal of inorganic contaminants by Cu or Mn oxides and their derivatives due to their strong ability of adsorption or oxidation, respectively[29-30].

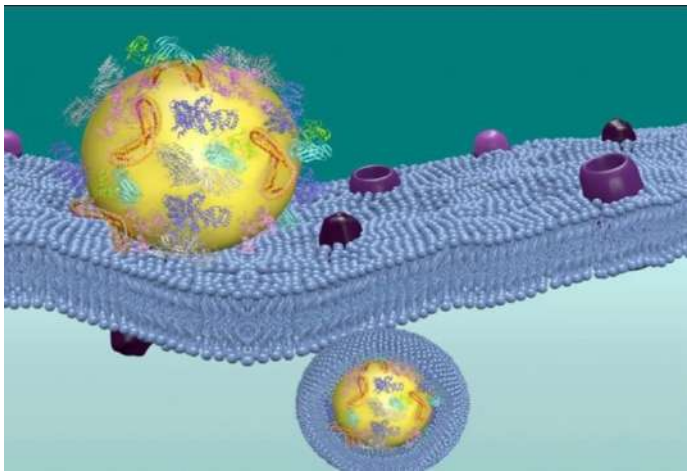

Fig8: Nanocarriers(yellow) are coated with a complex multitude of proteins before they interact with cell membranes and are adhered

Chapter Two

Litterer’s review and methods

2.1 Synthesis methods CuO nanoparticles

In recent years, researchers have been interested in copper oxides prepared with nanotechnology and applying them in various fields, especially medicines, and their effect has been proven in improving the effectiveness of drugs against diseases. The following table shows a summary of the efforts of some researchers in the manufacture of copper oxides and their medical and pharmaceutical applications

Table 2.1 summary of CuO pharmaceutical applications

NO.	Synthesis method	Characterization	Application	Refs.
1	Hydrothermal method	XRD: 21.79nm FTIR: TEM:	Antibacterial agents (E.coli)	[31]
2	Biosynthesis of CuO NPs from pumpkin seed	FTIR TEM: CuO NPs possess circular shapes with an average size of 20 nm.	Anti cancer(induction of apoptosis, increased formation of ROS, and loss of MMP). Breast cancer. Different tumor cells, such as lung adenocarcinoma (A549), leukemia monocytic cells (THP-1), and colon cancer (HCT-116), have exhibited substantial toxicity to these nanoparticles.	[32]
3	Biogenic synthesis of Cu2O NPs from the plant (Syzygium jambos (L.))	XRD: 4-10nm SEM AND TEM: octahedral particles	antibacterial activities against E. coli, S. aureus K. pneumoniae, P. mirabilisand B. cereus bacteria	[33]

5	Fabrication of CuO NPs coated gloves	CuO nanoparticles have the potential to be toxic to mammalian cells, as well as vertebrates and invertebrates 2-Copper oxide is a semiconductor metal having distinctive optical, electrical, and magnetic properties	Antibacterial activity of CuO nano Particles zone of inhibition against (a) E.coli (b) Calibicans. T	[35]
6	Biosynthesis of CuO nanoparticles and nanorod	Optical properties Magnetic properties Electrical Conductivity	superior antibacterial agent for wound healing.	[36]
7	Synthesis of Beetroot Extracts Copper Oxide Nanoparticles (BvCuONPs)	The two and three-dimensional surface topography (size and shape) of BvCuONPs was observed using; Transmission electron microscopy (TEM). UV-spectrum absorbance around 310 nm is a characteristic feature of CuONPs.	Antibacterial Activity	[7]
8	Extracellular Synthesis of CuONPs	TEM: average dimensional size of 1.72–13.49 nm, The XRD range of particles size value 20–80	Antimicrobial Activity	[38]
9	Sol-gel method	XRD SEM	Anticancer activity	[39]
10	Biogenic synthesis	XRD SEM (EDX)	potent microbicidal agents	[40]

2.2 The synthesis of CuNPs

The synthesis of CuNPs was carried out using the modification of a typical chemical reduction procedure. Copper acetate (0.05 wt %) was mixed with sodium hydroxide (1 wt %) and subjected to constant stirring using a magnetic stirrer at 90˚ C to attain a clear solution. The reducing agent, L-ascorbic acid (2.5 wt %) was added drop-wise to the mixture. With the increase in time, the color of the solution gradually turned from colorless to yellow, reddish brown and finally to dark brown. The occurrence of yellow color denoted the initiation of the reduction reaction. Reduction was permitted to continue for 15 min so as to ensure complete reduction of copper acetate. The precipitated CuNPs were centrifuged, pelleted and washed with distilled water and later using ethanol. CuNPs were finally dried in a hot air oven at 100 °C for 2 h. Optimization studies were then conducted to explore the size and shape of CuNPs[41].

2.3 loading Oxytetracycline (OTC) on CuO NPs

The loaded CuO nanoparticles Oxytetracycline (OTC) . To prepare the CuO / OTC , approximately 30 mg of OTC were dissolved in 50 mL of a mixture of OTC and 24.5 mg CuO. which was used as the drug carrier. The reaction was sonicated for 30 min. After that, it was processed under stirring for 24 h [42].

Chapter three

Results and Discussion

3.1 Scanning electron microscope (SEM) analysis

SEM images of the nanoparticles prepared via both the routes are shown in Fig. 9. Fig. 9a shows the SEM image of chemical reduction derived nanoparticles. Clear nanostructures can be seen having grain size of rang (25-30 nm) by Image J software employed to calculate particles and average size For SEM zoom 200 nm .

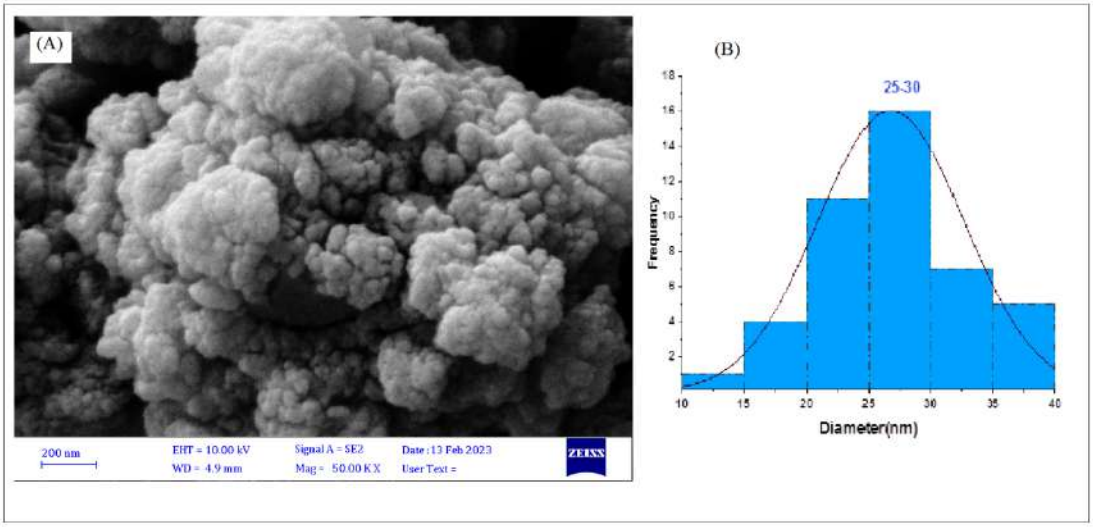

Fig 9:SEM of (A) CuO powder synthesized ,and (B) Histogram show the distribution of CnO-NPs according to their diameter

Fig. 10 displays the SEM morphology of CuO-NPs of Oxytetracycline(OTC) . This suggests that the concentrations of the drug have appropriate viscosity to synthesize NPs. Consequently, constant and regular NPs were formed effectively. This indicates that adding Oxytetracycline results in an increased diameter of the CuO-NPs.

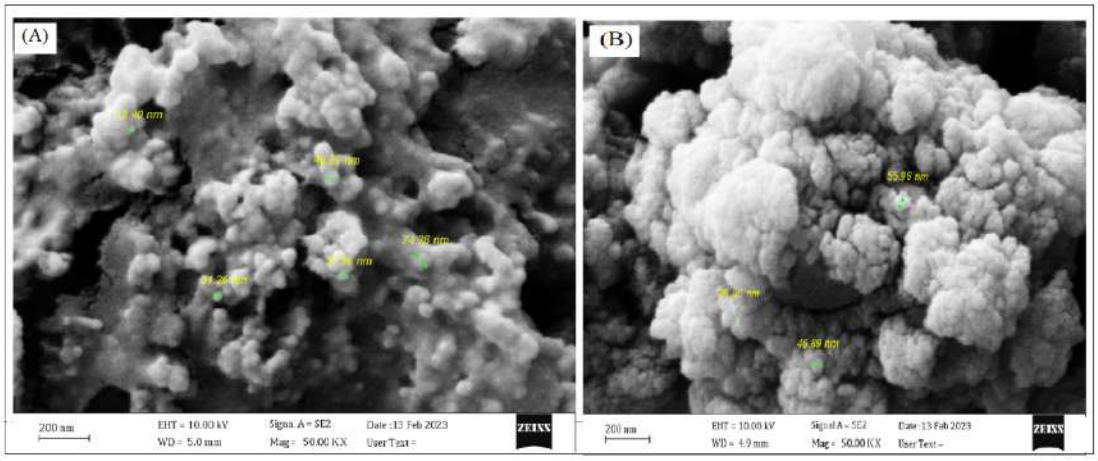

Fig 10:SEM of (A)Loading OTC /CuO , and (B) CuO powder synthesized

3.2 X-ray diffraction pattern (XRD)

The powder XRD patterns of the as-synthesized nanoparticles are shown in Fig. 11. For CuO, all diffraction peaks can be indexed to a crystalline monoclinic structure of CuO nanoparticles . The XRD reflection peaks become broader as the particle size decrease, which is a general size-dependent phenomenon in nanoparticles, because of their lattice imperfections, stacking faults and other reasons. The power XRD pattern of as-prepared Cu_2O nanoparticles is well consistent with cubic phase Cu_2O . All XRD peaks show obvious size-broading effects, indicating the finite size of these nanoparticles, which is similar to the case reported in the literature. The estimated values of the nanopartices size deduced from the full width at half maximum of the (1 1 1), (2 0 0), and (2 2 0) diffraction peaks is about 19.7 nm[43].

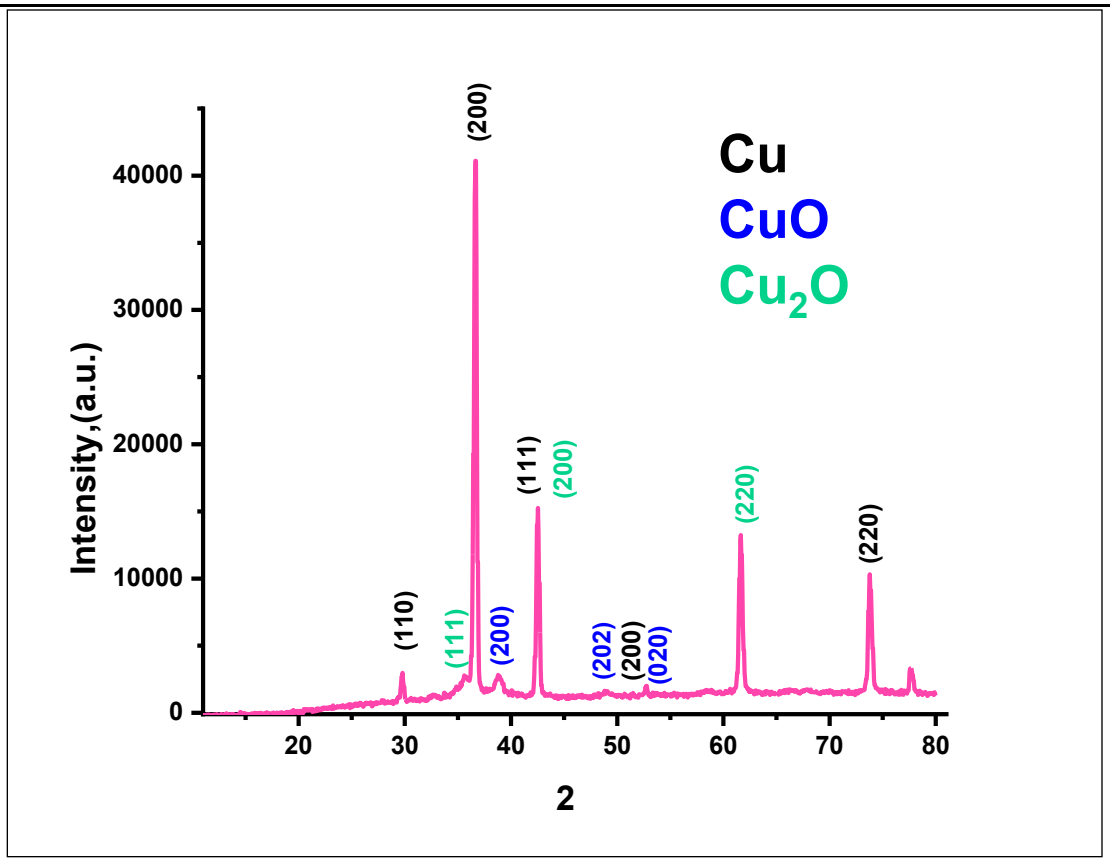

Fig 11: X-ray diffraction (XRD) spectra of CuO powder synthesized

In particular, the XRD pattern can show changes in the peak intensity and peak position of the diffraction peaks, which can be used to identify any changes in the crystal structure of CuO caused by the drug loading. Additionally, XRD can provide information on the degree of crystallinity of the material, which can help to quantify the loading of the drug onto the CuO.

Overall, XRD is a valuable tool for investigating the crystallographic structure of materials and can be used to study the loading of oxytetracycline on CuO[44].

On the other hand, the crystal phases of the prepared films were identified by XRD (Fig. 12). The main diffraction peaks are clearly observed besides the peaks . Besides, two weak diffraction peaks of CuO appear in the XRD pattern of Cu_2O sample, owing to the loaded of oxytetracycline on CuO[45].

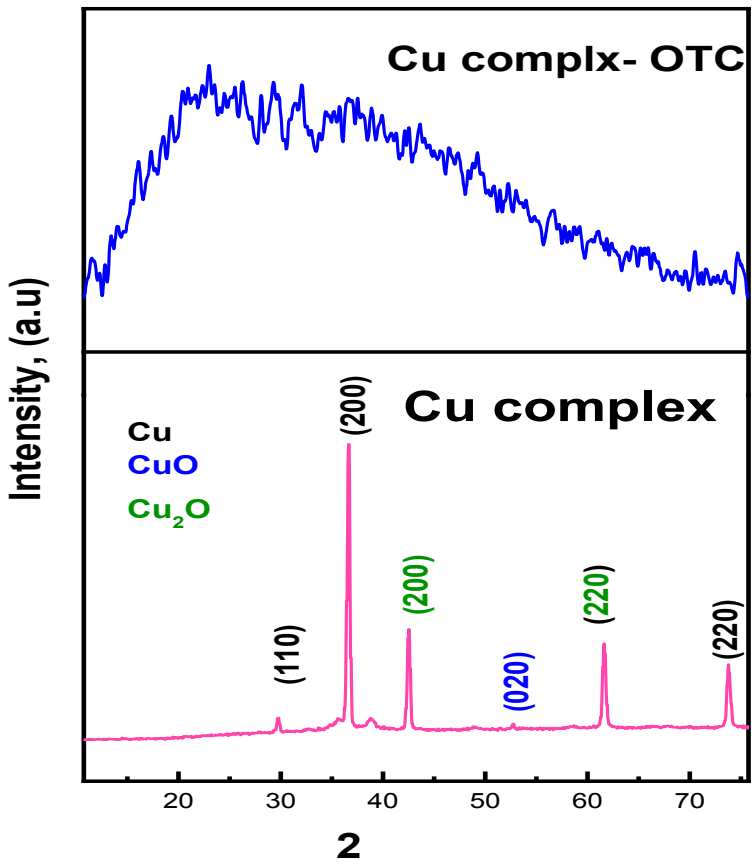

Fig 12: X-ray diffraction (XRD) spectra of Cu complex synthesized, and loaded Cu complex/OTX

References

[1] Bergeson, Lynn L., and Carla N. Hutton. "FDA's Final and Draft Nanotechnology Guidance Documents: No Big Surprises." *Nanotechnology Law & Business* 12.1 (2015).

[2] Kumar, Raj, et al. "Advances in nanotechnology and nanomaterials based strategies for neural tissue engineering." *Journal of Drug Delivery Science and Technology* 57 (2020): 101617.

[3] Qiu, Meng, et al. "Biocompatible and biodegradable inorganic nanostructures for nanomedicine: silicon and black phosphorus." *Nano Today* 25 (2019): 135-155.

[4] Chen, Qian, Chunyan Li, and Qiangbin Wang. "Multifunctional Nano-Biomaterials for Cancer Therapy via Inducing Enhanced Immunogenic Cell Death." *Small Methods* (2023): 2201457.

[5] Liu, Q. "Das M. Liu Y. Huang L." *Adv. Drug Delivery Rev* 127 (2018): 208-221.

[6] Qazi, Umair Yaqub, and Rahat Javaid. "A review on metal nanostructures: preparation methods and their potential applications." *Advances in Nanoparticles* 5.1 (2016): 27-43.

[7] Chen, Xiaoqing, et al. "Preparation of ZnO photocatalyst for the efficient and rapid photocatalytic degradation of azo dyes." *Nanoscale research letters* 12 (2017): 1-10.

[8] Guo, Ting, et al. "A comprehensive review on synthesis methods for transition-metal oxide nanostructures." *CrystEngComm* 17.19 (2015): 3551-3585.

[9] Khan, Ibrahim, Khalid Saeed, and Idrees Khan. "Nanoparticles: Properties, applications and toxicities." *Arabian journal of chemistry* 12.7 (2019): 908-931.

[10] Roy, Arpita, et al. "Biological synthesis of nanocatalysts and their applications." *Catalysts* 11.12 (2021): 1494.

[11] Zughaibi, Torki A., et al. "Evaluation of anticancer potential of biogenic copper oxide nanoparticles (CuO NPs) against breast cancer." *Journal of Nanomaterials* 2022 (2022): 1-7.

[12] Mohammed, Mohammed Razeeth Shait, et al. "Prospective of nanoscale metal organic frameworks [NMOFs] for cancer therapy." *Seminars in Cancer Biology*. Vol. 69. Academic Press, 2021.

[13] Coates, Anthony RM, and Gerry Halls. "Antibiotics in phase II and III clinical trials." *Antibiotic resistance* (2012): 167-183.

[14] Butler, Mark S., and Matthew A. Cooper. "Antibiotics in the clinical pipeline in 2011." *The Journal of antibiotics* 64.6 (2011): 413-425.

[15] Misra, Ranjita, Sarbari Acharya, and Sanjeeb K. Sahoo. "Cancer nanotechnology: application of nanotechnology in cancer therapy." *Drug discovery today* 15.19-20 (2010): 842-850.

[16] Deamer, David W. "From" banghasomes" to liposomes: a memoir of Alec Bangham, 1921-2010." *The FASEB Journal* 24.5 (2010): 1308.

[17] Drulis-Kawa, Zuzanna, and Agata Dorotkiewicz-Jach. "Liposomes as delivery systems for antibiotics." *International journal of pharmaceutics* 387.1-2 (2010): 187-198.

[18] Kim, Jang Kyoung, et al. "Natural and synthetic biomaterials for controlled drug delivery." *Archives of pharmacal research* 37 (2014): 60-68.

[19] Kim, Jang Kyoung, et al. "Natural and synthetic biomaterials for controlled drug delivery." *Archives of pharmacal research* 37 (2014): 60-68.

[20] Liu, Dongfei, et al. "Formulation and characterization of hydrophilic drug diclofenac sodium-loaded solid lipid nanoparticles based on phospholipid complexes technology." *Journal of liposome research* 24.1 (2014): 17-26.

[21] Astruc, Didier, Elodie Boisselier, and Catia Ornelas. "Dendrimers designed for functions: from physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine." *Chemical reviews* 110.4 (2010): 1857-1959.

[22] Zhang, Xi-Feng, et al. "Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches." *International journal of molecular sciences* 17.9 (2016): 1534.

[23Jeevanandam, Jaison, et al. "Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations." *Beilstein journal of nanotechnology* 9.1 (2018): 1050-1074.

[24Khan, Ibrahim, Khalid Saeed, and Idrees Khan. "Nanoparticles: Properties, applications and toxicities." *Arabian journal of chemistry* 12.7 (2019): 908-931.

[25] Shantkriti, S., and P. Rani. "Biological synthesis of copper nanoparticles using Pseudomonas fluorescens." *Int J Curr Microbiol App Sci* 3.9 (2014): 374-383.

[26] Raina, Swati, Arpita Roy, and Navneeta Bharadvaja. "Degradation of dyes using biologically synthesized silver and copper nanoparticles." *Environmental Nanotechnology, Monitoring & Management* 13 (2020): 100278.

[27] Kraemer, Susanne A., Arthi Ramachandran, and Gabriel G. Perron. "Antibiotic pollution in the environment: from microbial ecology to public policy." *Microorganisms* 7.6 (2019): 180.

[28] Mezzelani, Marica, Stefania Gorbi, and Francesco Regoli. "Pharmaceuticals in the aquatic environments: evidence of emerged threat and future challenges for marine organisms." *Marine environmental research* 140 (2018): 41-60.

[29] Kumar, Mohit, et al. "Antibiotics bioremediation: perspectives on its ecotoxicity and resistance." *Environment international* 124 (2019): 448-461.

[30] Dao, Thi Huong, et al. "Adsorption behavior of polyelectrolyte onto alumina and application in ciprofloxacin removal." *Polymers* 12.7 (2020): 1554.

[31] Chen, Nan-Fu, et al. "Investigation of the characteristics and antibacterial activity of polymer-modified copper oxide nanoparticles." *International Journal of Molecular Sciences* 22.23 (2021): 12913.

[32] Zughaibi, Torki A., et al. "Evaluation of anticancer potential of biogenic copper oxide nanoparticles (CuO NPs) against breast cancer." *Journal of Nanomaterials* 2022 (2022): 1-7.

[33] Yadav, Sushma, Arti Jain, and Priti Malhotra. "A review on the sustainable routes for the synthesis and applications of cuprous oxide nanoparticles and their nanocomposites." *Green Chemistry* 21.5 (2019): 937-955.

[34] Grigore, Madalina Elena, et al. "Methods of synthesis, properties and biomedical applications of CuO nanoparticles." *Pharmaceuticals* 9.4 (2016): 75.

[35] Zheng, Xiangyun, Li Wang, and Hong Xue. "Copper oxide nanoparticles impregnated antibacterial surgical gloves for potential application in prevention of nosocomial transmission infections during nursing." *Materials Research Express* 9.11 (2022): 115004.

[36] Babitha, N., et al. "Enhanced photocatalytic and Antibacterial Activity of Copper oxide Nanoparticles Synthesized by Facile Combustion methods from Mussaendafrondosa Plant Extract." *Physics and Chemistry of Solid State* 23.3 (2022): 443-449.

[37] Chandrasekaran, Rajkuberan, Sangilimuthu Alagar Yadav, and Sivaramakrishnan Sivaperumal. "Phytosynthesis and characterization of copper oxide nanoparticles using the aqueous extract of Beta vulgaris L and evaluation of their antibacterial and anticancer activities." *Journal of Cluster Science* 31.1 (2020): 221-230.

[38] Bukhari, Sara I., et al. "Biosynthesis of copper oxide nanoparticles using Streptomyces MHM38 and its biological applications." *Journal of Nanomaterials* 2021 (2021): 1-16.

[39] Sankar, Renu, et al. "Anticancer activity of Ficus religiosa engineered copper oxide nanoparticles." *Materials Science and Engineering: C* 44 (2014): 234-239.

[40] Rubilar, Olga, et al. "Biogenic nanoparticles: copper, copper oxides, copper sulphides, complex copper nanostructures and their applications." *Biotechnology Letters* 35 (2013): 1365-1375.

[41] Fathima, J.B., Pugazhendhi, A., Oves, M. and Venis, R., 2018. Synthesis of eco-friendly copper nanoparticles for augmentation of catalytic degradation of organic dyes. *Journal of Molecular Liquids*, *260*, pp.1-8.

[42] Cheng, Ya-Fei, et al. "Resistance of anammox granular sludge to copper nanoparticles and oxytetracycline and restoration of performance." *Bioresource technology* 307 (2020): 123264.

[43] Liu, Xiaowang, et al. "Temperature-controlled self-assembled synthesis of CuO, Cu_2O and Cu nanoparticles through a single-precursor route." *Materials Science and Engineering: A* 448.1-2 (2007): 7-14.

[44] Cheng, Ling, Yulu Tian, and Jingdong Zhang. "Construction of pn heterojunction film of Cu_2O/α-Fe2O_3 for efficiently photoelectrocatalytic degradation of oxytetracycline." *Journal of colloid and interface science* 526 (2018): 470-479.

[45] Singh, Jaspal, et al. "Sunlight mediated enhanced photocatalytic activity of TiO_2 nanoparticles functionalized CuO-Cu_2O nanorods for removal of methylene blue and oxytetracycline hydrochloride." *Journal of Colloid and Interface Science* 590 (2021): 60-71.